factors. Thus the generation of autoreactive CTLs involves at least two steps: (1) activation of F_1 CTL precursors by nonspecific diffusible mediators, and (2) selection and differentiation of CTL clones driven by self immunogen.

Autochthonous or spontaneous *in vitro* generation of anti-self H–2 CTLs by lymphoid cells of inbred or F_1 mice has been described by several investigators[32–36]. Deliberate stimulation was either not applied or was provided for by irradiated syngeneic spleen cells. In the context of the suggested model, allogeneic effect factor[32,33] and fetal calf serum[34–36] may have provided directly or indirectly the activating stimuli. Autologous or syngeneic cells present in the culture may have been the source of immunogen. In the absence of potent helper factors, or in conditions less favourable than those of mixed F_1/parent spleen-cell cultures, the generation of autoreactive CTLs is likely to be erratic and part of a broader, polyclonal lymphocyte activation[37–39]; such CTLs may be restricted by, rather than directed against, self H–2. In an interesting model of spontaneous generation of CTLs[36], the age of F_1 mice donating lymphoid cells was a critical variable, and the expression of target determinants on parental and syngeneic F_1 lymphoblasts was transient after stimulation with mitogen. Endogenous viral antigens that become expressed on stimulated lymphocytes[40–42] may have been recognized by these CTLs in the context of self H–2 determinants.

The induction of F_1 anti-parent CTLs has its closest parallel in the production of anti-self H–2 antibodies by F_1 hybrid mice immunized with cells of a parental Abelson virus-induced lymphoma[43]. The antibodies were specific for H–2D and H–2K antigens expressed on normal and neoplastic cells of H–2 homozygous and heterozygous mice. The successful induction of autoreactive B-lymphocyte effectors specific for self H–2 antigens apparently depended on the heterozygosity of responder mice, homozygosity of immunizing cells and probably on the diffusible mediators that virus-infected lymphoma cells release or induce. Hybrid resistance to parental grafts of normal or malignant haematopoietic cells[9,44–46] may represent yet another response to self H–2 determinants mediated by effectors of the natural killer (NK) type[47,48]. Like the autoreactive T- and B-lymphocyte responses, this F_1 host resistance involves recognition of, and reactivity against, the products of H–2-associated genes of homozygous parental target cells. However, no evidence has been obtained for cytotoxic or other activity (for example, cytostatic) of NK-like effectors against syngeneic targets.

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Production of anti-self H–2 antibodies by hybrid mice immune to a viral tumour

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During the course of immunization of hybrid mice (genotype $H\text{–}2^{b/k}$*) with a parental Abelson virus-induced lymphoma (genotype* $H\text{–}2^{b/b}$*), antibodies were produced to the* $H\text{–}2K^b$ *or* $H\text{–}2D^b$ *antigens of the immunizing cells. Such 'anti-self H–2' antibodies demonstrate the existence of autoreactive B-cell clones in hybrid mice, and pose intriguing questions for the nature of self-tolerance.*

THE success or failure of tissue grafts among inbred mice has been found to conform to certain empirically derived laws, one being that grafts from an inbred homozygous parent are accepted by hybrid offspring made between the donor strain and another inbred strain[1,2]. A corollary of this law is that hybrids do not generate cell-mediated or humoral immune responses to the parental graft, that is, they are tolerant to grafts of parental tissues[3–5]. The major histocompatibility complex (MHC) of the

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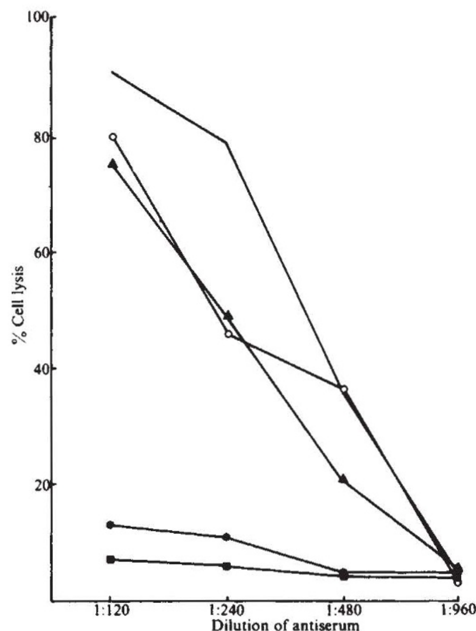

Fig. 1 Absorption of (C3H × B6)F_1 anti-B6T1 (S41 no. 2) antiserum with normal spleen cells from *H-2* congeneic mice. Cytotoxicity tests were performed on B6T1 cells as described[15] using preselected rabbit serum (dilution 1:7) as a complement source. Absorption experiments were performed as described elsewhere[15] by incubating 5×10^7 nucleated spleen cells with 120 μl of antiserum (dilution 1:120). Cells used for absorption: —, none; ●, B6 ($H\text{-}2^b$); ■, B10 ($H\text{-}2^b$); ○, B10.BR ($H\text{-}2^k$), and ▲, B6.C-H-2^d ($H\text{-}2^d$). Absorption with $H\text{-}2^b$ cells removed cytotoxic antibodies, whereas absorption with congeneic $H\text{-}2^d$ or $H\text{-}2^k$ cells did not.

mouse, *H-2*, controls these cell interactions, partly by specifying the genetically dominant expression of polymorphic transplantation antigens at the surfaces of all somatic cells[1,2,6].

Exceptions to the law of hybrid acceptance of parental grafts were noted in early tumour transplantation studies[7,8] and have also been observed in spleen and bone marrow cell transfers in certain strain combinations[9,10]. In the latter case it has been proposed that these exceptions reflect the expression of antigens by homozygous but not heterozygous mice[10]. A locus for such haematopoietic histocompatibility or Hh antigens displayed on parental cells and recognized by F_1 mice has been mapped to chromosome 17 of the mouse in the *H-2D* region[11]. The search for *in vitro* correlates of hybrid histocompatibility led to the discovery of induced and spontaneous F_1 anti-parent cytotoxic T-lymphocyte responses[12], the determinants of which also map to *H-2D* or, in some instances, to *H-2K*[13]. Because the recognition of a variety of antigens by cytotoxic T lymphocytes has been shown to require *H-2K* or *H-2D* identity on target and effector cells[14], it is unclear whether these F_1 anti-parent cytotoxic T-lymphocyte responses reflect responses to some additional antigen (perhaps Hh or viral) whose recognition is *H-2* restricted or true anti-MHC autoreactivity.

During the course of immunizations of various hybrid mice with a parental transplanted lymphoma induced by the Abelson murine leukaemia virus, we observed a serological correlate of hybrid rejection of parental tumour cells. Here we characterize this serological response and demonstrate that it is directed to the H-2K and H-2D molecules of the parental cells, although such products can also be found on cells of the responding hybrid.

A high titre of cytotoxic antibodies was produced when [C3H/HeJ × C57BL/6(B6)]F_1 mice were hyperimmunized with the transplanted B6 Abelson virus lymphoma B6T1[15]. Antiserum from one such hyperimmune mouse (S41 no. 2) was cytotoxic for the immunizing cell with a mid-point titre of 1:320. However, when antiviral antibodies were removed from the antiserum by absorption with tissue culture cells (non-$H\text{-}2^b$) producing virus, cytotoxic antibodies remained that reacted with the immunizing lymphoma cell. When the serum was absorbed a second time with bone marrow cells from each of the seven BALB/c × B6 recombinant inbred strains of Bailey[16], cells from strains carrying the $H\text{-}2^b$ allele of B6 removed cytotoxic reactivity for B6T1 but cells from the strains carrying $H\text{-}2^d$ of the BALB/c allele did not (data not shown). These results suggested that at least one antibody present in antiserum from mouse S41 no. 2 detected a cell-surface antigen on B6T1 that was shared with normal spleen cells carrying the $H\text{-}2^b$ allele. To confirm that such antibodies detected antigens controlled by the $H\text{-}2^b$ haplotype, unabsorbed antiserum was diluted 1:120 then absorbed once with spleen cells from uninfected B6, C57BL/10 (B10), congeneic B6.C-H-2^d or congeneic B10.BR ($H\text{-}2^{k/k}$) mice and tested in cytotoxic tests with B6T1 target cells. The results of this experiment, shown in Fig. 1, demonstrated that the major antigens detected on B6T1 cells in cytotoxic tests with this antiserum were also expressed on normal spleen cells of B6 or B10 mice but not on spleen cells of $H\text{-}2^d$ or $H\text{-}2^k$ congeneic partner strains.

If such antibodies were directed to the H-2 determinants expressed on normal cells, then they would be expected to lyse normal spleen cells from H-2^b-carrying mice. This antiserum lysed 95% of spleen cells from B6, B10, 129 and (C3H × B6)F_1 mice but did not lyse spleen cells from C3H/HeJ, B10.BR, B10.A or B6.C-H-2^d mice, a result that clearly demonstrated the reactivity of this serum for determinants specified by the $H\text{-}2^b$ haplotype[17] (Fig. 2).

Response of hybrid mice to immunization with B6T1

The unusual nature of the reactivities found in the preceding antiserum prompted us to examine several other hybrid mice for production of anti-self H-2 antibodies. To do this we screened for antibodies that lyse 95% of spleen cells from B6 or B10 mice but show low (0–25%) lysis of spleen cells from B6.C-H-2^d or B10.D2n($H\text{-}2^d$) mice in direct cytotoxic tests with these cells. The results of this survey (Table 1) indicated that several hybrid mice which survived hyperimmunization produced cytotoxic

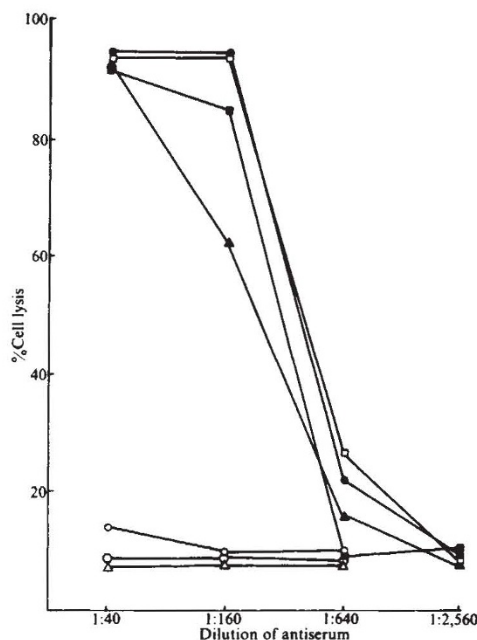

Fig. 2 Direct cytotoxic tests with (C3H × B6)F_1 anti-B6T1 (S41 no. 2) antiserum were performed as described[15] on spleen cells of: ●, B6; □, B10; ▲, 129 ($H\text{-}2^b$); ■, (C3H × B6)F_1 ($H\text{-}2^{k/b}$); ⬡, B10.BR; △, B6.C-H-2^d; and ○, C3H ($H\text{-}2^k$) mice using preselected rabbit serum (dilution 1:15) as a complement source. Normal spleen cells of mice bearing only the $H\text{-}2^b$ haplotype were lysed by this antiserum.

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Table 1 Immunization and antibody response of hybrid mice

Mouse	Immunogen	No. mice immunized	No. surviving 10^8 cells	No. producing αH-2^b/ No. screened
(C3H×B6)F_1 10–13 months	—	—	—	0/11
(BALB/c×B6)F_1 15 months	—	—	—	0/6
(C3H×B6)F_1	B6 bone marrow	10	10	0/10
B6	B6T1	113	15	0/15
(C3H×B6)F_1	B6T1	16	5	3/5
(C57BR×B6)F_1	B6T1	11	3	1/3
(BALB/c×B6)F_1	B6T1	15	2	1/2
(SEA×B6)F_1	B6T1	11	1	0/1

Female mice 8–12 weeks old were inoculated subcutaneously with 10^2 B6T1 cells, and tumours that developed were allowed to regress before a second intraperitoneal challenge of 10^2 cells was given. In alternate weeks mice were immunized with 10-fold increasing dose of cells until a plateau of 10^8 cells per mouse was achieved, and this immunization dose was maintained for the life of the animal. The initial immunization with bone marrow cells was 10^6 cells. Mice were bled individually 10 days after immunization, and antiserum, diluted 1 : 20, was screened for cytotoxic activity on B6 and B6.C–H–2^d, or B10 and B10.D2n spleen cells. Mice were considered positive for H–2^b antibody if their sera lysed 95% of spleen cells from the H–2^b strain. No antiserum sample lysed >30% of spleen cells from the respective congeneic (B6.C–H–2^d or B10.D2n) strains.

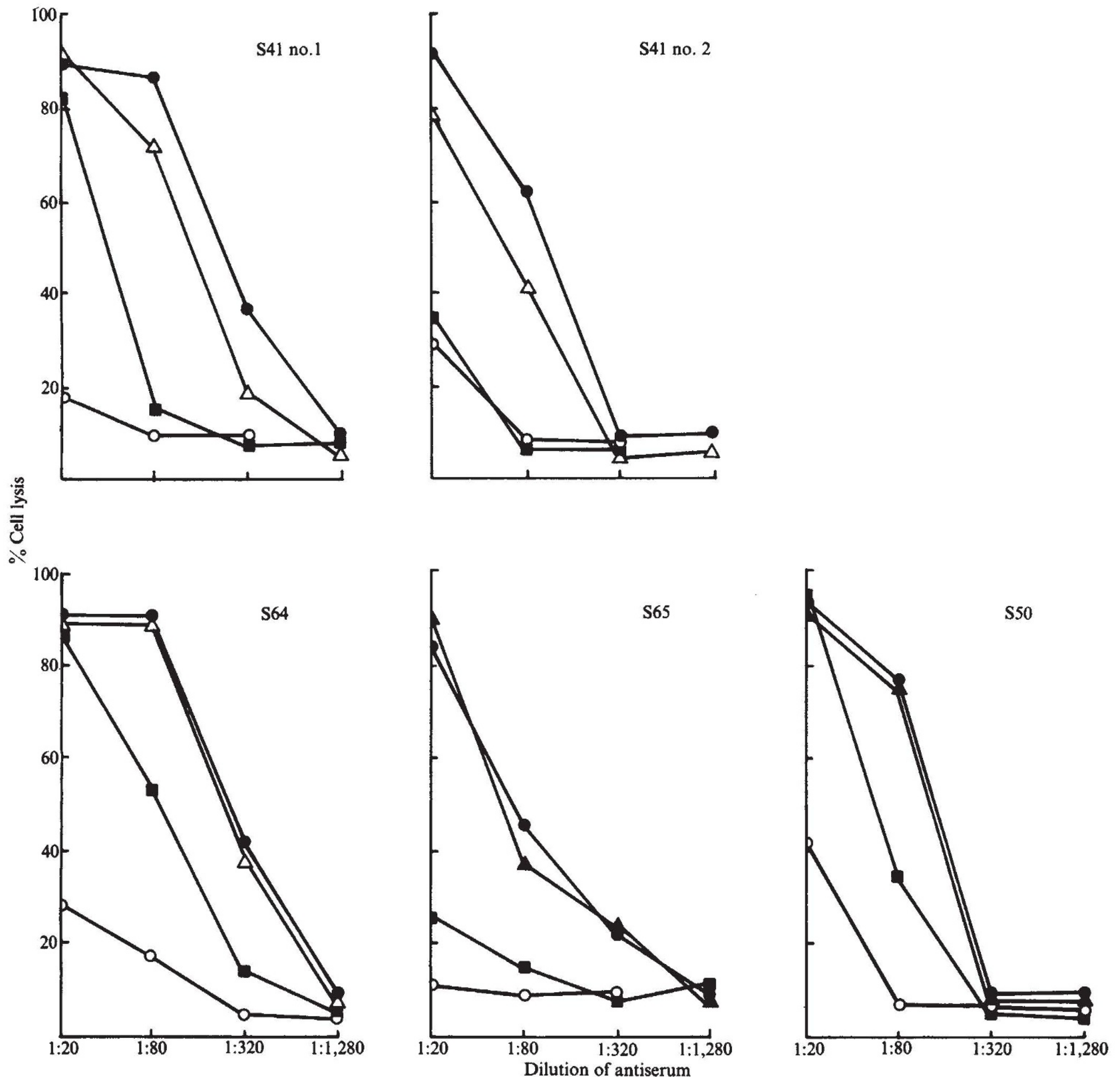

Fig. 3 Genetic mapping of antigens recognized by anti-self H–2 antisera. Antisera were assayed in direct cytotoxic tests on: ●, B10; △ and ▲, B10.A(5R) (H-$2K^b$, D^d); ■, B10.A(2R) (H-$2K^k$, D^b); and ○, B10.A (H-$2K^k$, D^d) cells as described elsewhere[15] using preselected rabbit serum (dilution 1 : 15) as a complement source. All five hybrid sera which contained anti-H–2^b antibodies recognized H–$2K^b$ antigens, and three of these sera (S41 no. 1, S64, S50) also recognized H–$2D^b$ antigens.

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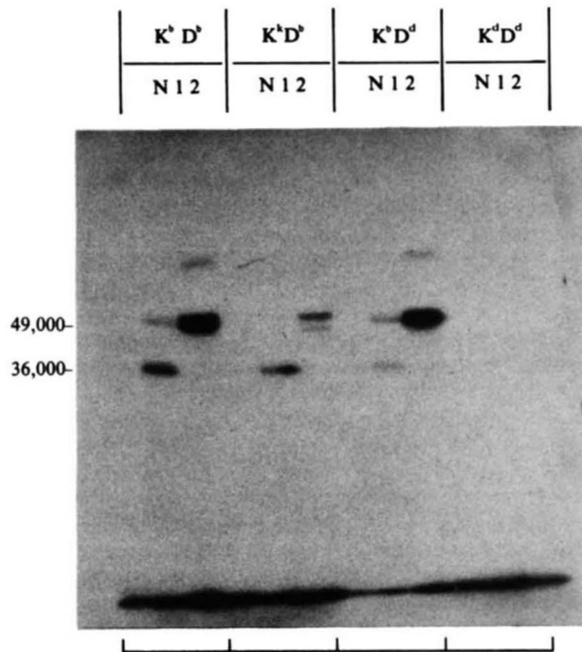

Fig. 4 Reactivity of antisera from hybrid mice with parental H-2K^b and/or H-2D^b molecules. 3×10^7 spleen cells from B10, B10.A(2R), B10.A(5R) and B10.D2n mice in 1 ml phosphate-buffered saline (PBS) were radiolabelled with 2 mCi Na^{125}I by the lactoperoxidase method[31,32]. Cells were washed with PBS, resuspended in 0.8 ml hypotonic buffer (10 mM Tris-HCl, *p*H 7.4, 10 mM NaCl, 1.5 mM $MgCl_2$, 1% Trasylol [protease inhibitor, FBA]) and lysed by the addition of 0.2 ml lysis buffer (50 mM $NaHPO_4$, *p*H 7.2, 4.5% NaCl, 5% Triton X-100, 2.5% sodium deoxycholate, 0.5% SDS)[33]. Lysates were centrifuged at 1,500*g* for 20 min to remove nuclei. Each lysate was then mixed with 3 ml of a 10% suspension of formalin-fixed *Staphylococcus aureus* bacteria (Cowan I strain)[34] and centrifuged at 120,000*g* for 90 min. This procedure removed all iodinated immunoglobulin molecules capable of binding the protein A-bearing bacteria. 10 µl of each antiserum was incubated with 2×10^6 c.p.m. of each lysate and immune complexes were collected by the addition of 200 µl of the *S. aureus* suspension. The immunoprecipitates were washed four times in RIPA buffer[35] (10 mM Tris-HCl, *p*H 7.2, 150 mM NaCl, 1% sodium deoxycholate, 1% Triton X-100, 0.1% SDS, 1% Trasylol), solubilized by heating to 100 °C for 5 min in sample buffer (0.0625 M Tris-HCl, *p*H 6.8, 10% glycerol, 5% 2-mercaptoethanol, 3% SDS, 0.001% bromophenol blue) and electrophoresed through a discontinuous 10% SDS-polyacrylamide slab gel according to the method of Laemmli[36]. For visualization of protein markers, gels were fixed and stained in 0.05% Coomassie brilliant blue in a solution containing ethanol/acetic acid/water (5:1:5) and destained in 7.5% acetic acid/5% ethanol. Dried gels were exposed to Kodak X-Omat R film in the presence of an intensifying screen. K^bD^b, K^kD^b, K^bD^d and K^dD^d refer to the *H-2* genotypes of the spleen cells from the B10, B10.A(2R), B10.A(5R) and B10.D2n strains, respectively. Tracks labelled N, 1 and 2 refer to the antisera used: N, normal mouse serum; 1, (C3H×B6)F_1 anti-B6T1, S41 no. 2; and 2, (BALB/c×B6)F_1 anti-B6T1, S50.

anti-H-2^b antibodies. As the data in Table 1 indicate, most of the mice challenged with this tumour do not survive, thus insufficient numbers were available to determine whether the production of anti-self H-2 antibodies was detrimental to the health of the animals. We can say, however, that the five hybrid mice which produced anti-H-2^b antibodies survived at least six challenges with 10^8 B6T1 cells, and one mouse, a (C57BR× B6)F_1, lived 15 months (20 challenges of 10^8 B6T1 cells) after its initial anti-H-2^b response was detected. This mouse (S65) was killed because it seemed to be lymphomatous. Autopsy revealed a massive lymphoma involving the mesenteric lymph node, liver and thymus, and histological examination also revealed glomerulonephritis. Washed cells from the tumour were lysed in cytotoxicity tests with conventional anti-H-2^b and anti-H-2^k antisera as well as the anti-self H-2 antiserum of that mouse, thus the lymphoma was of recipient and not immunizing cell origin. It remains to be determined whether the tumour, which was of recipient genotype, arose as a consequence of virus infection or autoimmune reactions.

Sera from 11 aged non-immune (C3H×B6)F_1 or six aged non-immune (BALB×B6)F_1 mice did not show appreciable reactivity (≤15% lysis) for B6 spleen cells, nor did antisera from 10 (C3H×B6)F_1 mice hyperimmunized with B6 bone marrow cells show reactivity for H-2^b spleen cells. Furthermore, antisera from 15 B6 mice hyperimmunized with the same B6T1 lymphoma cells did not have significant cytotoxic activity for B6 spleen cells, although all of these antisera contained antibody cytotoxic for B6 Moloney murine leukaemia virus (MuLV) leukaemias (recognition of the Friend-Moloney-Rauscher antigen[18]), and several contained antibody specifically cytotoxic for B6 Abelson lymphomas (recognition of the Abelson lymphoma antigen[15]).

Genetic mapping of determinants recognized by anti-self H-2 antibodies

Antisera from each of the five mice that produced anti-H-2^b antibodies were tested for direct cytotoxic activity on spleen cells of B10, B10.A, B10.A(2R) and B10.A(5R) mice (Fig. 3). All antisera reacted well with B10 and B10.A(5R) cells as >95% of spleen cells were lysed with titration mid-points of 1:160-1:640. Two antisera also reacted well with B10.A(2R) cells, and one antiserum (S64) reacted weakly with B10.A(2R) (95% lysis with a titration mid-point of 1:40). None of the antisera lysed more than 30% of spleen cells from B10.A. These results indicate that antigens controlled by the *H-2K-IA-IB* or *H-2K-IA-IB* plus *H-2D* subregions of the *H-2^b* but not *H-2^d* nor *H-2^k* haplotypes are recognized by these anti-self H-2 antisera. The lack of reactivity with cells of the *H-2^d* or *H-2^k* haplotypes excludes many public specificities of *H-2^b* haplotype from these antisera. We have also tested spleen cells of SWR (*H-2^q*), RIII/2J (*H-2^r*), PL (*H-2^u*) and B10.M (*H-2^f*) mice for sensitivity to lysis by these antisera, because these haplotypes also share public specificities[17] with *H-2^b*. As none of these spleen cells was lysed to an appreciable extent by anti-self H-2 antisera, we conclude that the major reactivity of these antisera is directed to the private specificities controlled by *H-2^b*.

Molecular identity of species recognized by anti-self H-2 antisera

To identify the molecule(s) on spleen cells that was recognized by these antisera, cells from B10, B10.A(2R), B10.A(5R) and B10.D2n were radiolabelled by the ^{125}I-lactoperoxidase technique, lysates prepared and immunoprecipitated with anti-self H-2 antiserum or normal mouse serum. Immunoprecipitates were electrophoresed through polyacrylamide gels containing SDS, and the results analysed autoradiographically. Each of the antisera showed strong reactivity with molecules of molecular weights (MW) ~49,000 and ≤13,000 (the dye front) on B10 or B10.A(5R) cells (Fig. 4). One antiserum, which reacted well in cytotoxic tests with B10.A(2R) cells, showed strong precipitating activity for molecules of MW 49,000 and ≤13,000 on these cells, whereas another antiserum (S64) that had a lower cytotoxic titre (1:40) on B10.A(2R) cells precipitated less material of these molecular weights. Reactivity for molecules of MW 37,000 was also noted with B10 and B10.A(5R) cells. No reactivity was observed with any molecule on B10.D2n cells.

Because the major molecules precipitated by these antisera co-migrate with the H-2K or H-2D heavy chains[19], we sought to

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confirm that the major protein recognized by anti-self H-2 sera was the *H-2K^b* product. We did this by removing the H-2K^b molecule from lysates by immunoprecipitation with conventional antisera, and then determining if molecules recognized by anti-self H-2 sera remained in the lysates. The results of this 'preclearing' experiment, presented in Fig. 5, demonstrate that preclearing of B6 radiolabelled lysates with conventional anti-H-2K-IA-IB^b antisera removed the major molecules reactive with anti-self H-2 sera, whereas preclearing with normal mouse serum (Fig. 5) or conventional anti-H-2^k (data not shown) did not. Therefore we conclude that the major species recognized by the antiserum of mouse S64 [(C3H × B6)F_1 anti-B6T1] on normal B6 spleen cells is the molecule carrying the H-2K^b transplantation antigens. Similar experiments have confirmed the identity of the principal molecules recognized by other anti-self H-2 antisera as being the *H-2K^b* product.

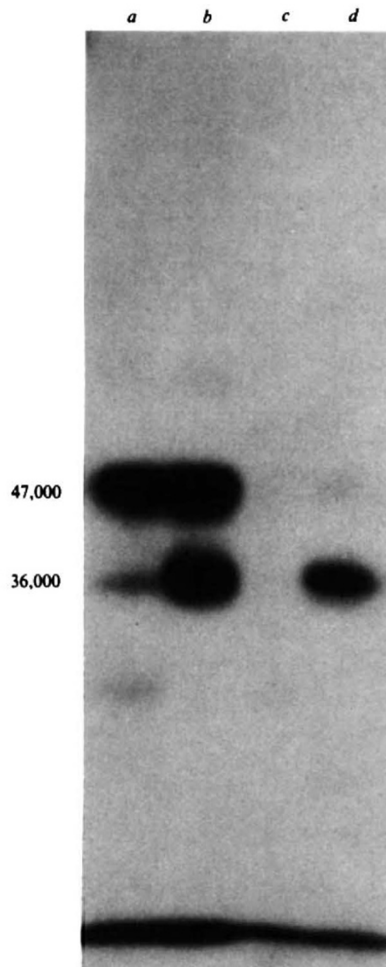

Fig. 5 Hybrid anti-self H-2 antisera and conventional anti-H-2K^b antisera recognize the same molecules. A single-cell suspension of B6 spleen cells was prepared and iodinated by the lactoperoxidase method. The cells were lysed and precleared with 3 ml of a 10% suspension of *S. aureus* bacteria as described in Fig. 4 legend. The lysate was then divided into two equal parts. One aliquot (lanes *a*, *b*) was incubated with 25 μl of normal mouse serum and the other (lanes *c*, *d*) was incubated with 25 μl of conventional anti-H-2K^b serum [(A × B10.D2)F_1 anti-B10.A(5R)]. The immune complexes were removed by the addition of 600 μl of the bacterial suspension followed by centrifugation. The supernatant of each reaction was further subdivided into two equal portions and incubated with 25 μl of anti-H-2K^b antiserum (lanes *a*, *c*) or 25 μl of (C3H × B6)F_1 antiserum (lanes *b*, *d*). These immunoprecipitation reactions were analysed by SDS-polyacrylamide gel electrophoresis as described in Fig. 4 legend.

Discussion

We have demonstrated that hybrid mice can generate a brisk humoral immune response to the major histocompatibility antigens presented on a parental virally induced leukaemia, although the hybrid mice also express those same histocompatibility antigens on their somatic cells. These results establish unambiguously the existence of autoreactive anti-MHC B-cell clones in hybrid mice. Earlier studies showed that not all immunizations of hybrid mice with parental tumours elicit anti-self H-2 antibodies. The Moloney cell-surface antigen (MCSA)[20], the X.1 antigen[21], and the Meth A antigen[22] were each detected by antisera from F_1 mice immunized with parental tumour cells, and no anti-self H-2 antibodies were detected in those sera. Anomalous anti-H-2 reactions are not without precedent, however, as demonstrated by the recent description of anti-H-2^k cytotoxic antibodies in the sera of mutant BALB/c mice immunized with normal BALB/c lymphoid cells[23].

The phenomenon of hybrid humoral response to parental antigens is reminiscent of the phenomenon of hybrid resistance to parental tumour grafts[10]. Two major features distinguish the humoral response described here from the *in vivo* phenomenon of F_1 rejection of parental bone marrow grafts or tumour grafts. First, the antigens detected on B6 cells by hybrid resistance map to the *H-2D* region, whereas those detected by anti-self H-2 antibodies map to the *H-2K* and *H-2D* regions. Second, the antigens detected in this serological study are expressed on heterozygous cells, whereas those detected in graft rejection do not seem to be. Furthermore, we did not detect anti-H-2^b antibody in (C3H × B6)F_1 hybrid mice immunized with B6 bone marrow, a strain combination that displays hybrid resistance[11].

Recent work on the induction of F_1 anti-parent cytotoxic T-lymphocyte responses by co-cultivation of F_1 spleen cells with homozygous parental cells[24,25], by treatment with allogeneic effect factor[26], or spontaneously[24,25] provide considerable evidence for the existence of autoreactive cytotoxic T-cells in the generation of cellular immune responses. Although the antigens recognized by F_1 anti-parent cytotoxic T-lymphocytes mapped to *H-2K* or *H-2D*, this apparent autoreactivity may reflect an H-2 compatibility requirement in conjunction with some other, perhaps endogenous viral, antigen and not cytotoxic function directed to the MHC product itself. Our present serological observations have no such ambiguity, and clearly demonstrate the possibility of humoral immune functions directed to self MHC products.

Ample evidence supports the physical and functional association of *H-2* products with oncornavirus glycoproteins[27-30], and it may be that the H-2 molecules of the tumour are recognized as foreign only in conjunction with unusual viral determinants. The failure to obtain anti-self H-2 antibodies from B6 mice immunized with this tumour might reflect the need for a proper immune response gene to recognize the unusual viral determinant. Note, however, that B6 mice are quite capable of responding to several viral antigens expressed on these cells[15]. Moreover, when anti-self responses were observed in hybrid mice, such anti-self H-2 antibodies were the major cytotoxic activities of the serum as demonstrated by absorption tests.

The observation here that normal bone marrow immunization did not elicit anti-self H-2 antibodies and the failure of earlier workers to observe such antibodies[20,21] in F_1 antiviral tumour immunizations may indicate that the anti-self response is only triggered by some novel, non-viral property of this tumour cell. Considered in that context, the failure of homozygous B6 mice to produce anti-self H-2 antibodies raises two questions regarding this response. First, whose antibodies are reactive with the H-2^b molecules, those of C3H or B6? Second, which genes must be heterozygous for this response to occur? It is possible, although unlikely, that self-reactive antibodies are absent from the repertoire of immunoglobulin genes of an inbred strain. More intriguing however, is the possibility that autoreactivity is itself generated by MHC heterozygosity. The

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resolution of the questions posed by the observation of anti-self H-2 antibodies will be of importance in the study of the generation and regulation of the immune response.

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LETTERS

γ-Ray observations of Cygnus X-3 at energies of 10^{12}eV

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The Crimean Observatory group have detected γ rays from the direction of the X-ray source Cyg X-3 (refs 1–5): rays of energy 10^{12}eV were observed using the atmospheric Cerenkov technique. Measurements with satellite-borne instruments in the 10^8 eV region have shown effects from Cyg X-3 in one case[6], but not in another[7]. Similarly, γ rays have been seen in some balloon flights[8], but not in those of McKechnie *et al.*[9] nor of White *et al.*[10]. A study using the atmospheric Cerenkov technique at 10^{11} eV showed no effect[11], though it has been shown to be compatible with the Crimean result[4]. We report here observations taken at Mount Hopkins Observatory, Arizona (altitude 2.3 km) in April, May and June 1980 using a similar system to that of Stepanian *et al.*[3], which seem to confirm, at least qualitatively, the Crimean results.

The Crimean group found a periodic signal with the same 4.8-h period as the X rays, and having two peaks: a narrow main one centred at 0.18 periods after X-ray minimum, and a broader interpulse at 0.7–0.8 periods after X-ray minimum. They have also reported a sporadic non-periodic effect. Recently (Stepanian, personal communication) they have found that the interpulse peak dominates over the main one.

In the present series of observations two mirrors of diameter 1.5 m were used, with a 5-cm photomultiplier and aperture stop at the focus of each, giving a full field of view of 2.0°. Coincidences were taken between the two detectors with a resolving time of 7 ns, and were recorded on magnetic tape together with pulses from the single detectors, and random coincidences. Current controlled servo lamps kept the background light constant. Observations were made in drift scans lasting 30 min each: 10 min before the source entered the field (OFF), 10 min in the field (ON), and a final 10 min outside the field (OFF). A scan was rejected if (1) the OFF parts of the scan divided into 120 10-s intervals failed a Poisson homogeneity test at the 1% level, (2) the two OFF observations differed by more than two Poisson standard deviations, or (3) if in any 10-s interval, the count rate was sufficiently high to give a 4σ departure from the mean. Of 139 scans on Cyg X-3, 95 were acceptable. These were taken at various phases on the 4.8-h period.

Figure 1 shows the ratio of ON/OFF rates plotted in 10 bins corresponding to the particular phases of X-ray emission. Standard errors have been calculated experimentally, for each phase bin and are $\sim 14\%$ higher than the Poisson values, consistent with previous experience with the Cerenkov technique. Phases have been calculated using Stepanian's constants and formulae. The ratios are clearly close to unity except at the interval from 0.7 to 0.8, which has a 3.5σ deviation from unity. The number of counts involved are: ON 799, OFF 662 before and 668 after transit. Three drift scans were available. Random coincidences and single rates were normal during the scans. The apparent flux corresponding to the effect at the interpulse is $\sim 1.5 \times 10^{-10}$

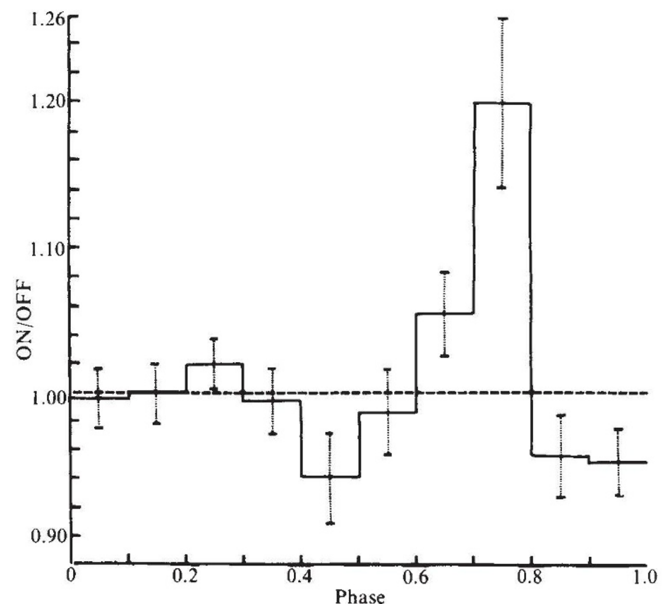

Fig. 1 Phase histogram of γ-ray emission from Cyg X-3.

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