Table 2 Influx of bone marrow-derived APC into thymuses

APC source	^{3}H-TdR incorporation (c.p.m. $\times 10^3$) by clone C.l.e −KLH	+5 μg ml^{-1} KLH
Normal (B6 × CBA)F_1 thymus	0.4 (±0.3)	14.7 (±1.1)
Normal B6 thymus	0.3 (±0.1)	0.5 (±0.2)
16-day B6 fetal thymus grafted 2 weeks in normal F_1	0.2 (±0.2)	11.2 (±0.9)
F_1 → B6 thymus 2 weeks after irradiation (1,100 rad) and reconstitution	0.5 (±0.1)	8.3 (±0.8)
B6 → F_1 thymus 2 weeks after irradiation (1,100 rad) and reconstitution	0.2 (±0.01)	0.4 (±0.1)

To test the origin of APC in the thymus of chimaeras, thymus cell suspensions were fractionated on a discontinuous Percoll gradient[29]. Most cells with APC function were found in the specific gravity 1.05–1.06 and 1.06–1.07 interfaces. Cells pooled from these fractions (2–4% of total thymocytes) were irradiated (1,500 rad) and placed in culture (5×10^5 cells per well) with a T-cell clone, C.l.e, specific for KLH seen in the context of 1-E^k molecules; the C.l.e. clone, added to the cultures at 10^5 cells per well, was derived from an F_1 → B6 chimaera primed with KLH. Soluble KLH was added to cultures where indicated. Cultures were pulsed with 1 μCi ^{3}H-TdR on day 2 and collected on day 3. Data are mean (±s.d.) of triplicate cultures. B6 → F_1 thymus APC, while unable to present KLH to clone C.l.e., are able to stimulate CBA lymph node T cells in mixed-lymphocyte cultures, though weakly (data not shown).

Jenkinson *et al.*[8], electron microscopy showed that dGuo-treated thymuses contained almost no lymphocytes or macrophages and consisted almost entirely of epithelial cells (Fig. 1) (confirmed in all of three experiments with two B6 and one CBA dGuo-treated thymuses). Despite the extreme paucity of MØ/DC in these thymuses, T cells differentiating in dGuo-treated thymus grafts exhibited strong restriction to thymic H-2 determinants (Table 1, groups *e*, *h*).

Although we cannot at present explain the contradictory data of Longo *et al.*[4,5], the above results seem to constitute a strong case that epithelial cells rather than MØ/DC are responsible for imprinting the H-2-restricted specificity of T_h cells; using a very similar approach to that of Longo and Schwartz[4], Zinkernagel[23] and Fink and Bevan[24] found no evidence that MØ/DC induce H-2 restriction of T_c cells. In the case of tolerance induction, however, there is accumulating evidence from both *in vivo*[6,7] and *in vitro*[25–27] studies that only MØ/DC and not epithelial cells induce tolerance across H-2 barriers. The only evidence against this notion is that tolerance to thymic H-2 determinants has been observed with T cells differentiating in thymuses precultured at low temperature[28], another procedure that selectively destroys bone marrow-derived cells. It remains possible, however, that culture at low temperature is less effective than dGuo treatment in removing MØ/DC. Note that, in agreement with the findings of others[6], we have confirmed that allografts of dGuo-treated fetal thymuses are accepted for prolonged periods and do not induce tolerance as measured by mixed-lymphocyte reactions (unpublished data), implying that our dGuo-treated thymus grafts were indeed depleted of MØ/DC.

According to the view that different cell types in the thymus control the induction of tolerance as opposed to H-2 restriction, T-cell specificity might be shaped as follows. Receptor-bearing thymocytes make initial contact with H-2 determinants on epithelial cells in the cortex, a region largely free of MØ/DC. After 'learning' self-H-2-restricted specificity, the T cells then migrate from the cortex to the cortico-medullary junction. Here, contact with MØ/DC deletes those T cells expressing high affinity for self-H-2 determinants. Low-affinity T cells then pass through this filter and migrate into the periphery.

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1. Klein, J. (ed.) *Immunology: The Science of Self-Nonself Discrimination* 1–687 (Wiley, New York, 1982).
2. Zinkernagel, R. M. *Immun. Rev.* **42**, 224–270 (1978).
3. Bevan, M. J. & Fink, P. J. *Immun. Rev.* **42**, 3–19 (1978).
4. Longo, D. L. & Schwartz, R. H. *Nature* **287**, 44–46 (1980).
5. Longo, D. L. & Davis, M. L. *J. Immun.* **130**, 2525–2527 (1983).
6. Ready, A. R., Jenkinson, E. J., Kingston, R. & Owen, J. J. T. *Nature* **310**, 231–233 (1984).
7. von Boehmer, H. & Schubiger, K. *Eur. J. Immun.* **14**, 1048–1052 (1983).
8. Jenkinson, E. J., Franchi, L., Kingston, R. & Owen, J. J. T. *Eur. J. Immun.* **12**, 583–587 (1982).
9. Sprent, J. *J. exp. Med.* **147**, 1838–1842 (1978).
10. Kappler, J. W. & Marrack, P. *J. exp. Med.* **148**, 1510–1522 (1978).
11. Singer, A., Hathcock, K. S. & Hodes, R. J. *J. exp. Med.* **153**, 1286–1301 (1981).
12. von Boehmer, H. & Haas, W. *J. exp. Med.* **150**, 1134–1142 (1979).
13. Bevan, M. J. *Nature* **269**, 417–418 (1977).
14. Zinkernagel, R. M. *et al. J. exp. Med.* **147**, 882–896 (1978).
15. Matzinger, P. & Mirkwood, G. *J. exp. Med.* **148**, 84–92 (1978).
16. Kruisbeek, A. M., Sharrow, S. O. & Singer, A. *J. Immun.* **130**, 1027–1032 (1983).
17. Wagner, H. *et al. J. exp. Med.* **153**, 1517–1532 (1981).
18. Corradin, G., Etlinger, H. M. & Chiller, J. M. *J. Immun.* **119**, 1048–1053 (1977).
19. Robinson, J. H. *J. Immun.* **130**, 1592–1595 (1983).
20. Barclay, A. N. & Mayrhofer, G. *J. exp. Med.* **153**, 1666–1671 (1981).
21. Kyewski, B. A., Fatham, C. G. & Kaplan, H. S. *Nature* **308**, 196–199 (1984).
22. Jordan, R. K., Bentley, A. L., Perry, G. A. & Crouse, D. A. *J. Immun.* **134**, 2155–2160 (1985).
23. Zinkernagel, R. M. *J. exp. Med.* **156**, 1842–1847 (1982).
24. Fink, P. J. & Bevan, M. J. in *Recognition and Regulation in Cell Mediated Immunity* (eds Watson, J. D. & Marbrook, J.) 107–125 (Dekker, New York, 1985).
25. Robinson, J. H. & Owen, J. J. T. *Nature* **271**, 758–760 (1978).
26. Good, M. F., Pyke, K. W. & Nossal, G. J. V. *Proc. natn. Acad. Sci. U.S.A.* **80**, 3045–3049 (1983).
27. Jenkinson, E. J., Jhittay, P., Kingston, R. & Owen, J. J. T. *Transplantation* **39**, 331–333 (1985).
28. Jordan, R. K., Robinson, J. H., Hopkinson, N. A., House, K. C. & Bentley, A. L. *Nature* **314**, 454–456 (1985).
29. Nathanson, S. D., Zamfirescu, P. L., Drew, S. I. & Wilbur, S. *J. immun. Meth.* **18**, 225–234 (1977).

Selective rejection of H–2-deficient lymphoma variants suggests alternative immune defence strategy

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Metazoan organisms may discriminate between self and non-self not only by the presence of foreign antigens but also by the absence of normal self markers[1]. Mammalian adaptive immune responses use the first strategy, with the additional requirement that foreign antigens are recognized in the context of self-major histocompatibility complex (MHC) products at the cell surface[2]. Aberrant cells which fail to express MHC products adequately can therefore avoid detection[2–4]. A more primitive but complementary defence system, eliminating such cells on the basis of absent self-markers, is suggested by a re-interpretation[5,6] of phenomena associated with metastasis and natural resistance. We now show that murine lymphoma cells selected for loss of H–2 expression are less malignant after low-dose inoculation in syngeneic hosts than are wild-type cells, and that the rejection of such cells is non-adaptive. On the basis of our data, we suggest that natural killer cells are effector cells in a defence system geared to detect the deleted or reduced expression of self-MHC.

More specifically, the hypothesis[5,6] stated that self-MHC genes influence the expression or recognition of inhibitor signals in interactions between potential target cells and natural effector cells. Our experimental approach was to test the prediction that selection for loss of H-2 in tumour lines should be accompanied by an increased sensitivity to natural resistance *in vitro* and *in vivo*. Three independent anti-H-2-resistant sublines from two lymphomas were selected. Such variant cells not only were able to escape adaptive alloreactivity *in vitro* and *in vivo*, but, as predicted by our hypothesis, had also become unable to grow

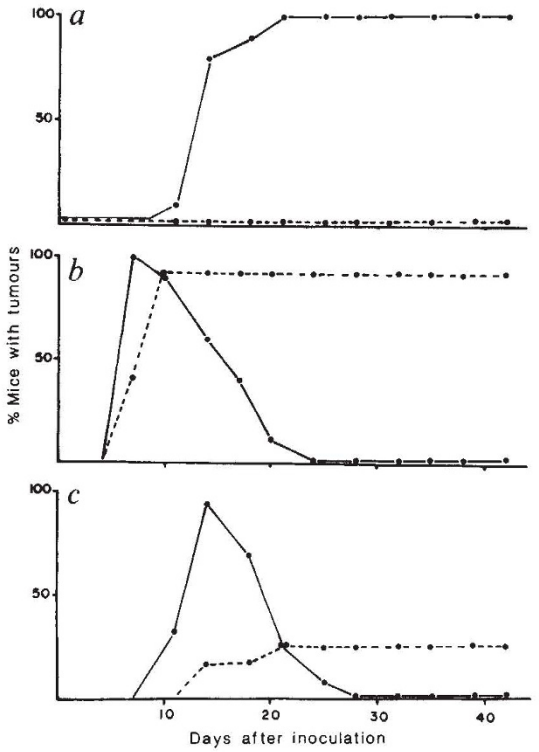

Fig. 1 Proportion of syngeneic and allogeneic mice with palpable tumours at different time points after subcutaneous injection of RMA (RBL-5, mutagenized but not selected; solid line) and RMA H-2 sel. (mutagenized, selected, H-2-resistant subline; dashed line). *a*, 10^4 cells in syngeneic C57BL mice: summary of two experiments with a total of 12 mice for each tumour. *b*, 10^6 cells in allogeneic A.BY mice; summary of four experiments with a total of 18–21 mice for each tumour. *c*, 10^3 cells in allogeneic A.BY mice; summary of four experiments with a total of 18–21 mice for each tumour.

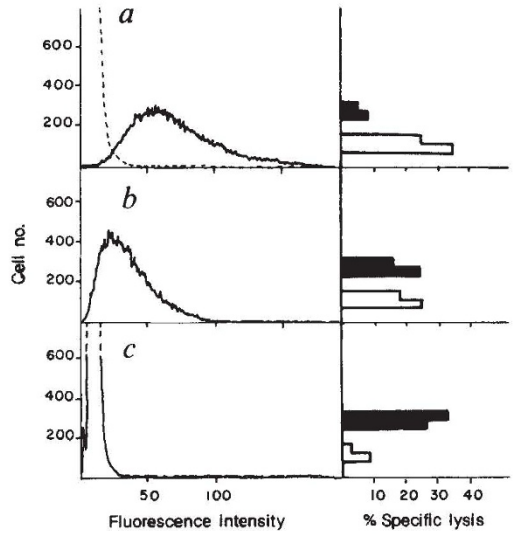

Fig. 2 H-2 expression, susceptibility to allospecific cytotoxic T lymphocytes (CTL), and poly-I:C-induced NK cells of RBL-5 sublines established after mutagenization, before selection (= RMA, *a*), after two selections with alloantiserum + complement (*b*) and after the completed series of five selections (= RMA H-2 sel., *c*). Left-hand panels show H-2^b expression by FACS analysis after indirect staining with hyperimmune A/Sn anti-A.BY alloantiserum (1/20) and fluorescent conjugated rabbit anti-mouse immunoglobulin (Dakopatts). The fluorescence profiles were determined on a FACS IV using a laser light output of 200 mV, photomultiplier at 450 V and fluorescence gain × 2. Nonspecific background staining, estimated by using the reciprocal antiserum A.BY anti-A/Sn (anti-H-2^a) as the first antibody, was similar for the three lines and is shown here for the nonselected line only (dashed curve, *a*). Right-hand panels show the sensitivity of the corresponding cell lines to anti-H-2^b cytotoxic T cells derived from A/Sn anti-A.BY mixed leukocyte cultures, and tested at an effector/target ratio of 6/1 (open bars, with the upper and lower part corresponding to the same two independent tests for all cells lines) and splenocytes from H-2-syngeneic C57BL or (A.BY × C57BL)F_1 mice injected with poly-I:C (150 μg intraperitoneally 18 h before test), tested at an effector/target ratio of 100/1 (solid bars, with the upper and lower part corresponding to the same two independent tests for each of the cell lines). The mixed lymphocyte cultures and the 4–6-h ^{51}Cr-release assays were set up as described previously[39] and all three lines were tested in parallel in each of the experiments.

out from small inocula in the normal syngeneic host, and switched from the natural killer (NK)-resistant to the NK-sensitive phenotype as a consequence of the selection against H-2 expression.

We used the RBL-5 lymphoma (H-2^b) for these studies because it readily expresses H-2 and is highly malignant in the normal syngeneic host. In contrast to two other C57BL-derived lymphomas, the number of tumour takes after a threshold inoculum was not significantly increased in NK-cell-defective mice[7]. This lymphoma is therefore probably regarded as close to 'normal self' by natural resistance mechanisms in the syngeneic or H-$2^{b/b}$-compatible hosts. In contrast, the subline that had been selected for loss of H-2 expression (RMA H-2 sel) failed to grow in such mice, even after a 10-fold increase in the dose required for 100% takes of the wild-type tumour (Table 1). The subline had been derived after mutagenization and repeated treatment with alloantiserum and complement. It was completely resistant to this treatment, due to a selective loss or reduction in expression of MHC class I gene products (Table 1) and β_2-microglobulin; it did not stain significantly above normal control serum with alloantibodies on analysis by fluorescence-activated cell sorter (FACS) (Fig. 1), while binding and complement-dependent killing with anti-Thy 1.2 antibodies were unaffected (Table 1). The wild-type and the selected line were both negative for monoclonal antibodies against TL and I-A^b antigens (not shown).

In view of the proposed hypothesis, it was important first to study natural resistance in syngeneic (C57BL) or at least semi-syngeneic (A.BY × C57BL) H-$2^{b/b}$ homozygous hosts, in order to avoid combinations producing strong T-cell reactivity. In an attempt to obtain the latter situation in subsequent studies, high-dose inocula were given to inbred A.BY mice (rather than outcrossed to C56BL), presenting a minor histocompatibility barrier to the RBL-5 lymphoma[8]. The pattern of progressive tumour growth then changed to the opposite: H-2-variant lymphoma cells grew progressively once they had formed a tumour, whereas all wild-type tumours were rejected after temporary growth (Fig. 1). High-dose challenge (1×10^6 cells) of preimmunized syngeneic C57BL mice gave a similar picture: complete protection was seen with the wild-type line, while H-2-resistant cells failed to immunize against themselves (Table 1). These results and observations on tumour outgrowth in pre-irradiated hosts (Table 1) indicated that the reduced tumorigenicity of H-2-lacking variant cells was not due to an intrinsic inability to grow *in vivo*, but rather to an active host defence, dependent on radiosensitive (precursor) cells, but independent of tumour immunogenicity in the classical sense. It was important to establish that this was the case, in view of reports that low malignant mutant or transfectant tumour lines can acquire the ability to elicit strong adaptive T-cell responses[9–12]. Indeed, the variants seemed to escape T-cell recognition *in vivo*, probably because minor histocompatibility antigens and tumour antigens are recognized only in the context of self-MHC products[2,13] and thus fulfilled one of the criteria for action by the postulated defence system. The variant cells were also able to escape anti-H-2^b

Table 1 Summary of experimental results

Sublines			Serology								Tumour growth *in vivo*					
			A/Sn→A.BY alloantiserum		20-8-4S K^bD^b mab		β_2M mab	β_2M rabbit	F7DG Thy 1.2 mab							
Original line	Mutagenization series selection	Designation	CX	FACS	CX	FACS	CX	FACS	CX	FACS	Untreated 10^3	10^4	10^5	10^6	Pre-immunized 10^6	Pre-irradiated 10^4
RBL-5	A—none	RMA	++	++	++	++	+	++	++	++	7/7	16/16	10/10	9/9	1/9	9/9
RBL-5	A—A/Sn→A.BY	RMA H-2 sel.	−	−	−	(±)	−	(±)	++	++	0/9	0/14	NT	5/5	5/5	4/4
RBL-5	B—A/Sn→A.BY	RMB H-2 sel.	−	−	−	(±)	−	(±)	++	++	1/14	0/16	1/12	4/5	5/5	5/5
EL-4	A—none	EL-4MA	++	++	++	++	NT	NT	NT	NT	3/10	6/10	7/10	NT	NT	NT
EL-4	A—A/Sn→A.BY	EL-4MA H-2 sel.	−	(±)	−	(±)	NT	NT	NT	NT	0/10	1/13	1/14	NT	NT	NT

Sublines: RBL-5 is a Rauscher virus-induced lymphoma and EL-4 a benzpyrene-induced leukaemia, both originating in the C57BL strain. The cells, maintained in RPMI with 10% fetal calf serum and penicillin-streptomycin, were mutagenized with ethanemethanesulphonate (EMS, Sigma), 200 μg ml^{-1} for 24 h. After washes, the cells were allowed to recover for 1 week and then treated with alloantiserum (A/Sn→A.BY diluted 1:20, 10^7 cells ml^{-1}) followed by rabbit complement (Pel Freez, 1/10) for 1–4 h. The cells were cultured without any attempts to remove the dead cells, and the survivors (<0.25%) were expanded. The treatment was repeated at least four times. **Serology:** The A/Sn→A.BY (anti-H-2^b) serum was produced in our animal colony at the Karolinska Institute. The monoclonal antibody (mab) 20-8-4S (ref. 38) was obtained from the American Type Culture Collection. The anti-β_2-microglobulin (β_2M) reagents were kindly sent by Drs Shen and Boyse (Sloan Kettering Memorial Institute, New York) (mab) and Dr Tanigaki (Roswell Park Memorial Institute, Buffalo) (rabbit antiserum). The F7D5 reagent was a gift from Dr E. Clark (Genetic Systems, Seattle). CX, complement-dependent cytotoxicity in a two-step assay evaluated by trypan blue exclusion. ++, 95–100% of cells killed, reciprocal titre no less than 100. +, 70–95% of cells killed, reciprocal titre 10–50; −, below background killing with complement alone. FACS, indirect fluorescence, after a two-step staining procedure using a FACS IV (Becton & Dickinson) with a laser output of 200 mV, photomultiplier at 600 V and a constant fluorescence gain for each antibody. The settings used gave a background median fluorescent intensity (MFI) below 10 on a linear scale (using normal mouse of rabbit serum, C57BL antisera to other H-2 haplotypes or T-lymphoma supernatant instead of the specific antibody in the first incubation). ++, MFI>100 under the same conditions; +, MFI 20–100; (±) MFI, 5–19; −, below background. Secondary reagents: fluorescein isothiocyanate-conjugated rabbit-anti-mouse immunoglobulin or swine anti-rabbit immunoglobulin (Dakopatts). The cells were incubated at 10^7 ml^{-1}, 30 min on ice in both steps. **Tumour growth *in vivo*:** All tests were with ascites tumours, serially passaged in 400R-pre-irradiated mice to avoid immunoselection *in vivo*. Different numbers of tumour cells were injected subcutaneously to groups of three to six H-2-syngeneic hosts, either C57BL or (A.BY×C57BL)F_1 for all tumours in each experiment. The two types of mice gave consistent results, and the number of mice with tumour takes (out of totally injected) have therefore been pooled from several different experiments, each one including wild-type and variant cells injected to parallel groups. Two experiments with 10^4 cells included parallel groups of preirradiated mice (400R, 24 h before tumour challenge). The data with 10^6 injected cells represent two experiments where half of the mice had been preimmunized with the challenging tumour (three weekly injections of 2×10^7 10,000 R irradiated RMA or RMA H-2 sel. cells). NT, not tested.

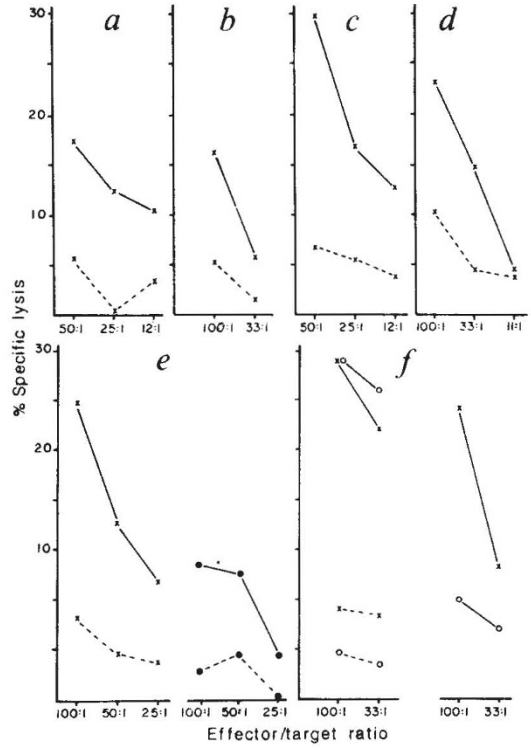

Fig. 3 Susceptibility of wild-type and H-2-selected sublines to killing by spleen cells from poly-I:C-treated (150 μg per mouse intraperitoneally 18 h before injection) or untreated mice. *a*, *b*, Two independent experiments with effector cells from poly-I:C-treated C57BL mice tested against EL-4MA (dashed line) and EL-4MA H-2 sel (solid line). *c*, *d*, Two independent experiments with effector cells from poly-I:C-treated C57BL (*c*) and (A.BY×C57BL)F_1 (*d*) mice tested against RBL-5 wild-type (dashed line) and RMB H-2 sel. (solid line). *e*, Nylon wool column-passed[35] spleen cells from normal C57BL (×) and A.BY (○) mice tested against RMA (dashed line) and RMA H-2 sel. (solid line) in a 16-h ^{51}Cr release assay. *f*, Left, effector cells from poly-I.C.-treated (A.BY×C57BL)F_1 mice tested against RMA (dashed lines) and RMA H-2 sel. (solid lines), with (○) and without (×) pretreatment of targets with β-interferon (Stratech Scientific Ltd, 3.6×10^7 immunoreactive units per mg protein, cat. no. 83001), 5×10^3 immunoreactive units ml^{-1}, 18 h; *f*, right, effector cells from poly-I:C-treated A/Sn mice tested against YAC-1 cells with (○) and without (×) pretreatment of targets with β-interferon as described above. Median H-2 fluorescence intensity by FACS analysis: RMA, 105, β-interferon-treated RMA, 180; RMA H-2 sel.<15 before as well as after β-interferon treatment; YAC-1, 28; β-interferon-treated YAC-1, 71 (staining procedure as in Fig. 2, except that A.BY anti-A/Sn H-2^a serum was used as the first antibody with YAC-1 cells; background was 6–11 for all lines. All cytotoxicity tests were performed with the ^{51}Cr-release assay, as described previously[39,40], with an incubation time of 4–6 h, except in *e* (16 h).

cytotoxic lymphocytes *in vitro* (Fig. 2) as a result of the selection. However, at the same time, they had switched from the NK-resistant to the NK-sensitive phenotype (Fig. 2).

Although the *in vivo* results presented here do not prove definitively that the natural resistance was mediated by NK cells, the non-adaptive nature of the rejection and the results on irradiated hosts are consistent with this interpretation. The dose of 400R reduces splenic cellularity by 95%; even if some functional NK cells remain, the total activity in terms of lytic units per animal is reduced within 24 h, and falls to zero within the following weeks, that is, the period between tumour injection and outgrowth in our experiments[14]. This enhanced sensitivity to natural resistance *in vitro* and *in vivo* was observed with an independently derived H-2-resistant RBL-5 subline, as well as with an EL-4 subline selected in a similar fashion (Table 1, Fig. 3). The general lysability of the wild-type control line was also confirmed in the case of EL-4, which was efficiently killed (>50% lysis at effector target/ratio 5:1) by anti-H-2-specific, mixed lymphocyte culture-derived effector cells (data not shown).

According to the proposed model, the tumour cells would be unable to inhibit triggering of NK lysis after complete or partial loss of self H-2[5,6]. This would be consistent with several observations on NK cells, including high sensitivity of *in vitro*-grown YAC-1 lymphoma cells, immature thymocytes and teratocarcinoma cells, all of which have low or defective MHC expression[15,16]. The corresponding cells show reduced sensitivity when H-2 is induced (by differentiation[17,30], interferon[19] or

growth *in vivo*[2]). NK-cell-mediated rejection of H-2-disparate bone marrow and lymphoid grafts, including the F_1 hybrid resistance phenomenon[18,21,22] and early elimination of allogeneic lymphocytes[23], are other examples that could be reinterpreted to reflect recognition of incomplete self-H-2 expression: allogeneic grafts lack H-2 genes of the recipient, and parental grafts fail to match one haplotype, that is, they lack 50% or more of the total 'self'-H-2 molecules expressed in the F_1 recipient environment. This predicts that the relationship between reduced self expression and NK susceptibility should be quantitative rather than dependent on complete deletion of H-2 products, as is indeed supported by findings with a subline of RBL-5 derived after the two first selections with anti-H-2 + complement. These cells had an average loss on H-2 fluorescence corresponding to more than 50% of the total H-2 expression in the original line, and a clear increase in NK sensitivity (Fig. 2).

The hypothesis would also explain why it is difficult to select $H\text{-}2^-$ variants *in vivo*[24], and predict a selection for increased H-2 expression during tumour progression. The correlation between metastatic potential and increased expression of all[25] or certain (D-end encoded[26,27]) MHC class I alleles observed for several experimental tumours could therefore be reinterpreted as selection of phenotypes able to escape NK cells. However, preferential outgrowth of cells with reduced or deleted expression of certain MHC alleles (often H-2K-end encoded) has been observed after large-dose inoculation ($\geq 10^5$ cells) of some virally induced, strongly antigenic tumours[12,28,29]. T cells presumably represented the major surveillance mechanism in those systems, as they would in the present experiments, where H-2-deficient sublines grew out preferentially after transplantation of large-dose inocula to preimmunized C57BL or histoincompatible A.BY mice (Table 1, Fig. 1).

Burnet pointed out the fundamental difference between strategies for self-non-self-discrimination in invertebrates and mammals[1]. In the colonial tunicate *Botryllus*, rejection of allogeneic cells as well as control against self-fertilization is thought to operate by a mechanism scanning for the presence or absence of self-markers, encoded by a single locus with considerable polymorphism[1,31]. Such a system may also have been fixed in mammals, despite the development of adaptive immunity. The selective pressures would have required not only a back-up system eliminating aberrant (MHC loss) cells escaping detection by T lymphocytes, but also a rapid first-line defence with a certain selectivity. Examples exist of lytic as well as transforming viruses that cause a profound but selective reduction of H-2 expression in infected cells[29,32]. The latter would be eliminated by the proposed defence mechanism, while sparing of normal cells in the tissues would be guaranteed by an increase in their H-2 expression due to interferon stimulation early in immune responses and NK-cell activation. Interferons do reduce the NK susceptibility of many targets[33]; the mechanisms involved are unknown, but may be related to MHC gene regulation as suggested by observations on variant cells in this (Fig. 3*f*) and another study[41].

The present findings may also be relevant to previous speculations by Snell[34] and Dausset[35] concerning the primordial functions of MHC genes, that is, functions that would not be related to T-cell restriction. The possibility of a common regulation of H-2 expression, NK-cell-resistant phenotype and malignancy properties in lymphoma cells is also very interesting in relation to recent advances in the research on regulation of MHC class I genes, particularly in tumour cells[36,37].

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1. Burnet, F. M. *Nature* **232**, 230-235 (1971).
2. Zinkernagel, R. M. & Doherty, P. C. *J. exp. Med.* **141**, 1427-1436 (1975).
3. Doherty, P. C., Knowles, B. B. & Wettstein, P. J. *Adv. Cancer Res.* **42**, 1-66 (1984).
4. Sanderson, A. R. & Beverley, P. C. L. *Immun. Today* **4**, 211-213 (1983).
5. Kärre, K. thesis, Karolinska Inst. (1981).
6. Kärre, K. in *Mechanisms of Cytoxicity by Natural Killer Cells* (eds Herberman, R. B. & Callewaert, D.) 81-91 (Academic, Orlando, 1985).
7. Kärre, K., Klein, G. O., Kiessling, R., Klein, G. & Roder, J. C. *Int. J. Cancer* **26**, 789-797 (1980).
8. Klein, J. in *Biology of the Mouse Histocompatibility-2 Complex*, 36-37 (Springer, New York, 1975).
9. Boon, R. *Adv. Cancer Res.* **39**, 121-151 (1983).
10. Frost, P., Kerbel, R. S., Bauer, E., Tartanella-Biondo, R. & Cefalu, W. *Cancer Res.* **43**, 125-132 (1983).
11. Hui, K., Grosvels, F. & Festenstein, H. *Nature* **311**, 750-752 (1984).
12. Hämmerling, G. J. *et al. Nature* **315**, 301-306 (1985).
13. Bevan, M. J. *J. exp. Med.* **142**, 1349-1364 (1975).
14. Cudkowicz, G. & Hochman, P. S. *Immun. Rev.* **44**, 13-41 (1979).
15. Cikes, M., Friberg, S. Jr & Klein, G. *J. natn. Cancer Inst.* **30**, 347-361 (1973).
16. Stern, P. *et al. Nature* **285**, 341-342 (1982).
17. Hansson, M. *et al. J. Immun.* **123**, 765-771 (1979).
18. Carlson, G. A., Melnychuk, D. & Meeker, M. J. *Int. J. Cancer* **25**, 111-122 (1980).
19. Hansson, M., Kiessling, R., Andersson, B. & Welsh, R. M. *J. Immun.* **125**, 2225-2231 (1980).
20. Becker, S., Kiessling, R., Lee, N. & Klein, G. *J. natn. Cancer Inst.* **61**, 1493-1498 (1978).
21. Cudkowicz, G. & Bennet, M. *J. exp. Med.* **134**, 1513-1528 (1971).
22. Klein, G. O., Klein, G., Kiessling, R. & Kärre, K. *Immunogenetics* **6**, 561-569 (1978).
23. Rolstad, B. & Ford, W. L. *Immun. Rev.* **73**, 87-114 (1983).
24. Möller, E. *J. natn. Cancer Inst.* **33**, 979-987 (1964).
25. Haywood, G. R. & McKhann, C. *J. exp. Med.* **133**, 1171-1187 (1971).
26. Katzav, S., De Baetselier, P., Tarbakovsky, B., Feldman, M. & Segal, S. *J. natn. Cancer Inst.* **71**, 317-324 (1983).
27. Eisenbach, L., Segal, S. & Feldman, M. *Int. J. Cancer* **32**, 113-120 (1983).
28. Gooding, L. R. *J. Immun.* **129**, 1306-1312 (1982).
29. Schrier, P. I., Bernards, R., Vaessen, J., Houweling, A. & van der Eb, A. J. *Nature* **305**, 771-775 (1983).
30. Gidlund, M. *et al. Nature* **292**, 848-850 (1981).
31. Scofield, V. L., Schlumpberger, J. M., West, L. A. & Weissman, I. L. *Nature* **295**, 499-502 (1982).
32. Hecht, T. & Summers, D. *J. Virol.* **10**, 378-385 (1972).
33. Welsh, R. M., Kärre, K., Hansson, M., Kunkel, L. A. & Kiessling, R. *J. Immun.* **126**, 219-225 (1981).
34. Snell, G. *Transplantn Proc.* **8**, 147-156 (1976).
35. Dausset, J. *Devl comp. Immun.* **5**, 1-4 (1981).
36. Brickell, P. M., Latchman, D., Murphy, D., Wilson, K. & Rigby, P. W. J. *Nature* **306**, 756-761 (1984).
37. Baldacci, P., Pozo, F., Gisselbrecht, S. & Kourilsky, P. *J. exp. Med.* **158**, 1294-1306 (1981).
38. Ozato, K. & Sachs, D. H. *J. Immun.* **126**, 317-321 (1981).
39. Kärre, K., Seeley, J., Eriksson, E., Burton, R. & Kiessling, R. *J. exp. Med.* **156**, 385-403 (1983).
40. Julius, M. H., Simpson, E. & Herzenberg, L. A. *Eur. J. Immun.* **3**, 645-651 (1973).
41. Piontek, G. *et al. J. Immun.* **135**, 4281-4288 (1985).

Expression of apamin receptor in muscles of patients with myotonic muscular dystrophy

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Myotonic muscular dystrophy, or Steinert disease, is a dominantly inherited disease of muscle which occurs with a frequency of between 1 in 18,000 and 1 in 7,500 people (refs 1, 2). One of the prominent clinical manifestations is muscle stiffness and difficulty in relaxation of mucles after voluntary contractions. Electrophysiological signs of myotonia include increased excitability with a tendency to fire trains of repetitive action potentials in response to direct electrical and mechanical stimulation. Most experimental and clinical data suggest that myotonic muscular dystrophy arises from genetically induced alterations of the muscle membrane[3]. We show here for the first time that muscle membranes of patients with myotonic muscular dystrophy contain the receptor for apamin, a bee venom toxin known to be a specific and high-affinity blocker of one class of Ca^{2+}-activated K^+ channels in mammalian muscle[4-6]. The apamin receptor is completely absent in normal human muscle as well as in muscles of patients with spinal anterior horn disorders.