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Hybrid Resistance Against a Natural Killer (NK) Cell-Resistant Lymphoma (YWA) is Mediated by a T Cell-Dependent Mechanism*

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Abstract—*Rejection of the Moloney virus-induced YAC lymphoma of strain A origin by semisyngeneic F_1 hybrids has previously been shown to correlate with the levels of natural killer (NK) cell activity in the same F_1 hybrids against this target cell line* in vitro. *In the present study, YAC and another Moloney virus-induced lymphoma, YWA, derived from the A congenic A.SW strain, were tested for F_1 hybrid resistance after s.c. inoculation of small numbers of cells into syngeneic and semisyngeneic F_1 mice. While YAC cells invariably grew progressively once they formed a palpable tumor, regression of YWA tumors was frequently observed in both susceptible and resistant genotypes. The hybrid resistance pattern for YAC and YWA differed in one important respect: outcross of the syngeneic host to the A-congenic A.BY strain introduced a strong H-2^b-associated resistance factor against YWA, but not against YAC. Compared to YAC, which is highly NK-sensitive and rapidly eliminated from mice with high NK activity, YWA was insensitive to NK-mediated lysis* in vitro *and* [^{125}I]*UdR-labelled YWA cells were not eliminated more efficiently from the highly resistant (A.SW × A.BY) F_1 than from the parental strain in short-term* (4-18*h*) in vivo *rejection assays. It was therefore concluded that the H-2^b-associated resistance against YWA was independent of NK cells or other rapidly acting effector mechanisms. Moreover, thymectomy, followed by irradiation and fetal liver reconstitution, completely abolished the resistance against YWA but left the resistance against YAC virtually intact. These data suggest that two lymphomas induced by the same agent can be rejected by different effectors. The NK-resistant YWA lymphoma is rejected by a T-dependent mechanism, while the resistance against the inoculation of the highly NK-sensitive YAC line is T-independent and, in all probability, mediated by NK cells.*

INTRODUCTION

RESISTANCE against parental tumor grafts in semisyngeneic F_1 hybrids was first described by Snell and Stevens [1]. The hybrid resistance effect has later been confirmed by others and has been regarded as an immunological reaction sometimes influenced by H-2-linked genes [2, 3].

In previous studies on the highly NK-sensitive Moloney virus-induced YAC lymphoma of strain A origin, we have found a close parallelism between the resistance of semisyngeneic F_1 hybrids to small tumor cell inocula and their NK cell activity against the same target cell line *in vitro* [4]. (A × C57BL) × A backcross mice were tested individually for *in vivo* resistance and *in vitro* NK cell-mediated cytoxicity. There was a close correlation between these two parameters which appeared to be under polygenic control, but with a strong H-2^b-linked component [5].

In a recent *in vivo* study on hybrid resistance [6] we tested a larger collection of lymphomas. The resistance pattern was influenced by different H-2-linked genes depending on the genotype of the lymphoma. Another Moloney virus-induced lymphoma, YWA (of A.SW origin), showed a hybrid resistance pattern that differed significantly from

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the pattern against YAC. Outcross of the syngeneic host to the A congenic, H-2^b-carrying, A.BY strain resulted in a highly resistant F_1 hybrid against YWA, but in no detectable resistance against YAC [4].

These preliminary observations suggested that the genetic control of hybrid resistance against different lymphomas of highly similar origin may differ considerably, depending on the effector cell that mediates the resistance.

In the present study we have compared YWA and YAC directly, and in relation to both resistant and susceptible F_1 hybrid hosts. We have characterized the effector responsible for resistance by both *in vitro* and *in vivo* methods. The relative significance of NK and T cell-dependent resistance was evaluated and was found to differ markedly between the two tumors.

MATERIALS AND METHODS

Animals

The following inbred strains and their F_1 hybrids were used: A/Sn, A.SW, A.CA, A.BY, C57BL/6, CBA and C3H/St. All animals were obtained from our own breeding nucleus and were between 5 and 8 weeks of age when tested.

Tumors

YWA [6] and YAC [7] are Moloney virus-induced lymphomas, derived from strains A.SW and A respectively. The tumor cells were passaged weekly in syngeneic animals in the ascites form. YAC-1 is a tissue culture-adapted line of YAC [8]. In the *in vitro* assays we used a subline of YWA that had been adapted from the corresponding ascites line and grown in tissue culture medium (RPMI 1640 supplemented with 100% heat inactivated FCS and antibiotics) for more than six months.

In vivo *transplantation tests*

YWA and YAC were harvested from the ascitic fluid of syngeneic mice and washed once in balanced salt solution (BSS). Various numbers of trypan-blue-excluding cells (as indicated in the tables and figures) were then inoculated subcutaneously in the flank of syngeneic and semisyngeneic F_1 mice. Tumor growth and regression was followed by weekly palpations during observation periods of between 35 and 49 days. The cumulative take incidence was recorded as per cent tumor takes.

In vitro *cytotoxic assay*

Tumor cells ($2–5 \times 10^6$) were labelled with 50 μCi ^{51}Cr (sodium chromate; New England Nuclear) for 60 min at 37°C, 5% CO_2. Effector cell suspensions were prepared from 3 pooled spleens and mixed with 10^4 [^{51}Cr]-labelled and washed tumor cells in a total volume of 200 μl per well in a microtiter plate (Falcon). Effector: target ratios were in the range between 100 : 1 and 25 : 1, as indicated in the text. The assay was interrupted by a short centrifugation of the plate and 100 μl aliquots of the supernatants were harvested. Radioactivity released into the supernatant was measured in a gamma counter and calculated according to the following formula:

$$\text{percentage specific } [^{51}\text{Cr}] \text{ release} = \frac{\text{test cpm} - \text{spontaneous cpm}}{\text{total cpm} - \text{spontaneous cpm}}.$$

Spontaneous release was determined by incubating target cells in RPMI 1640 10% FCS, and total release by resuspending the target cells in the well before harvesting.

In vivo *rejection assay with* [^{125}I]*UdR-labelled cells*

Tumor cells were labelled by intraperitoneal injection of 50 μCi [^{125}I]-5-iodo-2-deoxyuridine ([^{125}I]UdR) (Radiochemical Centre, Amersham, Bucks, UK) 3 days after the injection of 10^7 viable tumor cells i.p. into a syngeneic mouse. The cells were harvested and washed after 6hr. Syngeneic and F_1 mice were injected via a lateral tail vein with 1×10^6 [^{125}I]UdR-labelled tumor cells in 0.25 ml BSS. The mice were killed at different time intervals and the remaining radioactivity was measured in the whole body and in spleen, liver and lungs separately. Remaining radioactivity was expressed as per cent of injected.

Thymectomy

Thymectomy was performed at 4 weeks of age. Two weeks later the mice received 650 R whole body irradiation and were reconstituted intravenously with $5–15 \times 10^6$ suspended fetal liver cells of the same genotype. In order to check the completeness of the T cell depletion, the antibody response against a T-dependent antigen was measured. Two weeks after reconstitution with fetal liver each mouse was challenged i.p. with 0.1 ml of a 10% solution of horse red blood cells (HRBC) in BSS. All mice were bled 1 week later and thereafter used for *in vivo* rejection studies. Control mice were given the same treatment except for the thymectomy. Sera from all thymectomized and randomly selected control animals were tested for hemolytic antibodies against HRBC in the presence of GPC. All thymectomized mice found to be posi-

tive (titers > 1/10) for antibodies against HRBC (varying between 10 and 30% in the different groups) were excluded from the tests. The control mice all had titers exceeding 1/80.

Statistical analysis

The frequency of takes in F_1 hybrids was compared with the takes in the parental strain by the χ^2-test. Student's *t*-test for grouped observations was used for comparing the remaining radioactivity in F_1 hybrids injected with [^{125}I]UdR-labelled tumor cells. *P* values above 0.1 were considered as non-significant.

RESULTS

In vivo F_1 hybrid resistance against YWA and YAC

Inoculation of 10^4 YWA cells into syngeneic A.SW mice gave rise to palpable tumors in approximately 70% of the animals within two weeks after injection. Interestingly, a large fraction of these tumors regressed and had disappeared completely at the end of the observation period (35–49 days after inoculation). The remaining tumors grew progressively and resulted in death of the animals. Table 1 shows a comparison between A.SW and semisyngeneic F_1 hybrids with regard to the fraction of animals with tumor growth at the time when the maximum number of tumors appeared, total numbers of mice with palpable tumors and the fraction of regression tumors. Most tumors appeared within 14 days after tumor cell inoculation. At this time point, F_1 hybrids between the sygeneic host and the A.BY, C57BL, CBA and C3H strains showed lower tumor take frequencies compared to A.SW. This was highly significant ($P < 0.0005$) for the (A.SW × A.BY), (A.SW × C57BL) and (A.SW × C3H), but of only borderline significance ($P < 0.1$) for (A.SW × CBA). The (A.SW × A) and (A.SW × A.CA) F_1 hybrids showed susceptibilities similar to A.SW. This pattern was also maintained when the cumulative incidence of takes were compared at the end of the observation period. All genotypes showed regression of YWA tumors in a fraction of the cases. In only one F_1 hybrid, (A.SW × A.BY), was the relative number of regressing tumors significantly higher than in the parental strain (100% compared to 47% for A.SW, $P < 0.005$).

The cumulative incidences of progressively growing YWA tumors are shown in Fig. 1a. As indicated by the numbers in Table 1, 41% of the tumors grew progressively in the syngeneic host. The (A.SW × A) and (A.SW × A.CA) F_1 hybrids were not more resistant than syngeneic recipients. The regression in (A.SW × A.BY) led to complete resistance against progressively growing tumors.

Since A.BY is congenic with the A strain, only differing at the segment of chromosome 17 containing H-2, but otherwise sharing the genetic background, these findings suggest that the fate of small YWA inocula is governed by a H-2^b-associated resistance factor. In line with this conclusion, a second H-2$^{s/b}$ F_1 hybrid, (A.SW × C57BL), was also highly resistant against YWA (14% progressively growing takes, $P < 0.005$) compared to A.SW. A comparison between (A.SW × C57BL) and (A.SW × A.BY) revealed a higher resistance in the latter F_1 hybrid, both when the numbers of progressively growing tumors ($P < 0.1$) and the numbers of regressor mice ($P < 0.005$) were com-

*Table 1. Growth and regression of YWA cells in syngeneic and semisyngeneic mice**

Genotype	Number of mice with tumors 14 days after inoculation (%)	Total number of mice with tumors (%)	Number of regressor mice (%)
A.SW	55/75 (73)	58/75 (77)	27/58 (47)
A.SW × A	15/20 (75)	15/20 (75)	5/15 (33)
A.SW × A.CA	20/29 (69)	25/29 (86)	10/25 (40)
A.SW × A.BY	8/33 (24)†	13/33 (39)	13/13(100)§
A.SW × C57BL	8/51 (16)†	10/51 (20)	3/10 (30)‖
A.SW × CBA	31/56 (55)‡	24/56 (43)	10/24 (42)
A.SW × C3H	14/41 (34)†	20/41 (49)	14/20 (70)

*Pooled tests with 10^4 YWA cells inoculated s.c.
†$P < 0.0005$, compared to A.SW.
‡$P < 0.1$, compared to A.SW.
§$P < 0.005$, compared to A.SW.
‖$P < 0.005$, compared to (A.SW × A.BY).

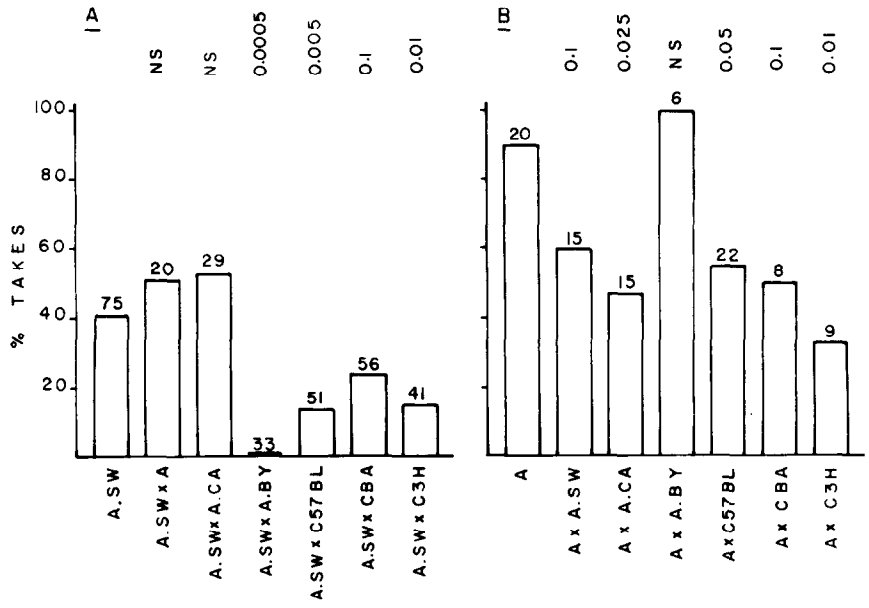

Fig. 1. Incidence of tumor takes after inoculation of (a) 10^4 YWA and (b) 10^3 YAC tumor cells into syngeneic and semisyngeneic F_1 recipients. Horizontal numbers at the top of the columns designate the number of mice inoculated. Vertical numbers denote the statistical significance of the difference between the take incidence in the F_1 hybrid and the syngeneic parental strain in a χ^2-test. NS: not significant.

pared. These differences suggested influence from the C57BL background on the degree of resistance. Both the (A.SW × CBA) and (A.SW × C3H) F_1 hybrids showed a low frequency of takes, but only the latter was significantly ($P < 0.01$) more resistant than A.SW.

The YAC lymphoma showed a different behaviour compared to YWA in that once a palpable tumor was formed it continued to grow progressively. The hybrid resistance pattern against YAC (Fig. 1b) differed from that against YWA in one important respect: (A × A.BY) hybrids were not significantly more resistant to small tumor grafts (10^3 cells) than the A parent. Thus, the H-2^b haplotype does not introduce a resistance factor *per se* against this tumor. However, we have previously found a strong H-2^b linkage of the resistance to YAC in segregating (A × C57BL) × A and (A × C57L) × A backcross mice [4, 5]. This discrepancy may be due to the combined effect of H-2^b-linked and other C57BL-derived genes in determining resistance against YAC. Alternatively, it is also possible that the H-2^b-linked resistance gene against YAC demonstrated in C57BL is actually located on chromosome 17 although outside the H-2 complex, and thus may not be present in A.BY.

As another difference between the two lymphomas, outcross of the syngenic host to the A congenic A.SW and A.CA strains produced resistant hybrids against YAC, whereas the corresponding hybrids were as susceptible to [YWA] as the syngeneic host itself.

In vitro *sensitivity to NK cell-mediated lysis*

YAC ascites tumor cells are moderately sensitive to NK cell-mediated lysis, and the sensitivity increases after 3–4 weeks in tissue culture [9]. We found that YWA was relatively resistant to NK lysis even if a tissue culture adapted line, carried for more than 6 months *in vitro*, was used. In short-term (4–6 hr) assays with effector cells derived from a variety of mouse strains, YWA showed no or only very low levels of lysis compared to the highly NK sensitive YAC-1 line (Table 2). In a 16-hr assay with effector cells from (A.SW × C3H), YAC-1 showed 71% lysis but YWA only 15% lysis (Table 2). In spite of the relative insensitivity of YWA, a similar genetic pattern could be discerned as against YAC-1: C57BL and (A.SW × C3H) were somewhat more active than A.SW and (A.SW × A.BY).

The relative resistance of YWA cells to NK-mediated *in vitro* lysis made it rather unlikely that *in vivo* hybrid resistance against this lymphoma was mediated by NK cells. This conclusion was also reinforced by the fact that the highly resistant (A.SW × A.BY) F_1 hybrid showed low NK activity *in vitro* against YAC-1 and YWA.

In vivo *rejection of* (^{125}I)*UdR-labelled cells*

Radiolabelled lymphoma cells injected into

normal allogeneic and semisyngeneic mice are rapidly eliminated by a cytotoxic mechanism provided there are H-2 differences between the lymphoma cells and the recipient [10, 11]. Puccetti *et al.* and Ricardi *et al.* [12, 13] have found that the NK activity of different strains *in vitro* can be correlated with their ability to eliminate radiolabelled YAC-1 lymphoma cells. We have also shown that the NK-sensitive EL 4 lymphoma is more efficiently eliminated from C57BL mice with normal NK activity than from the partially NK-deficient littermates which are homozygous at the beige locus [14]. In this study we have used the *in vivo* short-term rejection assay with [^{125}I]UdR-labelled tumor cells to compare the elimination of YWA in relation to the hybrid resistance and NK activity pattern against this cell line.

In 5 out of 6 experiments there was no demonstrable difference in the total residual radioactivity, measuring the elimination of 10^6 YWA cells from the susceptible A.SW and the highly resistant (A.SW × A.BY) F_1 hybrid. In one representative experiment, shown in Table 3, approximately 50% of the injected radioactivity was already cleared after 4 hr, with no significant difference between the genotypes tested. After 18 hr, 23% of the label was retained both in A.SW and (A.SW × A.BY).

Table 4 (experiment 1) shows that the organ distribution of the radioactive label was similar for the *in vivo* resistant (A.SW × A.BY) F_1 hybrid and the syngeneic A.SW host. However, in a second experiment a more rapid elimination was seen from the lungs of the (A.SW × C3H) mice compared to A.SW, (A.SW × A.BY) and (A.SW × A.CA) mice (Table 4). The elimination from the lungs was in line with the NK activities of the genotypes tested. The highly NK-active (A.SW × C3H) eliminated somewhat more radioactivity than the low NK-active A.SW, (A.SW × A.BY) and (A.SW × A.CA) (compare Table 2).

Effect of thymectomy on hybrid resistance

Table 5 shows the effect of thymectomy, whole body irradiation and fetal liver reconstitution on the resistance of A.SW, (A.SW × C3H) and (A.SW × C57BL) F_1 hybrid mice against YWA cells. Take incidence was much greater (100%) in the thymectomized animals, compared to the irradiated and reconstituted

Table 2. In vitro *sensitivity of YWA and YAC-1 to natural killer cells*

Experiment No.	Assay duration (hr)	Genotype of effector cells	Percentage lysis* YWA	YAC-1
1	4	A.SW	−3.7	3.3
		A.BY	−1.2	6.8
		C57BL	5.7	30.0
2	6	A.SW	−3.6	8.5
		A.SW × C3H	0.8	24.4
3	16	A.SW	7.2	44.7
		A.SW × A.BY	7.6	38.0
		A.SW × C3H	15.4	70.2

*Effector to target cell ratio 100 : 1.

Table 3. Elimination of intravenously injected [^{125}I]*UdR-labelled YWA cells from syngeneic and semisyngeneic mice**

Genotype	Percentage remaining radioactivity† after 4hr	18hr
A.SW	56.7(7.2)	23.5(6.7)
A.SW × A.BY	53.4(9.5)	23.4(4.8)
A.SW × C57BL	51.1(6.8)	N.T.‡

*Each mouse was injected with 10^6 [^{125}I]UdR-labelled tumor cells into a lateral tail vein. At different time intervals the mice were killed, and the remaining radioactivity was determined by whole body counting in a gamma counter. Each group consisted of 5 mice.

†Percentage of injected cpm(± S.D.).

‡Not tested.

control groups. With the YAC lymphoma, the results were the opposite: thymectomy led to only a slight reduction of hybrid resistance (Table 5). (A × C57BL) and (A × CBA) F_1 hybrids were significantly more resistant to an inoculum of 10^3 YAC cells than syngeneic A mice, no matter whether they were thymectomized or not.

DISCUSSION

In previous studies [4, 5] we have found close parallelisms between hybrid resistance *in vivo* and NK activity *in vitro* against the Moloney virus-induced lymphoma of strain A origin. Later tests on a larger collection of lymphomas showed that YAD, another Moloney virus-induced lymphoma of strain A origin, was not rejected by any of the F_1 hybrids tested [6]. Since YAD was NK-resistant, this finding was in line with the hypothesis that the hybrid effect is mediated by NK cells. A third Moloney-induced lymphoma, YWA of A.SW origin, showed a different pattern compared to YAC, manifested by the complete resistance of the (A.SW × A.BY) F_1 hybrid. The A.BY strain, congenic with both A and A.SW, fails to introduce hybrid resistance against YAC in the (A × A.BY) cross. Both the (A × A.BY) and the (A.SW × A.BY) F_1 hybrids are low in NK activity. Two main alternatives were considered in order to explain this difference in the F_1 resistance pattern against the two lymphomas: (1) the genetic control of NK activity against YWA differs from the pattern characteristic for YAC [15] as well as against tumors of other genotypes [6]; (2) hybrid resistance against YWA is mediated by a different (non-NK) mechanism.

The present study confirms the strong resistance of the (A.SW × A.BY) hybrid and a number of other F_1 hybrids. Our data suggest that resistance against YWA is influenced by both H-2-associated and non-H-2-associated factors. The latter is based on the fact that F_1 hybrids with the same H-2 genotype but differing in their background (non-H-2) genome differed with regard to the degree of their resistance. While the (A.SW × A.BY) F_1 ($H\text{-}2^{s/b}$) was completely resistant to 10^4 tumor cells, the H-2 identical (A.SW × C57BL) hybrid was somewhat more susceptible to progressively growing tumors (14% takes). The basis

Table 4. Organ distribution of residual radioactivity after intravenous injection of [^{125}I]UdR-labelled YWA cells into syngeneic and semisyngeneic mice

Experiment* No.	Genotype	No. of mice	Percentage of injecting cpm remaining in: Spleen	Liver	Lungs
1	A.SW	5	1.7(0.6)	11.1(2.7)	9.0(4.3)
	A.SW × A.BY	3	1.6(0.1)	11.7(0.7)	8.6(3.9)
2	A.SW	4	1.1(0.8)	1.9(1.4)	0.9(0.9)
	A.SW × A.BY	5	1.0(0.5)	0.9(0.4)	0.5(0.2)
	A.SW × A.CA	5	0.9(0.4)	1.9(0.6)	0.7(0.4)
	A.SW × C3H	5	0.8(0.2)	1.3(0.4)	0.2(0.1)

*Experiment 1 was terminated after 18 hr and experiment 2 after 12 hr. The elimination of radioactivity from the lungs of (A.SW × C3H) was significantly lower than for (A.SW × A.CA) ($P < 0.01$). The values within parentheses represent the standard deviation (± S.D.).

Table 5. Incidence of progressively growing tumors after inoculation of small doses of YWA and YAC cells into intact and thymectomized mice

Tumor	Recipient host	Control†	Thymectomized†
YWA	A.SW‡	10/12(83)	7/7(100)
	A.SW × C57BL‡	5/33(15) $P < 0.0005$	12/12(100)
	A.SW × C3H§	2/7(29) $P < 0.1$	5/5(100)
YAC‖	A	12/14(86)	16/19(84)
	A × C57BL	0/11(0) $P < 0.0005$	2/10 (20) $P < 0.005$
	A × CBA	2/11(18) $P < 0.005$	2/8 (25) $P < 0.025$

*Incidence of progressively growing tumors after s.c. inoculation.
†As described in Materials and Methods.
‡10^4 cells.
§Pooled results from tests with 10^4 and 10^5 cells.
‖10^3 cells.

for the complete resistance introduced by the outcross to A.BY was the higher frequency of regressing tumors in this hybrid (100%) compared to (A.SW × C57BL) (30%, $P < 0.005$). This suggests that the genetic background of C57BL modifies the expression of the H-2^b-associated resistance.

YWA showed a very low sensitivity to NK cell-mediated lysis *in vitro* compared to YAC-1 (Table 2). Significant lysis was only obtained in a long-term assay (16 hr). The genetic control of NK activity against these two lymphomas was similar, however. It is therefore unlikely that NK cells could be responsible for the resistance of the (A.SW × A.BY) hybrid against YWA, since this hybrid has an equally low NK activity as the parental A.SW host.

The *in vivo* elimination studies confirmed our *in vitro* results and demonstrated no difference between the susceptible and the resistant genotypes in 4 to 18 hr rejection assays with [^{125}I]UdR-labelled YWA cells (Table 3). Elimination of radiolabelled cells of the NK-sensitive YAC-1 and EL 4 lymphomas from the lungs of mice has been shown to correlate with the NK activity *in vitro* for the mouse strain[12–14]. Apparently, the low NK sensitivity of YWA does not permit any major NK-mediated elimination of labelled cells, since there was no difference between low and high NK-reactive F_1 hybrids with regard to the total residual radioactivity. However, it cannot be excluded that NK cells may play a role in determining the fate of intravenously injected YWA cells, since one of the resistant F_1 hybrids, (A.SW × C3H), with high NK activity *in vitro*, showed a more efficient elimination of radioactivity from the lungs compared to low-reactive genotypes. It has been demonstrated that cytotoxic cells with the same characteristics as NK cells can be recovered from the lungs of normal mice [17].

In contrast to the results of Carlson *et al.* [11], we were not able to demonstrate any H-2-associated *in vivo* rejection. This may, however, reflect the uniqueness of the YWA tumor that we have used in this study.

The generation of cytotoxic T cells [18] or a T-dependent humoral response [19] provides alternatives to NK-mediated resistance to small tumor cell inocula. A T cell-dependent rejection of YWA could be demonstrated by the thymectomy–irradiation–reconstitution experiments, which totally abrogated the F_1 resistance against YWA but did not influence the resistance against YAC (Table 5). The nature of the thymus-dependent mechanism cannot be established from these data, however. The frequent regression observed for YWA tumors suggested that the induction of an immune response was required in instances where the first line of defence against tumor growth had been unsuccessful. Against YAC this first line of defence is T-independent and in all probability is mediated by NK cells [20]. Since YWA is NK-resistant and the *in vivo* rejection pattern, in addition to being T-dependent, does not correlate with the pattern of NK activity for the F_1 hybrids, some other type of effector has to be postulated. Cytotoxic T cells are favoured by our recent demonstration of a highly specific cytotoxic response in resistant F_1 hybrids after challenge with YWA cells (F. Merino, manuscript in preparation). Cell-mediated immunity is implicated in the resistance against YWA also by the fact that we have failed to induce cytotoxic antibodies by extensive immunization of (A.SW × A.BY) with irradiated YWA cells (unpublished observation).

In a study on regulation of killer T cell activity against the BALB/c leukemia, RL♂1, [21] it was shown that responsiveness was controlled by a dominant Ir gene but with a non-H-2 gene also required for full expression of anti-RL♂1 cell-mediated immunity. Since A.SW and A.BY are H-2 congenic sublines of strain A, it is likely that the complete resistance of this hybrid to the transplantation of YWA is mediated by Ir genes introduced by the A.BY parent. Modification of the action of immune response genes by a non-H-2 associated gene similar to that described by Duprez *et al.* [21] could presumably be responsible for the somewhat lower resistance in (A.SW × C57BL) compared to (A.SW × A.BY). Both hybrids are H-$2^{s/b}$, but differ in their background genome. A more precise mapping of these genes would be of great interest.

Since both YAC and YWA are Moloney virus-induced lymphomas of T cell origin (Dr B. Åsjö, personal communication) it was of interest to note the striking difference in NK sensitivity and T cell-dependent rejection for the two cell lines. A somewhat parallel situation was described by Becker and Klein [22], showing preferential T cell killing of RBL-5, compared to the antigenically cross-reactive and more NK-sensitive YAC line, when MSV-immune effector cells were used. A general high susceptibility to immune T cell-mediated lysis may not be the only explanation for a T-dependent rejection of YWA. The existence of a unique cell surface antigen remains to be demonstrated, however.

Taken together, the difference in the sensitivity of the YWA and YAC lymphomas to NK versus T cell-mediated rejection is reflected in the genetic patterns of resistance *in vivo*.

This emphasizes that the role of immune responsiveness genes (or other genes that influence the various components of the immune response) in tumor resistance must be evaluated in relation to the genetics of the host, the immune effectors involved, the immunogenicity of the tumor and the sensitivity of the target cell.

REFERENCES

1. SNELL GD, STEVENS LC. Histocompatibility genes of mice. III. H-1 and H-4, two histocompatibility loci in the first linkage group. *Immunology* 1961, **4** 366–379.
2. OTH D, DONNER N, BURG C. Measurement of the antitumoral immune reactions against a strain-specific chemically induced sarcoma in syngeneic and F_1 hybrid mice. *Eur J Cancer* 1971, **7**, 479–482.
3. SATO H, BOYSE EA, AOKI T, IRITANI C, OLD LJ. Leukemia associated transplantation antigens related to murine leukemia virus. The X1 System: immune response controlled by a locus linked to H-2. *J Exp Med* 1973, **138**, 593–606.
4. KIESSLING R, PETRANYI G, KLEIN G, WIGZELL H. Genetic variation of *in vitro* cytolytic activity and *in vivo* rejection potential of non-immunized semi-syngeneic mice against a mouse lymphoma line. *Int J Cancer* 1975, **15**, 933–940.
5. PETRANYI GG, KIESSLING R, POVEY S, KLEIN G, HERZENBERG L, WIGZELL H. The genetic control of natural killer cell activity and its association with *in vivo* resistance against a Moloney lymphoma isograft. *Immunogenetics* 1976, **3**, 15–28.
6. KLEIN G, KLEIN GO, KÄRRE K, KIESSLING R. "Hybrid resistance" against parental tumors: one or several genetic patterns? *Immunogenetics* 1978, **7**, 391–404.
7. KLEIN E, KLEIN G. Antigenic properties of lymphomas induced by the Moloney agent. *J Natl Cancer Inst* 1964, **32**, 547–568.
8. CIKES M, FRIBERG S, Jr, KLEIN G. Progressive loss of H-2 antigens with concomitant increase of cell-surface antigens determined by Moloney leukemic virus in cultured murine lymphomas. *J Natl Cancer Inst* 1973, **50**, 347–362.
9. BECKER S, KIESSLING R, LEE N, KLEIN G. Modulation of sensitivity to natural killer cell lysis after *in vitro* explanation of a mouse lymphoma. *J Natl Cancer Inst* 1978, **61**, 1494–1498.
10. CARLSON GA, WEGMAN TG. Rapid *in vivo* destruction of semisyngeneic and allogeneic cells by nonimmunized mice as a consequence of nonidentitiy at H-2. *J Immunol* 1977, **118**, 2130–2137.
11. CARLSON GA, MELNYCHUK D, MEEKER MJ. H-2 associated resistance to leukemia transplantation: natural killing *in vivo*. *Int J Cancer* 1980, **25**, 111–122.
12. PUCCETTI P, SANTONI A, RICCARDI C, HERBERMAN RB. Rapid *in vivo* assay of mouse natural killer cell activity. *J Natl Cancer Inst* 1979, **63**, 1041–1045.
13. RICCARDI C, SANTONI A, BURLOZZARI T, HERBERMAN RB. Role of NK cells in rapid *in vivo* clearance of radiolabelled tumor cells. *In*: *Natural Cell-mediated Immunity Against Tumors*. New York, Academic Press, 1980, 1121–1139.
14. KÄRRE K, KLEIN GO, KIESSLING R, KLEIN G, RODGER JC. *In vitro* NK-activity and *in vivo* resistance to leukemia: studies of *beige, beige/nude* and wild-type hosts on C57BL background. *Int J Cancer* 1980, **26**, 789–797.
15. PETRANYI GG, KIESSLING R, KLEIN G. Genetic control of "natural" killer lymphocytes in the mouse. *Immunogenetics* 1975, **2**, 53–61.
16. KLEIN GO, KLEIN G, KIESSLING R, KÄRRE K. H-2 associated control of natural cytotoxicity and hybrid resistance RBL-5. *Immunogenetics* 1978, **6**, 561–569.
17. PUCCETTI P, SANTONI A, RICCARDI C, HERBMAN RB. Cytotoxic effector cells with the characteristics of natural killer cells in the lungs of mice. *Int J Cancer* 1980, **25**, 153–158.
18. MERUELO D, DEAK B, MCDEVITT HO. Genetic control of cell-mediated responsiveness to an AKR tumor-associated antigen. *J Exp Med* 1977, **146**, 1367–1379.
19. MERUELO D, FLIEGER N, SMITH D, MCDEVITT HO. *In vivo* or *in vitro* treatments with anti-I-J alloantisera abolish immunity to AKR leukemia. *Proc Natl Acad Sci U.S.A.* 1980, **77**, 2178–2182.
20. KIESSLING R, PETRANYI G, KLEIN G, WIGZELL H. Non-T-cell resistance against a mouse Moloney lymphoma. *Int J Cancer* 1976, **17**, 275–281.
21. DUPREZ V, BELUCCI S, LEVY JP. Cell-mediated anti-tumor response in the RL♂1 system. II. Control of the T killer cells by an H-2-linked Ir gene interfering with non-H-2 factors. *Eur J Immunol* 1980, **10**, 26–30.
22. BECKER S, KLEIN E. Decreased "natural killer" effect in tumor-bearing mice and its relation to the immunity against oncorna virus-determined cell surface antigens. *Eur J Immunol* 1976, **6**, 892–898.