mouse strains displayed Ly-1^b and Ly-2^b as common features [2]. The significance of the differentiation in Ly haplotype, however, remains unclear.

It was deemed interesting to determine how a living body recognizes and rejects those tumor cells which lack the H-2 antigen on their cell surface. In this experiment, the susceptibility of H-2 congenic or Ly congenic mice to EAT was compared with their standard strains to see how the shift restricted in H-2 or in Ly loci affects the mouse strain susceptibility to EAT.

MATERIALS AND METHODS

Laboratory animals used

C57BL/10(=B10)(H-2^b, Ly-1^b, Ly-2^b), B10.D2(H-2^d), B10.BR (H-2^k) and C57BL/6(=B6) (H-2^b, Ly-1^b, Ly-2^b), mice were purchased from SLC Inc. (Shizuoka, Japan). B10.Y/Sn (H-2^{pa}), B10. RIII(71NS) /ola (H-2^r) and B10. SM (70NS) / Sn (H-2^v) mice were donated by the National Institute of Genetics (Mishima, Japan). B6-Ly-1^a (H-2^b, Ly-1^a, Ly-2^b), B6-Ly-2^a (H-2^b, Ly-1^b, Ly-2^a), B6-Ly-2^a, 3^a (H-2^b, Ly-1^b, Ly-2^a, Ly-3^a), B6-Tla^a (H-2K^k • D^b) and B6. C-H-2^{bm12}(H-2A_β: bm 12) congenic mice were donated by Aichi Cancer Center Research Institute (Nagoya, Japan).

All mice were used under specific pathogen free conditions. Three to four mice were housed in plastic cages (14.3×29.3×14.8 cm, Charles River Japan Inc., Atsugi, Japan) with bedding (cedar shavings) and fed a cube diet (CE-2, CLEA Japan Inc., Tokyo, Japan) and water *ad libitum*. All the cages and bedding were autoclaved before use and stored in a separate room. The environmental conditions of the animal room were controlled at a constant temperature (23±1℃) and humidity (45 to 75%). The room was ventilated 18 times per hour and was illuminated at 300 lx by daylight fluorescent lamps in a 12/12 -hour light/dark cycle.

All animal procedures conformed to established guidelines (ILAR)[3] and the Guidelines for the Regulation of the Animal Experimentation (JALAS 1987)[4]. The mice were killed by cervical dislocation.

Tumors

EAT, maintained by the intraperitoneal transfer of 10^7 cells to ddY mice (closed colony, H-$2^{s/q}$, 5 to 8 weeks old), were harvested on days 7 to 10 post transfer and washed in phosphate-buffered saline (pH 7.4). The tumor cells (2×10^7) were inoculated subcutaneously into the central portion of the dorsal skin of each mouse. EAT outgrowth was then monitored by measuring the length and width of the developing solid tumors.

EAT regression rates in the congenic mice were statistically analyzed in comparison with those in control mice with Fisher's exact test for fourfold tables.

RESULTS

Susceptibility of B10-H-2 congenic mice to EAT

The grades of EAT outgrowth in B10-H-2 congenic mice at 45 days after subcutaneous inoculation of the tumor cells are summarized in Table 1.

B10(H-2^b) as a standard was strongly EAT-regressive. Subcutaneously inoculated EAT (2×10^7 cells) regressed completely and disappeared within 10 days in this strain.

B10.D2(H-2^d), which is H-2 congenic between B10(H-2^b) and DBA/2(H-2^d), was EAT-regressive, though the H-2 locus of B10 shifted to the haplotype d of DBA/2. DBA/2 itself was EAT-progressive. This indicates that at least H-2K and D loci as class I are not simply related to the mouse strain susceptibility to EAT. B10.BR (H-2^k), which is congenic of B10(H-2^b) and C57BR (H-2^k), was EAT-progressive. C57BR itself is estimated to be EAT-progressive as are AKR (H-2^k), C3H (H-2^k), and CBA (H-2^k). In B10, the shift in the H-2 haplotype from b to k was significant in changing the susceptibility to EAT. B10.Y (H-2^{pa}) was EAT-regressive. B10.RIII(H-2^r) was almost EAT-regressive with some individual variations. B10. SM (H-2^v) was EAT-progressive, indicating that SM (H-2^v) itself is EAT-progressive. The shift in H-2 haplotype from b to v in B10 was also significant in changing the mouse strain susceptibility to EAT.

No significant difference in susceptibility between male and female within the strain was observed throughout the experiment.

Susceptibility of B6-Ly-congenic mice to EAT

The grades of EAT outgrowth in B6-Ly congenic mice at 45 days after subcutaneous inoculation of the tumor cells are summarized in Table 2.

B6 mice (H-2^b, Ly-1^b, Ly-2^b) were strongly EAT-regressive. In the same manner, B6-Ly-1^a, 2^a and/or 3^a congenic mice were all EAT-regressive. No change in B6 susceptibility to EAT was produced by the Ly haplotype shift from b to a. Subcutaneously inoculated EAT regressed completely within 10 days in the congenic mice. Although the Ly haplotypes of EAT-progressive mice classified were Ly-1^a (except

AKR) and Ly-2^a and those of EAT-regressive strains classified were all Ly-1^b and Ly-2^b as common features, the shift in Ly-1 or in Ly-2, or shifts in Ly-2 and 3 loci from b to a, are therefore, not related to the mouse strain susceptibility to EAT.

Susceptibility of other congenic mice to EAT

Other B6 congenic mice such as B6-Tla^a and B6.C-H-2^{bm12} were also examined. B6-Tla^a (H-$2K^k$ • D^b) is TL congenic. TL loci as class I in H-2 complex usually codes surface antigens of lymphoid cells. B6.C-H-2^{bm12}(H-$2A_\beta$: bm12) is characterized by I-A subregion (class II) mutation.

As shown in Table 3, they were both EAT-regressive. No change in susceptibility to EAT was produced. Subcutaneously inoculated EAT regressed completely within 10 days. The function of the

Table 1. Susceptibility of B10-H-2 congenic mice to EAT (2×10^7, s.c.)

Congenic mouse			EAT outgrowth at 45 days after inoculation					
Strain	H-2		−	+	++	+++	Total	Regression(%)
B10	b	♂	5[1)]				5	100.0
		♀	5				5	100.0
DBA/2	b	♂				5	5	0***
		♀				5	5	0***
B10.D2	d	♂	4				4	100.0
/SnSlc		♀	3				3	100.0
B10.BR	k	♂		1	1	2	4	0**
		♀	1	1		3	5	20.0*
B10.Y/Sn	pa	♂	5				5	100.0
		♀	5				5	100.0
B10.RIII	r	♂	14	1			15	93.3[2)]
(71NS)/ola		♀	10		1		11	90.9[3)]
B10.SM	v	♂		1	3		4	0**
(70NS)/Sn		♀		1	4		5	0***

[1)], number of mice, EAT outgrowth; −, completely regressive; +, solid tumor (~1cm diameter); ++, solid tumor (1~2cm diameter); +++, solid tumor (2~3 cm diameter); *, statistically significant in comparison with B10 standard with Fisher's exact test for fourfold tables ($p<0.05$); **, ibid. ($p<0.01$); ***, ibid. ($p<0.005$); [2)], not statistically significant ($p=0.75$); [3)], not statistically significant ($p=0.73$)

Table 2. Susceptibility of B6-Ly congenic mice to EAT (2×10^7, s.c.)

Ly congenic					EAT outgrowth at 45 days after inoculation			
Strain	Ly-1	Ly-2	Ly-3		−	+	Total	Regression(%)
B6	b	b	b	♂	5[1)]	0	5	100.0
				♀	5	0	5	100.0
B6-Ly-1^a	a	b	b	♂	3	0	3	100.0[2)]
				♀	7	0	7	100.0
B6-Ly-2^a	b	a	b	♂	4	0	4	100.0
				♀	3	0	3	100.0
B6-Ly-2^a,3^a	b	a	a	♂	5	0	5	100.0
				♀	5	0	5	100.0

[1)], Number of mice, EAT outgrowth; −, completely regressive; +, solid tumor (~1 cm diameter), [2)], each regression rate in Ly congenic mice was analyzed with Fisher's exact test for fourfold tables, showing no significant difference from that in the B6 standard.

TL loci or the A_β subregion was therefore thought to be insignificant for the determination of mouse strain susceptibility to EAT.

DISCUSSION

As initially described, EAT cells lack most H-2 antigens on their cell surface. This characteristic is the result of numerous serial selections *in vivo* of the tumor cell population since the beginning of this century through outbred mice with different genetic backgrounds[5]. The differentiation of EAT-regressive and EAT-progressive mouse strains is nevertheless apparently conditioned by their H-2 haplotypes with some exceptions. Antigens due to minor histocompatibility (H) genes possibly remain on the EAT cell surface because of weak rejection by the host defense mechanism. Data concerning the immunogenecity of EAT have been reported showing the existence of such tumor-associated transplantation antigens on the EAT cell surface[6-11]. EAT-regressive mice may recognize such minor H antigens remaining on their cell surface under the control of minor H genes frequently coordinated with a H-2 locus function. Other possibilities that some unknown tumor-resistant gene may exist within the H-2 region restricted in EAT-regressive mouse strains or a minor H gene near H-2 locus accompanied as passenger in some congenic strains, might be considered to explain such an incomplete H-2-associated phenomenon in congenic resistant strains. It has been suggested that spontaneous insulitis and thyroiditis in the BB/Wor rat develop through common immune defects involving T cell lymphopenia, but do not always segregate together due to disease-specific interactions with the MHC-class II-linked genes [12].

Tumor rejection in the present experiment resembles in part a weak and delayed rejection of the transplanted allogeneic skin graft which is under the control of minor H genes. It has been shown that skin grafts from MHC (class I and class II)-deficient mice are rapidly rejected by normal allogeneic recipients, and the MHC-deficient mice reject allogeneic skin grafts with little delay[13], suggesting plasticity or some compensatory mechanisms in the immune system. Under a weak MHC gene function, the recognition by minor H genes may play an important role in rejecting allografts. In a skin graft transplantation, B6 female mice reject syngeneic skin grafts from B6 male mice[14,15]. B6. C-H-2^{bm12} female mice, I-A subregion mutation (A_β: bm12), do not reject skin grafts from B6 male mice[16]. It has been suggested that minor H genes which function in the recognition of X-Y antigens are under the control of the Ir gene locus in the H-2 region. In comparison, B6.C-H-2^{bm12} mice, in both males and females, rejected transplanted EAT as strongly as B6 mice did. At least the A_β locus in the H-2 region was not functional in the process of EAT rejection in the present series of experiments. In our experimental system, functions of minor H genes might be considered under a linkage with H-2 loci to explicate the H-2 associated mouse strain susceptibility to EAT. From our previous data concerning ddY-drm and ddY-prg back cross breeding, the number of related gene loci to satisfy, $[(\text{ddY-drm} \times \text{ddY-prg})\ F_1 \times \text{ddY-drm}]: (0.5)^n = 0.27$ and F_2: $(0.75)^n = 0.72$ according to Snell's theory[17], was shown to be one or two[18].

On the other hand, shifts from haplotype b to a in Ly-1,2 and/or 3 loci left no perceptible influence on B6 mice susceptibility to EAT. At least, a single locus of Ly-1 or Ly-2, or Ly-2 and Ly-3 loci is not directly concerned with the susceptibility. The classification of Ly haplotypes into EAT-progressive and EAT-regressive mouse strains is thought to be coincidental at present, though we cannot deny the change in the susceptibility of mice with Ly-1^a and 2^a double shifts. Congenic mice with Ly-1^a, 2^a double shifts are not obtainable at present, but when they are, additional tests on their susceptibility will be

Table 3. Susceptibility of other B6 congenic mice to EAT (2×10^7, s.c.)

Others					EAT outgrowth at 45 days after inoculation			
Strain	H-2	Ly-1	Ly-2		−	+	Total	Regression(%)
B6-T1^a	k/b	2	2	♂	10[1)]	0	10	100.0[2)]
				♀	10	0	10	100.0
B6.C-H-2^{bm12}	b	2	2	♀	10	0	10	100.0
				♀	3	0	3	100.0

[1)], Number of mice, EAT outgrowth; −, completely regressive; +, solid tumor (~1 cm diameter), [2)], each regression rate in Ly congenic mice was analyzed with Fisher's exact test for fourfold tables, showing no significant difference from that in the B6 standard.

required.

Other Ly loci such as Ly-6, Ly-10, Ly-11, Ly-18, Ly-19, Ly-20, Ly-22 and Ly-31 do not show common differentiation into haplotype b or a classified, according to their EAT-regressive or EAT-progressive characteristics[2]. Although Ly-antigens on the mouse lymphocyte cell surface increase in number, initially characterized by complement-dependent cytotoxicity test[19,20] and later by monoclonal antibodies[21-25], the function of each Ly gene in tumor rejection remains unclear.

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