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Evaluation of Adoptive Immunotherapy for Ehrlich Tumors in Mice

Norimitsu L. SATO and Akiko KATO

Institute for Laboratory Animals, Niigata University School of Medicine, Niigata, Japan

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Summary. **Although the genetic relationship between donor and recipient is quite critical for obtaining real effectiveness in adoptive immune transfer against cancer, the essential prerequisite in their genetic correspondence is not completely understood. For an analysis, a combination of Ehrlich ascites tumor (EAT) and mouse strains as host comprises a desirable experimental system because some mouse strains are susceptible to EAT while others are resistant. In the present series of experiments, adoptive immunotherapeutic effects on EAT were examined in mice by transferring spleen cells from EAT-regressive mouse strains to EAT-progressive ones, with the following results obtained.**

Activated spleen cells from B6 (H-2^b) and its Ly congenic mice such as B6-Ly-1^a, B6-Ly-2^a, and B6-Ly-2^a, 3^a as donors were very effective in suppressing EAT outgrowth in B6-nu/nu mice. No graft versus host disease (GVHD) was observed in the recipient. Activated spleen cells from other B6 congenic mice such as B6-Tla^a (H-$2K^k$ • D^b) and B6.C-$H2^{bm12}$ (H-$2A_\beta$: bm12) were highly or moderately effective in suppressing EAT outgrowth in B6-nu/nu, and in the latter combination of B6.C-$H2^{bm12}$ and B6-nu/nu, the recipient showed GVHD. In contrast, activated spleen cells from B6 mice were ineffective in suppressing EAT outgrowth in ddY-prg (H-2^q) or in ICR-nu/nu (H-$2^{q/?}$) mice.

In other combinations such as B10 (H-2^b) and B10.BR (H-2^k), adoptive immune transfer was unsuccessful. Adoptive immune transfer from B10.D2 (H-2^d) to DBA/2 (H-2^d) was also unsuccessful, though the donor and recipient share the same H-2 genes. Transfer from BALB/c (H-2^d) to DBA/2 (H-2^d) also failed even though they have similar genetic profile.

On the other hand, activated spleen cells from B6 and from C3H were ineffective in suppressing EAT outgrowth in the hybrid; [B6×C3H] F_1. With this combination, the recipient showed GVHD.

The results indicated the following: 1) Adoptive immunity to EAT was successfully transferred from B6 mice to B6-nu/nu mice, and some genetic shifts in TL or Ly haplotypes in the donor interferred slightly with the effectiveness. 2) Between the allogeneic inbred mouse strains, adoptive immune transfer was unsuccessful even between a donor and recipient combination which share the same H-2 haplotype, suggesting interference by other genes such as the minor histocompatibility gene. 3) GVHD occurred in some ineffective recipients but not in other ineffective ones, indicating the existence of two different interactions between donor spleen cells and recipient defense mechanisms which disturb the immune transfer.

Key words—cancer immunotherapy, Ehrlich tumor, spleen cell transfer.

Correspondence: Norimitsu L. Sato, Institute for Laboratory Animals, Niigata University School of Medicine, Niigata 951-8510, Japan.

INTRODUCTION

Mouse strains show different susceptibility to Ehrlich ascites tumor (EAT) cells which lack most H-2 antigens on their cell surface.

Mice such as AKR/J (H-2^k), C3H/He (H-2^k), CBA/J (H-2^k), DBA/1 (H-2^q), DBA/2 (H-2^d), ddY-prg (H-2^q), ICR (closed colony, H-$2^{q/?}$) and B10.BR (H-2^k) were EAT-progressive, in which subcutaneously inoculated EAT cells grow and form a solid tumor[1,2]. On the other hand, mice such as A/J (H-2^a), BALB/c (H-2^d), C57BL/6 (H-2^b, Ly-1^b, Ly-2^b), C57BL/10 (H-2^b, Ly-1^b, Ly-2^b), NZB/N (H-2^d), SJL (H-2^s), ddY-drm (H-2^s), B10.D2 (H-2^d), B6-Ly-1^a (congenic, H-2^b, Ly-1^a, Ly-2^b), B6-Ly-2^a (congenic, H-2^b, Ly-1^b, Ly-2^a), B6-Ly-2^a, 3^a (congenic, H-2^b, Ly-1^b, Ly-2^a, Ly-3^a), B6-Tla^a (H-$2K^k$ • D^b), B6. C-H-2^{bm12} (congenic, H-$2A_\beta$: bm12) and A. SW (congenic, H-2^s) were EAT-regressive, in which subcutaneously inoculated EAT cells (2×10^7) regress[1,2].

The characteristics of the mouse strain susceptibil-

ity to EAT is a useful tool for evaluating adoptive immunotherapy against the tumor in an allogeneic donor-recipient combination. Although the classified genetic relationship between the donor and recipient is quite important in obtaining real effectiveness in the immune transfer, the essential prerequisite in their genetic correspondence is not completely understood. Previously we demonstrated a successful case of adoptive immune transfer of tumor-dormant disposition between the different H-2 haplotypes by using ddY-drm (H-2^s) and ddY-prg (H-2^q) mice[3]. This phenomenon encouraged us to search for any allogeneic combination of donor and recipient in which adoptive immune transfer against a tumor is achieved. While is quite interesting to generalize factors involved in producing a successful adoptive immune transfer against tumors because the recognition of tumor-associated antigens by allogeneic individuals is very strong, conversely histoincompatible immunocompetence in the recipient remarkably disturbs the anti-tumor function *in vivo*.

In this series of experiments, activated spleen cells from EAT-regressive mice were transferred to EAT-progressive mice in semi-syngeneic or allogeneic combinations and the adoptive immunotherapeutic effects on EAT were evaluated.

MATERIALS AND METHODS

Laboratory animals used

AKR/J (H-2^k), BALB/c (H-2^d), B10. D2 (H-2^d), B10. BR (H-2^k), C3H/He (H-2^k), C57BL/10 (=B10)(H-2^b, Ly-1^b, Ly-2^b), C57BL/6 (=B6)(H-2^b, Ly-1^b, Ly-2^b), and DBA/2 (H-2^d) were purchased from SLC Inc. (Shizuoka, Japan). B6-nu/nu mice were originally from the Jackson Lab. (Maine, USA), and have been maintained and bred in our laboratory. B6-Ly1^a (H-2^b, Ly-1^a, Ly-2^b), B6-Ly2^a (H-2^b, Ly-1^b, Ly-2^a), B6-Ly2^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-H2^{bm12} (H-$2A_\beta$: bm12) were donated by Aichi Cancer Center Research Institute (Nagoya, Japan). DBA/1 (H-2^q) was purchased from SEAC Inc. (Fukuoka, Japan). ICR (closed colony, H-$2^{q/r}$) and its athymic strain were purchased from Charles River Japan (Kanagawa, Japan). A. SW (congenic, H-2^s) was donated by Dr. J. Hayakawa, Institute for experimental animals, Kanazawa University (Kanazawa, Japan). B6C3F_1, which is a hybrid of C57BL/6(female) and C3H (male), was purchased from SLC Inc. (Shizuoka, Japan).

The ddY-drm (H-2^s) and ddY-prg (H-2^q) mice were established in our laboratory by a two-way selection of a closed colony stock of ddY mice as EAT-regressive (toward ddY-drm mice) or EAT-progressive (toward ddY-prg mice)[4].

All the 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 cages and bedding were autoclaved before use and stored in a separated 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 confirmed to established guidelines (ILAR)[5] and the Guidelines for the Regulation of the Animal Experimentation (JALAS, 1987)[6]. 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 (pH7.4) before inoculation. The cells (2×10^7) were subcutaneously inoculated into the central portion of the dorsal skin of each mouse.

Activation of spleen cells and adoptive transfer

EAT-regressive mice were immunized two times (at 0 and 20～30 days) with a subcutaneous injection of 2×10^7 EAT cells. On days 4～7 after the final immunization, spleen cells ("activated") were collected immediately after cervical dislocation. After filtration through a nylon mesh, the cells were washed and collected by centrifugation at 250×g for 5 min, and 10^8 cells were transferred intravenously or intraperitoneally into EAT-progressive mice. Immediately after these procedures, EAT cells (2×10^7) were subcutaneously inoculated into the central portion of the back skin of the recipient mice. EAT outgrowth was then monitored by measuring the length and width of developing solid tumors and compared with that without spleen cell transfer.

RESULTS

Adoptive immune transfer from ddY-drm to others

Activated spleen cells from ddY-drm mice were intravenously transferred to EAT-progressive mice. The efficiency of the adoptive immune transfer is shown in Table 1, including some of the previous data. Grades in suppression of EAT outgrowth in the recipient are shown in Fig.1. Activated spleen cells (10^8 cells) of ddY-drm mice (H-2^s) administered by the intravenous route were moderately effective in suppressing EAT outgrowth in ddY-prg mice (H-2^q). By the intraperitoneal route, their efficacy decreased to a lower grade (data not shown). As for the other types of recipients such as DBA/1 and ICR mice, the activated spleen cells from ddY-drm mice were ineffective in suppressing EAT although they have a similar H-2 haplotype q as ddY-prg. The activated spleen cells from ddY-drm mice were also ineffective in suppressing EAT outgrowth in BALB/c (H-2^d), DBA/2 (H-2^d) and AKR (H-2^k). Moreover, the activated spleen cells from ddY-drm were ineffective even in ICR-nu/nu (closed colony, H-$2^{q/?}$) and B6-nu/nu (H-2^b), which lack a T-cell population. In these experiments, graft versus host disease (GVHD) did not occur in any of recipients due to the spleen cell transfer from ddY-drm mice.

On the other hand, activated spleen cells from A. SW mice (congenic, H-2^s) were transferred intravenously to ddY-prg mice because they had a similar combination of H-2 haplotype s (donor) and q (recipient). The activated spleen cells from A. SW, however, were ineffective in suppressing EAT outgrowth in ddY-prg mice.

Adoptive immune transfer from B6 to others

Activated spleen cells from B6 mice and Ly congenic mice were intraperitonealy transferred to B6-nu/nu mice. In these donor-recipient combinations, the intraperitoneal transfer of spleen cells is as effective as transfer by the intravenous route. The efficiency of the immune transfer by the intraperitoneal route is shown in Table 2. The activated spleen cells from B6 mice (H-2^b, Ly-1^b, Ly-2^b) were very effective in suppressing EAT outgrowth in B6-nu/nu mice. Subcutaneous EAT did not grow at all in the athymic mice, showing successful immune transfer into the recipient. No GVHD was observed in the recipient. In comparison, the activated spleen cells from B6 mice were ineffective in suppressing EAT outgrowth in ddY-prg or in ICR-nu/nu mice.

On the other hand, activated spleen cells from B6-Ly-congenic mice such as B6-Ly-1^a, B6-Ly-2^a and B6-Ly-2^a,3^a were intraperitoneally transferred to B6-nu/nu mice. They were all as effective in suppressing EAT outgrowth in B6-nu/nu as in the B6 and B6-nu/nu combination. In these cases, those activated spleen cells which were intraperitoneally inoculated were as effective as those by the intravenous route (data not shown). GVHD in the recipient in any combination was not produced as a result of the spleen cell transfer.

Adoptive immune transfer in other combinations

Activated spleen cells from other B6 congenic mice such as B6-Tla^a (H-2K^k • D^b) and B6. C-H2^{bm12} (H-2A_β: bm12), were intraperitoneally transferred to B6-nu/nu mice. They were highly or moderately

Table 1. Anti-tumor (dormant) effect of spleen cells transferred from ddY-drm mice to other mice

Donor		Recipient		Suppression of	
Strain	H-2	Strain	H-2	EAT outgrowth	GVHD
ddY-drm	s	ddY-prg	q	++※)	−
		DBA/1	q	−	−
		ICR	q/?	−	−
		BALB/c	d	−	−
		DBA/2	d	−	−
		AKR	k	−	−
		ICR-nu/nu	q/?	−	−
		B6-nu/nu	b	−	−
A.SW	s	ddY-prg	q	−	−

Suppression of EAT outgrowth: −, no effect; +, slightly suppressive; ++, moderately suppressive; +++, strongly suppressive; ※), EAT-dormant; GVHD: −, no apparent symptom.

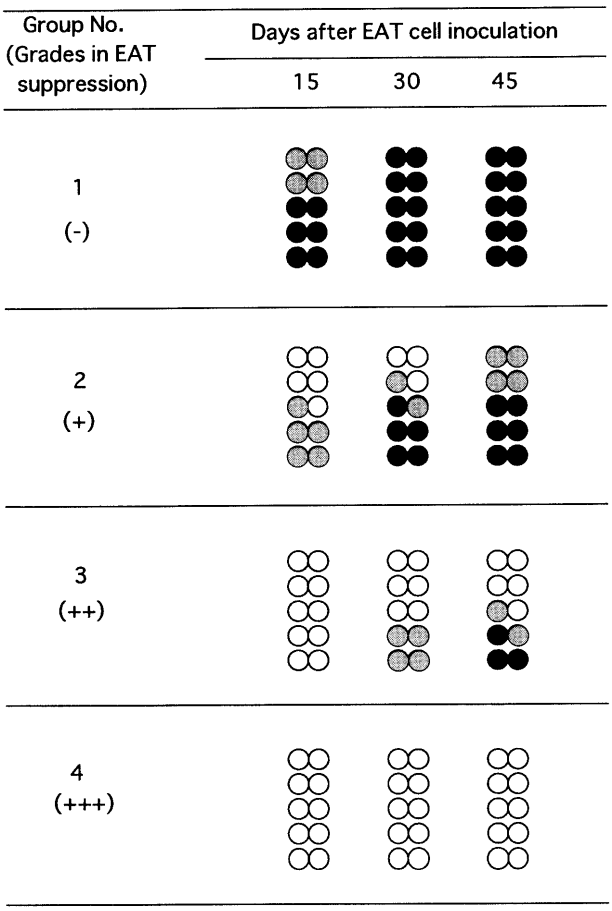

Fig. 1. Suppressive effect of transferred spleen cells on EAT outgrowth. Group: 1. EAT outgrowth in B6-nu/nu or in ddY-prg mice without spleen cell transfer; 2. EAT outgrowth in ddY-prg mice after non-activated spleen cell (10^8) transfer from ddY-drm mice; 3. EAT outgrowth in ddY-prg mice after activated spleen cell (immunized ×2, 10^8) transfer from ddY-drm mice; 4. EAT outgrowth in B6-nu/nu mice after activated spleen cell (immunized ×2, 10^8) transfer from B6 mice.

○, EAT regressed completely ; ◍, Solid tumor formation, 1~2 cm diameter ; ●, Solid tumor formation, 2~3 cm diameter.

effective in suppressing EAT outgrowth in B6-nu/nu, as shown in Table 3. In the latter combination of B6.C-H2^{bm12} and B6-nu/nu, however, severe GVHD was produced and all B6-nu/nu mice died within 11 to 16 days after the transfer of spleen cells.

On the other hand, activated spleen cells from B10 mice (H-2^b) were intraperitoneally transferred to B10.BR (H-2^k), which share the same genetic background with B10 except the H-2 gene loci. The activated spleen cells from B10 were ineffective in suppressing EAT outgrowth in B10.BR. In another combination of B10.D2 (H-2^d) as donor and DBA/2 (H-2^d) as recipient, adoptive immune transfer was unsuccessful even though B10.D2 (H-2^d) has same H-2 genes as DBA/2 (H-2^d). Activated spleen cells from BALB/c (H-2^d) were also ineffective in suppressing EAT outgrowth in DBA/2 even though they had a global similarity to each other in genetic profile.

Adoptive immune transfer from parent to hybrid

Activated spleen cells from B6 (male and female) or C3H (male and female) were transferred to [B6×C3H] F_1. As shown in Table 4, the activated spleen cells from B6 or from C3H were both ineffective in suppressing EAT outgrowth in the hybrid. In this combination, the recipient showed GVHD. Within 17 to 30 days after the spleen cell transfer, 9 out of 10 recipient mice in the former and 5 out of 8 recipient mice in the latter combination died of GVHD.

DISCUSSION

Successful immune transfer of the tumor-dormant disposition from ddY-drm to ddY-prg mice has already been demonstrated in the mechanism characterized by cellular immune functions[3]. The suppression of EAT outgrowth by adoptively transferred spleen cells in the present series of experiments may be explained by such a mechanism. The effector cells from donor mice are thought to be various types of cells such as T, B lymphocytes, natural killer (NK) cells, macrophages and so on. Cytotoxic T (Tc) lymphocytes have been reported to play an important role in tumor destruction[7-11].

The present results showed that adoptive immune transfer was successful only between strictly defined strains such as B6 and its athymic mice which share the same genetic profile except for the nu-gene. Shifts in B6-Ly-1, 2 and/or 3 haplotypes from b to a in the donor leave no perceptible influence on the effectiveness of the immune transfer.

In general, it was quite difficult to transfer the cellular immune function between the different H-2 haplotypes of mouse strains. In this connection, it is quite interesting that the EAT-dormant disposition of ddY-drm mice could be transferred to ddY-prg mice even though their H-2 haplotypes differ from each other. Since this substrain is thought to share a rather common genetic background through the history of two-way selection starting from the same basal stock colony, there might be a global similarity in surface antigens of the spleen cells which is acceptable in the recipient as a semi-syngenetic partner in

Table 2. Anti-tumor effect of spleen cells transferred from B6-Ly congenic mice to B6-nu/nu mice

Donor		Recipient		Suppression of	
Strain	H-2	Strain	H-2	EAT outgrowth	GVHD
B6	b	B6-nu/nu	b	+++	−
		ddY-prg	q	−	−
B6-Ly-1^a	b	B6-nu/nu	b	+++	−
B6-Ly-2^a	b	B6-nu/nu	b	+++	−
B6-Ly-2^a,3^a	b	B6-nu/nu	b	+++	−

Suppression of EAT outgrowth: −, no effect; +++, strongly suppressive; GVHD: −, no apparent symptom.

Table 3. Anti-tumor effect of spleen cells transferred in other combinations

Donor		Recipient		Suppression of	
Strain	H-2	Strain	H-2	EAT outgrowth	GVHD
B6-Tla^a	k/b	B6-nu/nu	b	+++	−
B6.C-H2^{bm12}	b	B6-nu/nu	b	++	+++
B10	b	B10.BR	k	−	−
B10.D2	d	DBA/2	d	−	−
BALB/c	d	DBA/2	d	−	−

Suppression of EAT outgrowth: −, no effect; ++, moderately suppressive; +++, strongly suppressive; GVHD: −, no apparent symptom; +++, death of all mice within 11 to 16 days after spleen cell transfer.

Table 4. Anti-tumor effect of spleen cells transferred from parents to the hybrid

Donor		Recipient		Suppression of	
Strain	H-2	Strain [hybrid]	H-2	EAT outgrowth	GVHD
B6	b	[B6×C3H] F_1	k/b	−	+++
C3H	k	[B6×C3H] F_1	k/b	−	++

Suppression of EAT outgrowth: −, no effect; GVHD: ++, death of 5 out of 8 mice within 17 to 30 days after spleen cell transfer; +++, death of 9 out of 10 mice within 17 to 24 days after spleen cell transfer.

spite of the difference between their H-2 haplotypes.

Activated spleen cells from B6-Tla^a (H-2K^k • D^b) were also effective in suppressing EAT outgrowth in B6-nu/nu mice (H-2^b), suggesting a more decisive role for the H-2D gene locus than H-2K in the recognition of the lymphocyte surface antigen by the athymic recipient.

The present results confirm that homogeneity in H-2K and D loci alone between donor and recipient are insufficient for successly transferring the cellular immunity to EAT. The reason why immune transfer from BALB/c (H-2^d) to DBA/2 (H-2^d) was unsuccessful in spite of their H-2 similarity may be discovered in future studies.

On the other hand, it became clear that shifts in Ly-1, 2 and/or 3 loci of donor mice did not disturb the adoptive immune mechanism. Other Ly loci such as Ly-6, Ly-10, Ly-11, Ly-18 and others also seemed to be unrelated to the adoptive immune transfer mechanism because no common features in the classification of types a and b in Ly haplotypes are seen in the EAT-progressive and EAT-regressive characteristics of the mouse strains.

In the shift in H-2A$_\beta$ of B6 mice from type b to type bm12, the cellular immune effect decreased when severe GVHD occurred in the recipient. The reason

for such GVHD provocation by the H-2A_β shift remains undetermined. One phenomenon in GVHD is the major potential complication of allogeneic lymphocyte transfer in the field of biotherapy for cancer.

Recently there has been tremendous progress in the clinical field of cellular immunotherapy for cancer with such materials as lymphokine-activated killer (LAK) cells, tumor-infiltrating lymphocytes (TIL), macrophages, and so on.[12-17]. Autologous or allogeneic bone marrow transplantation is also one of the strategies in this field[18-23]. While it will be best to use autologous cells for the biotherapy, autologous cells are slow to recognize the syngeneic tumor-associated antigens. Recognition of tumor-associated antigens by allogeneic individuals is very positive, but, conversely, histoincompatible immunocompetence in the recipient greatly disturbs the anti-tumor function *in vivo*. The elimination of such disadvantages will provide us in the future with a potent tool for tumor destruction *in vivo*.

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