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Passive Immune Transfer of Murine Tumor-dormant Disposition

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Summary. **1) subcutaneously inoculated Ehrlich Ascites Tumor (EAT) remained dormant in a ddY-drm mouse, while it grew and formed a solid tumor in a ddY-prg mouse. H-2 haplotype of ddY-drm was s and that of ddY-prg was q, which separated by two-way (EAT-dormant or EAT-progressive) selection over F+16 of a closed colony stock of ddY mice. Ly-1 and Ly-2 haplotypes, however, were not clearly distinct, showing a reduced expression of Ly-1.1 (11.0%), -1.2 (48.0%), Ly-2.1 (32.0%) and -2.2 (32.0%) in the ddY-drm mouse while revealing a multi-expression of Ly-1.1 (70.4%), -1.2 (75.8%), Ly-2.1 (65.5%) and -2.2 (67.7%) in the ddY-prg mouse.**

2) Adoptive immunotherapy against subcutaneous EAT outgrowth in the ddY-prg mouse was successfully undertaken beyond the difference of H-2 haplotype by transfer of ddY-drm spleen cells. Spleen cells from EAT-immunized ddY-drm mice were strongly effective. By combination with another mouse strain, such adoptive immunotherapy was unsuccessful except in a case of combination such as C57BL/6 (EAT-regressive) and C57BL/6-nu/nu (EAT-progressive). Some genetic background data were suggested for the success of the passive immune transfer of the murine tumor-dormant disposition.

INTRODUCTION

Tumor-dormancy has been experimentally developed. For example, intraportal inoculation of as few as 50 Walker 256 carcino-sarcoma cells has induced tumor dormancy in outbred rats.[1] Small implants of anaplastic Brown-Pearce carcinoma on the iris in susceptible rabbits remained dormant when they were not vascularized.[2] An estrogen-induced tumor in Nb rats was dormant in the absence of estrogen stimulation.[3] Methylcholanthrene-induced lymphoma cells, L5178Y, were dormant when they were transplanted into immunized murine hosts with the lymphoma cells.[4] BCL-1 tumors were dormant in lethally X-irradiated BALB/c mice with reconstituted C57BL/6 bone marrow.[5]

We have recently reported another type of tumor-dormancy in mice. Subcutaneously inoculated Ehrlich Ascites Tumor (EAT) cells were dormant in ddY-drm mice which were established by two-way selection of a closed colony stock of ddY mice for EAT-regressive (tumor-dormant) or an EAT-progressive mouse.[6]

In this report, we analyzed host genetic backgrounds of ddY-drm mice to induce tumor-dormancy and attempted to transfer the host disposition to tumor-progressive (ddY-prg) mice by the adoptive inoculation of the spleen cells from EAT-immunized ddY-drm mice. A successful immune transfer of the tumor-dormant disposition under the restricted genetic background beyond H-2 loci is presented.

MATERIALS AND METHODS

Monoclonal antibodies (mAbs)

All mAbs against lymphocytes surface antigens were purchased from Health Science Laboratories, Meiji-Nyugyo (Tokyo, Japan). The specificity of mAbs were against K^b, $K^b \cdot D^b \cdot H\text{-}2^q$, K^d, K^k, $K^k \cdot H\text{-}2^{q,r}$, $D^d \cdot H\text{-}2^s$, D^k as class I and $I\text{-}A^{b,f,k,p,q,r,s,u,v}$, $Ia^{d,j,u}$, $Ia^{d,f,j,p,q,v}$, $I\text{-}A^{d,f,j}$, $I\text{-}A^{f,j,(s)}$, $I\text{-}A^{k,r}$, Ia^k, $Ia^{j,q}$, $I\text{-}A^{j,r,s}$ as class II. mAbs against Ly-1 or -2 were also used. All mAbs were appropriately diluted to 1/100-1/1000 for use.

Experimental animals

This study utilized ddY-prg and ddY-drm mice, which were established by two-way selection of a closed colony stock of ddY mice for EAT-progressive or an

EAT-regressive (EAT-dormant) mouse.[6] Subcutaneously inoculated EAT is progressive and forms a solid tumor in the ddY-prg subline, but not in the ddY-drm subline showing the tumor-dormant state beyond two months after the tumor inoculation.

DBA/1JSea (inbred, H-2^q, Seiwa Experimental Animals Ltd., Fukuoka, Japan), C57BL/6(inbred, H-2^b, SLC Inc., Shizuoka, Japan), C57BL/6-nu/nu, ICR (closed colony, H-2^q major, Charles River, Atsugi, Japan) and ICR-nu/nu were also used. A. SW (congenic, H-2^s) were donated from Dr. J. Hayakawa, Institute for experimental animals, Kanazawa University (Kanazawa, Japan).

All animals used were specific-pathogen-free. Five 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 cubic diet (MF-1, Oriental Co., 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 kept at a constant temperature (23±1℃) and humidity (45 to 75%). The room was ventilated 18 times per hour and was illuminated with 300 lx by daylight fluorescent lamps on a 12/12-h light/dark cycle.

All animal procedures conformed to established guidelines[7] and the Guidelines for the Regulation of the Animal Experimentation (JALAS 1987).[8] Animals were euthanized by cervical dislocation.

Genetic analysis

Surface antigens of lymphoid cells of ddY-prg and ddY-drm mice, and of other strains, were examined by complement-dependent cytotoxicity test.[9] Briefly, 20 μl of 6×10^6 cells/ml and 20 μl of appropriately diluted mAbs were combined on ice. The mixture was augmented with appropriately diluted rabbit complement. After 45 min incubation at 37°C, the dead cells were stained with 0.25% trypan blue.

Tumor inoculation

Ehrlich Ascites Tumor cells, maintained by intraperitoneal transfer of 10^7 cells to ddY-prg mice (5 to 8 wks old), were harvested on Days 5 to 7 post transfer and washed in phosphate-buffered saline (pH 7.4). The cells (2×10^7) were inoculated subcutaneously into the central portion of the dorsal skin of the mice (5 to 8 wks old).

Adoptive spleen cells transfer

ddY-drm mice were immunized one or two times (at 0 and the 20-30th days) with 2×10^7 EAT cells. At 4 days after the final immunization, spleen cells were collected immediately after cervical dislocation. After filtration through nylon mesh, spleen cells were washed and collected by centrifugation at 250 xg for 5 min, and 2.5×10^7 to 2×10^8 cells were transferred intravenously into ddY-prg mice. Immediately, EAT cells (2×10^7) were subcutaneously injected into the central portion of the back skin of the recipients. Following this, solid EAT growth was monitored by measuring diameters lengthwise and crosswise.

On the other hand, spleen cells from ddY-drm mice immunized two times with EAT cells (2×10^7) were transferred into DBA/1(H-2^q, EAT-progressive), C57BL/10-nu/nu (H-2^b, EAT-progressive), ICR (closed colony, H-2^q major, EAT-progressive) or ICR-nu/nu (closed colony, EAT-progressive) to ascertain the effectiveness of the adoptive immunotherapy on the subcutaneous EAT growth in the recipients.

For extension, spleen cells from A. SW mice (congenic, H-2^s, EAT-regressive) immunized two times with EAT (2×10^7) were transferred into ddY-prg mice to determine the effectiveness of the adoptive immune transfer of the host EAT-regressive disposition. Furthermore, spleen cells from C57BL/6 mice (H-2^b, EAT-regressive) immunized with EAT were transferred into C57BL/6-nu/nu (EAT-progressive) to judge the effectiveness of the adoptive immunotherapy under the same genetical background except for nu-gene.

RESULTS

Genetic analysis of ddY-drm and ddY-prg mice

Spleen cells from ddY-drm and ddY-prg mice reacted with mAbs as shown in Table 1.

Concerning H-2 loci, spleen cells from ddY-drm mice reacted only with mAbs against D^d • H-2^s, I-$A^{b,f,k,p,q,r,s,u,v}$, and I-$A^{j,r,s}$, but not with those against K^d, $Ia^{d,f,j,p,q,v}$, I-$A^{k,r}$ and $Ia^{j,q}$. Hence, the H-2 haplotype of ddY-drm mice seemed 's' as class I and class II. On the other hand, spleen cells from ddY-prg mice reacted with mAbs against K^b • D^b • H-2^q, K^k • H-$2^{q,r}$, I-$A^{b,f,k,p,q,r,s,u,v}$, $Ia^{d,f,j,p,q,v}$ and $Ia^{j,q}$, but not with those against K^b, K^k, I-$A^{k,r}$ and I-$A^{j,r,s}$. Hence, the H-2 haplotype of ddY-prg mice seemed 'q' as class I and class II.

Concerning Ly-antigens, spleen cells from ddY-drm mice reacted by 11.0, 48.0, 32.0 and 32.0% against Ly-

Table 1. H-2 and Ly haplotypes of ddY-drm and ddY-prg mice.

mAbs specificity	Dead cells/counted (%)	
	ddY-drm	ddY-prg
K^b	0.2	0
$K^b \cdot D^b \cdot H\text{-}2^q$	0	94.2
K^d	0	0
K^k	0	0
$K^k \cdot H\text{-}2^{q,r}$	4.1	70.2
$D^d \cdot H\text{-}2^s$	73.2	0
$I\text{-}A^{b,f,k,p,q,r,s,u,v}$	67.4	63.7
$Ia^{d,f,j,p,q,v}$	0	61.1
$I\text{-}A^{k,r}$	6.2	0
$Ia^{j,q}$	1.0	73.1
$I\text{-}A^{j,r,s}$	70.0	8.4
Ly-1.1	11.0	70.4
Ly-1.2	48.0	75.8
Ly-2.1	32.0	65.5
Ly-2.2	32.0	67.7

1.1, Ly-1.2, Ly-2.1 and Ly-2.2 respectively. Spleen cells from ddY-prg mice reacted by 70.4, 75.8, 65.5, and 67.7% against Ly-1.1, Ly-1.2, Ly-2.1 and Ly-2.2 respectively. From the results, ddY-drm mice may be regarded as an example of a reduced expression of the Ly-genes products. In contrast, ddY-prg mice are regarded as an example of undissociated Ly haplotypes in spite of their H-2 haplotypes being clearly dissociated as q through two-way selection over F+16.

On the other hand, as shown in Table 2, reactivities of EAT cells with those mAbs against class I and II were all negative. This will show an absence or reduced expression of class I and II MHC gene products on the EAT cell surface. Reactivities of EAT cells with mAbs against Ly-1 and -2 antigens were also negative.

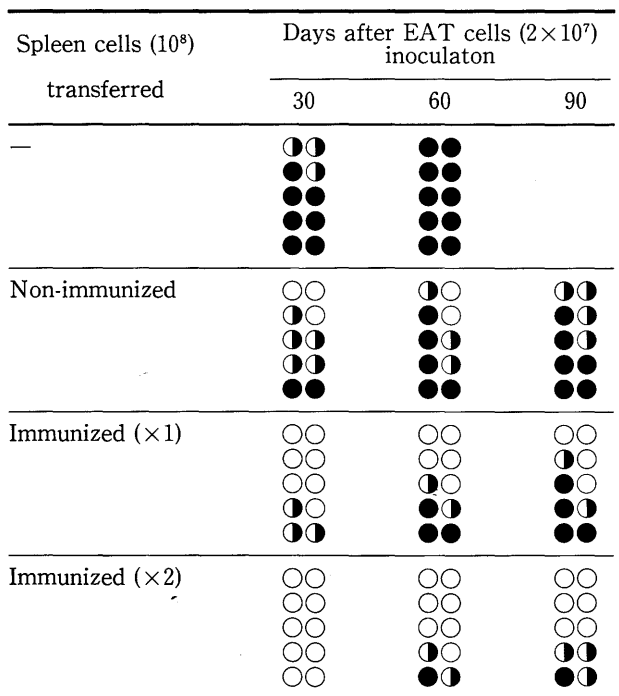
Immune transfer of tumor-dormant disposition from ddY-drm mice to ddY-prg mice

As shown schematically in Fig. 1, spleen cells (10^8) from ddY-drm mice which were immunized twice with EAT cells strongly suppressed EAT growth in the recipients (ddY-prg mice) and maintained them in a dormant state. Spleen cells from ddY-drm mice which were immunized once with EAT cells were moderately effective in suppressing EAT growth in ddY-prg mice. Spleen cells from non-immunized ddY-drm mice were slightly effective in suppressing the

Table 2. Surface antigenecity of EAT cells.

MAbs specificity	Dead cells/counted (%)	
	EAT	BAMC-1*
$K^b \cdot D^b \cdot H\text{-}2^q$	6.0	3.2
$K^k \cdot H\text{-}2^{q,r}$	7.8	7.6
$K^d \cdot H\text{-}2^s$	6.6	75.4
K^d	8.9	82.4
K^k	8.2	5.1
$I\text{-}A^{b,f,k,p,q,r,s,u,v}$	8.0	3.3
$I\text{-}A^{d,f,j}$	7.6	3.8
Ly-1.1	2.5	0
Ly-1.2	1.5	1.9
Ly-2.1	0.3	0
Ly-2.2	2.5	6.1

*) For positive control, BAMC-1: methylcholanthrene-induced fibroma which has been passed in syngeneic mice (BALB/c, $H\text{-}2^d$)



EAT growth in diameter (mm): ○ -7, ◑ 8-16, ● 17-
left-hand column (n=5): ♂ right-hand column (n=5): ♀

Fig. 1. Passive immune transfer of EAT-dormant disposition from ddY-drm mice to ddY-prg mice.

tumor growth. A number of spleen cells over 5×10^7/animal to be necessary to produce distinctly the adoptive immunotherapeutic effect (data not shown).

Spleen cells of EAT-immunized ddY-drm mice were also effective when they were subcutaneously injected into ddY-prg mice in the mixture condition with EAT cells. In this simultaneous inoculation method, the addition of blood serum from immunized ddY-drm mice or of the supernatant of the immunized spleen extract which was obtained in the course of spleen cells preparation failed to prove effective in suppressing EAT growth in ddY-prg mice. The results show that cell-dependent immunity mainly participates in the transmission of the donor tumor-dormant disposition.

Adoptive immunity between ddY-drm mouse and other mouse strains

For extension, adoptive immunotherapy was attempted with other strains of EAT-progressive mice: DBA/1(H-2^q), AKR (H-2^k), ICR (closed colony, H-2^q major), ICR-nu/nu, or C57BL/6-nu/nu (H-2^b). As shown in Table 3, the transfer of spleen cells (10^8) from EAT-immunized ddY-drm mice was completly ineffective in suppressing the tumor growth in those EAT-progressive recipients. This shows that adoptive immunotherapy against EAT is only successful under restricted genetical backgrounds. Namely, in spite of the strains, the ddY-prg mouse and DBA/1 mouse share the same H-2 haplotype q, though adoptive immunotherapy was highly successful in the former but not in the latter at all. In addition, the immunotherapy was ineffective in C57BL/6-nu/nu and ICR-nu/nu recipients in spite of T-cell depletion. Adoptive immunotherapy was also ineffective in the combination of A. SW (congenic, H-2^s, EAT-regressive) and ddY-prg mice (H-2^q) in spite of a similar H-2 s and q combination.

On the other hand, adoptive immunotherapy against EAT was quite successful in the combination of C57BL/6 (EAT-regressive) and C57BL/6-nu/nu (EAT-progressive).

These results are summarized as follows: 1) adoptive immunotherapy against EAT is possible among mouse strains, both normal and its athymic mouse of the same genetical background except for nu-gene; 2) among strains of different H-2 haplotypes, adoptive immunotherapy is rarely successful among mice of restricted genetic profile such as a combination of ddY-drm and ddY-prg which may share some common genetic background other than H-2 loci through the original closed colony history.

DISCUSSION

Previous studies have suggested that ddY-drm mice are genetically distinct from ddY-prg mice because of their rejection of cross-grafted skins.[6] The present study has clearly shown that H-2 haplotype of ddY-drm mice is s and that of ddY-prg mice is q, which successfully separated by selective breeding of closed colony stock for resistant or susceptible mice to subcutaneously inoculated EAT cells. Concerning Ly-haplotypes, however, the two types of ddY substrains did not show a clearly dissociated pattern. Namely, ddY-drm mice showed the reduced expression of Ly-1.1 (11.0%), Ly-1.2 (48.0%), Ly-2.1 (32.0%) and Ly-2.2 (32.0%). In contrast to these, ddY-prg mice showed a multi-expression of Ly-1.1 (70.4%),

Table 3. Efficiency of adoptive immunotherapy.

Donor		Recipient		Suppression of EAT growth
Strain	H-2	Strain	H-2	
ddY-drm	s	ddY-prg	q	+++
		DBA/1	q	—
		ICR	q major	—
		AKR	k	—
		ICRnu/nu		—
		C57BL/6-nu/nu	b	—
A. SW (congenic)	s	ddY-prg	q	—
C57BL/6	b	C57BL/6-nu/nu	b	+++

Spleen cells (10^8) from donor immunized with 2×10^7 EAT cells ($\times2$) were transferred intravenously into recipient before EAT (2×10^7) s. c. inoculation. Suppression of EAT growth in recipient was determined at the 60th day after EAT inoculation.

Ly-1.2 (75.8%), Ly-2.1 (65.5%), and Ly-2.2 (67.7%), respectively.

We previously pointed out that EAT-regressive mouse strains showed a common feature of Ly-1.2 and -2.2 as lymphocyte surface antigen while EAT-progressive mouse strains showed Ly-1.1 (with an exception) and -2.1 as a common feature.[10] Among the two groups of the distinct Ly-haplotypes, adoptive immunotherapy against EAT is quite impossible.

Based on our experience (data not shown), transfer of the tumor-resistant disposition by adoptive transfer of spleen cells among different mouse strains is generally unsuccessful. It is surprising that adoptive immunotherapy was successfully undertaken beyond a different H-2 haplotype between ddY-drm ($H\text{-}2^s$) and ddY-prg ($H\text{-}2^q$), where cross-grafted skins are rejected. When not only Ly-haplotypes but also H-2 haplotypes are different, adoptive immunotherapy against EAT is quite impossible even in the athymic recipient as shown in the present experiments. The reduced expression of Ly-1 and -2 in ddY-drm mice and/or multi-expression of the genes product in ddY-prg mice may be a key role to explain the successful immune transfer of tumor-dormant disposition between ddY-drm and ddY-prg mice beyond their distinct H-2 haplotypes, s and q.

Generally speaking, adoptive immunotherapy against tumors is only successful in the combination of normal mouse (tumor-regressive) and its nu congenic such as C57BL/6 and C57BL/6-nu/nu.

Ehrlich Ascites Tumor (EAT) may be regarded as an extreme example of a reduced expression of normal histocompatible antigens, which is also reflected in its ability to grow intraperitoneally in almost any mouse strain.[11] We also could not detect any H-2 class I and II surface antigens on EAT cells. Ly-1 and Ly-2 antigens were also negative. Numerous data concerning the immunogenecity of EAT, however, have been reported showing the existence of tumor-specific transplantation antigens on the EAT cell surface.[12–15] The antigen from EAT cells seemed to be macromolecules of 16S, free of murine mammary tumor virus protein such as gp55 and p28.[15] Antibody recognizing Ag of EAT-origin was reported as IgM which bridges macrophages to the tumor cells.[16]

It is evident that T cell-dependent immunity participates in the tumor-dormancy in ddY-drm mice. Understanding the recognition mechanisms against the EAT-specific transplantation antigen and to maintain tumors in a dormant state in living body has obvious importance.

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