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ESSENTIAL REQUIREMENT OF I-A REGION-IDENTICAL HOST BONE MARROW OR BONE MARROW-DERIVED CELLS FOR TUMOR NEUTRALIZATION BY PRIMED L3T4$^+$ T CELLS

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The antitumor activity of Meth A-hyperimmunized BALB/c mouse spleen cells (Meth A-Im-SPL) was assayed by the Winn test in H-2 incompatible bone marrow chimeras in closed colony CD-1(nu/nu), inbred DDD/1(nu/nu) (H-2^s), or inbred BALB/c(nu/nu) (H-2^d) mice as recipients. We found that Meth A-Im-SPL suppressed Meth A growth in the chimera nude mice which were reconstituted with bone marrow cells of the H-2^d haplotype (i.e., BALB/c, DBA/2 and B10.D2), but not in the chimeras which were reconstituted with bone marrow cells of the H-2^a, H-2^b, or H-2^k haplotype (i.e., B10.A, B10, and B10.BR). These results suggested that H-2 restriction occurred between Meth A-Im-SPL and bone marrow or bone marrow-derived cells in tumor neutralization. Furthermore, Meth A-Im-SPL did not suppress Meth 1 tumors (antigenically distinct from Meth A tumors) in the presence or absence of mitomycin C-treated Meth A in a Winn assay. These results suggested that there is tumor specificity in the "effector phase" as well as in the "induction phase". The phenotype of the effectors in the Meth A-Im-SPL was Thy-1.2$^+$ and L3T4$^+$, because Meth A-Im-SPL lost their antitumor activity with pretreatment with anti-Thy-1.2 monoclonal antibody (mAb) and complement or anti-L3T4 mAb and complement, but not with anti-Lyt-2.2 mAb and complement or complement alone.

Positively purified L3T4$^+$ T cells from Meth A-Im-SPL (Meth A-Im-L3T4), obtained by the panning method, suppressed the tumor growth in the chimera nude mice which were reconstituted with bone marrow cells of B10.KEA2 mice (that were I-A region-identical with Meth A-Im-L3T4 cells but not others in H-2) as well as B10.D2 cells (that were fully identical with Meth A-Im-L3T4 cells in H-2). We conclude that Meth A-Im-SPL (L3T4$^+$) neutralized the tumors in collaboration with I-A region-identical host bone marrow or bone marrow-derived cells, and the neutralization was not accompanied by the "bystander effect."

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It was reported that Lyt-1$^+$2$^-$ T cells are the primary effectors of murine skin graft (1) and tumor (2–4) rejection. These "helper" type effectors are not cytotoxic in vitro and so they may not kill the grafts directly in vivo. This suggests that these immunocytes collaborate with the host cells in skin graft and tumor rejection.

We previously showed (5) that in Meth A tumors and the BALB/c mouse experimental system, the effector cells i) had the phenotype of Lyt-1$^+$2$^-$ T cells, ii) showed no cytotoxic activity in vitro, iii) caused tumor-specific suppression in the "effector phase" (the bystander effect was not observed), and iv) expressed their antitumor activity in BALB/c(nu/nu) as well as BALB/c(+/+) mice as the recipients in a Winn assay. These results also suggest the cooperation of immune lymphocytes with the host cells.

Other investigators reported the possible cooperation of immunocytes with the host macrophages (6, 7), mast cells (8), or bone marrow cells (9, 10). However, there has been no report on genetic restriction between immunocytes and the host cells. To investigate the genetic restriction between the latter, we prepared an H-2 incompatible bone marrow chimera through the use of athymic nude mice. These chimera nude mice, which were reconstituted with the bone marrow of various strains of mice, could not react to third party antigens, because none of the T cells underwent differentiation in the chimera nude mice. Thus, we could analyze the interaction between immunoctyes and host bone marrow or bone marrow-derived cells in vivo from the genetic view point.

MATERIALS AND METHODS

Mice. Inbred BALB/c (H-2^d), C3H/He (H-2^k), C57BL/6 (H-2^b), DBA/2 (H-2^d) and closed colony CD-1(nu/nu) mice were obtained from Charles River Japan, Inc. (Astugi, Japan). Inbred B10 (H-2^b), B10.D2 (H-2^d), B10.BR (H-2^k), and B10.A (H-2^a) mice were obtained from the Shizuoka Laboratory Animal Center (Hamamatsu, Japan). Inbred BALB/c (nu/nu) (H-2^d) and DDD/1(nu/nu) (H-2^s) mice were kindly supplied by Dr. A. Matsuzawa, Institute of Medical Science, the University of Tokyo, Tokyo. B10.KEA2 (H-2^{w9}) mice were kindly supplied by Dr. J. Klein, Max-Plank-Institut fur Biologie, Tubingen, West Germany.

Tumors. Methylcholanthrene-induced sarcomas, Meth A (1) (kindly supplied by Dr. Y. Hashimoto, Tohoku University, Sendai, Japan) and Meth 1 (kindly supplied by Kyowa Hakko Co., Ltd., Tokyo), were both originally induced in BALB/c mice and were passaged in the ascitic form. They are antigentically distinct and not cross-reactive (12).

Mitomycin C treatment. Meth A cells (1×10^7/ml) suspended in

HEPES-buffered Hanks' minimal essential medium (MEM-H)[2] supplemented with 10% fetal calf serum were incubated with mitomycin C (100 μg/ml; Kyowa Hakko Co.) at 37°C for 1 hr. After washing with phosphate-buffered saline (PBS), the cells were resuspended in MEM-H.

Hyperimmune mice. Meth A (Meth 1)-hyperimmunized mice were obtained as follows. BALB/c mice were i.p. sensitized with the tumor cell vaccine (12), and those surviving after subsequent inoculation of the tumor cells were used as donors for the following expansion in immune mice. Spleen cells from these donor mice (5×10^6/mouse) and the tumor cells (5×10^5/mouse) were mixed and then inoculated intradermally into naive mice. These mice were repeatedly inoculated with the tumor cells, and the spleen cells of those surviving after i.p. inoculation of 2×10^6 tumor cells were used as the source of effector cells.

Bone marrow chimeras. Bone marrow chimera nude mice were prepared according to the method of Onoe et al. (13). In brief, female CD-1(nu/nu) and BALB/c(nu/nu) mice, and DDD/1(nu/nu) mice of both sexes were subjected to whole body x-irradiation, 900, 600, and 650 R, respectively, using Hitachi x-ray equipment (MBR-1505R; Hitachi Medical Corp., Tokyo). Twenty-four hours after the irradiation, the mice were given an i.v. injection of 2.5×10^7 bone marrow cells prepared from the tibias and femurs of the donor mice. The bone marrow cells were pretreated with anti-Thy-1.2 monoclonal antibody (mAb) (1/4000; Olac, Oxon, England) plus Low-Tox-Rabbit complement (1/10; Cedarlane Laboratories, Ontario, Canada) before i.v. injection. The chimeras are designated as (irradiated recipient←bone marrow donor).

Chimerism. Two to three months after the bone marrow transplantation, chimerism was confirmed by the presence of donor-type cells in the spleen. Donor cells were detected by the use of H-2 specific mAb (anti-H-2D^d, anti-H-2K^k, and anti-H-2K^b; mouse IgG2a; Meiji Institute of Health Science, Odawara, Japan), followed by fluorescein isothiocyanate-conjugated anti-mouse IgG (Fc) fragment-specific, F(ab')$_2$; Cappel Laboratories, Inc., Cochranville, PA). The number of fluorescence-positive cells was determined with a fluorescence-activated cell sorter (FACS IV, Becton Dickinson FACS Systems, Sunnyvale, CA).

Spleen cell suspension. Spleens were asceptically removed, pooled, and then gently dissociated in PBS in a cellulose tube. The cells were then centrifuged and resuspended in a buffered ammonium chloride solution at 37°C for 5 min to lyse the erythrocytes. The cells were then washed three times with PBS, filtered through a nylon mesh (#200), and then resuspended in MEM-H for the Winn assay and for purification by the panning method, or resuspended in the cytotoxicity medium (Cedarlane) for mAb and complement treatment.

Antibody and complement treatment. Meth A-hyperimmunized BALB/c mouse spleen cells (Meth A-Im-SPL) (1×10^7 cells/ml) were incubated with anti-Thy-1.2 mAb (1/2000; Olac), anti-L3T4 mAb (1/500; Sera-Lab., Crawley Down, Sussex, England) or anti-Lyt-2.2 mAb (1/20; Cedarlane) at 4°C for 1 hr. The antibody treated cells were then incubated with Low-Tox-Rabbit complement (1/10; Cedarlane) at 37°C for 1 hr.

Positive selection of L3T4$^+$ T cells from Meth A-Im-SPL. The details were given previously (5). Briefly, L3T4$^+$ T cells were positively selected from Meth A-Im-SPL by the panning method (14) (Meth A-Im-L3T4). Meth A-Im-SPL suspended in 5% fetal calf serum-MEM-H were incubated on petri dishes (Falcon #1001; Oxnard, CA) coated with rabbit anti-mouse IgG (Cappel) for B cell depletion and the non-adherent cells were recovered. After treatment with anti-L3T4 mAb (rat IgG2a; Becton Dickinson, Mountain View, CA), the cells were applied to anti-rat-IgG (Fc fragment specific; Cappel)-coated dishes. After washing out nonadherent cells with cold PBS, the adherent cells were recovered by vigorous pipetting. These procedures resulted in the recovery of a L3T4$^+$ cell population (Meth A-Im-L3T4:L3T4$^+$ (>98%), Lyt-2$^+$ (<0.1%)), as determined with a FACS IV.

Winn assay. In vivo antitumor activity was determined by means of the Winn tumor neutralization assay (15). Effector cells (5×10^6 cells) and Meth A cells (5×10^5 cells) were mixed in 0.1 ml of MEM-H and then inoculated intradermally into the right flank of chimera nude mice. Antitumor activity was scored against tumor size and the number of tumor-bearing mice/total mice on day 21. Tumor size was expressed as mean of the square root of tumor area (a × b) (mm) ± SD with tumor area being the product of the two perpendicular diameters (a × b).

[2] Abbreviations used in this paper: MEM-H, Hanks' minimal essential medium; Meth A-Im-SPL, Meth A-hyperimmunized BALB/c mouse spleen cells; Meth A1-Im-SPL, Meth 1-hyperimmunized BALB/c mouse spleen cells; Meth A-Im-L3T4, positively purified L3T4$^+$ cells from Meth A-Im-SPL by the panning method; mAb, monoclonal antibody; FACS, fluorescence-activated cell sorter; CTL, cytotoxic T lymphocytes; MAF, macrophage-activating factor.

RESULTS

Tumor-neutralizing activity of Meth A-Im-SPL in the chimeras. The CD-1(nu/nu) mice were closed colony nude mice, which did not have a specific H-2 haplotype. Thus we used three types of mAb against H-2D^d, H-2K^k, and H-2K^b, to be used to confirm their chimerism. These antibodies could clarify the donor H-2 haplotype, but not the recipient's. Figure 1 shows the FACS profiles for the splenocytes of CD-1(nu/nu) chimeras. [CD-1(nu/nu)←BALB/c(H-2^d)] and [CD-1(nu/nu)←DBA/2(H-2^d)] chimera splenocytes were stained only by anti-H-2D^d (>85%), i.e., not by the others (<5%). The splenocytes of [CD-1(nu/nu)←C3H/He (H-2^k)] and [CD-1(nu/nu)←C57BL/6(H-2^b)] were stained only by anti-H-2K^k and anti-H-2K^b mAb, respectively (>85%), i.e., not by the others (<5%). These results showed that these chimeras were fully reconstituted with the donor cells.

Using these chimeras, the tumor-neutralizing activity of Meth A-Im-SPL was assayed. Experiment A in Table 1 shows that Meth A-Im-SPL (H-2^d) suppressed Meth A growth in [CD-1(nu/nu)←BALB/c] and [CD-1(nu/nu)←DBA/2] chimeras, but not in [CD-1(nu/nu)←C3H/He] or [CD-1 (nu/nu)←C57BL/6] chimeras. In these chimeras, Meth A tumors grew equally well. On the other hand, in the chimeras which were reconstituted with B10 congenic bone marrow cells, Meth A-Im-SPL neutralized Meth A tumors in the [CD-1(nu/nu)←B10.D2(H-2^d)] chimeras, but not in the [CD-1(nu/nu)←B10(H-2^b)], [CD-1(nu/nu)←B10.BR(H-2^k)] or [CD-1(nu/nu)←B10.A(H-2^a)] chimeras (Table I, experiment B).

Similar results were obtained for the chimeras prepared with the use of inbred DDD/1(nu/nu) (H-2^s) mice as recipients (Table 2, experiment A). Meth A-Im-SPL suppressed Meth A growth in the [DDD/1(nu/nu)←B10.D2] chimeras, but not in the [DDD/1(nu/nu)←B10], [DDD/1(nu/nu)←B10.BR] or [DDD/1(nu/nu)←B10.A] chimeras. These results suggested that H-2 restriction occurred between Meth A-Im-SPL and the host cells in tumor neutralization.

We extended these results as to tumor neutralization to BALB/c(nu/nu) chimeras. Meth A-Im-SPL did not neutralize Meth A tumors in the [BALB/c(nu/nu)←B10], [BALB/c(nu/nu)←B10.BR] or [BALB/c(nu/nu)←B10.A] chimeras, but it did in the [BALB/c(nu/nu)←B10.D2] chimeras (Table 2, experiment B).

These results showed that H-2-restricted interaction between Meth A-Im-SPL and host bone marrow or bone marrow-derived cells was involved in tumor neutralization.

Phenotype of the effectors in Meth A-Im-SPL in the [CD-1(nu/nu)←BALB/c] chimeras. To determine the phenotype of the effectors responsible for the Meth A tumor regression in the H-2 identical [CD-1(nu/nu)←BALB/c] chimera nude mice, Meth A-Im-SPL were pretreated with mAb and complement in vitro before the Winn assay (Table III). In these experiments, the activity of Meth A-Im-SPL was completely abolished by treatment with anti-Thy-1.2 mAb and complement. Anti-Lyt-2.2 mAb and complement had no effect on the neutralizing activity of Meth A-Im-SPL. Anti-L3T4 mAb and complement abrogated the neutralizing activity of Meth A-Im-SPL. These

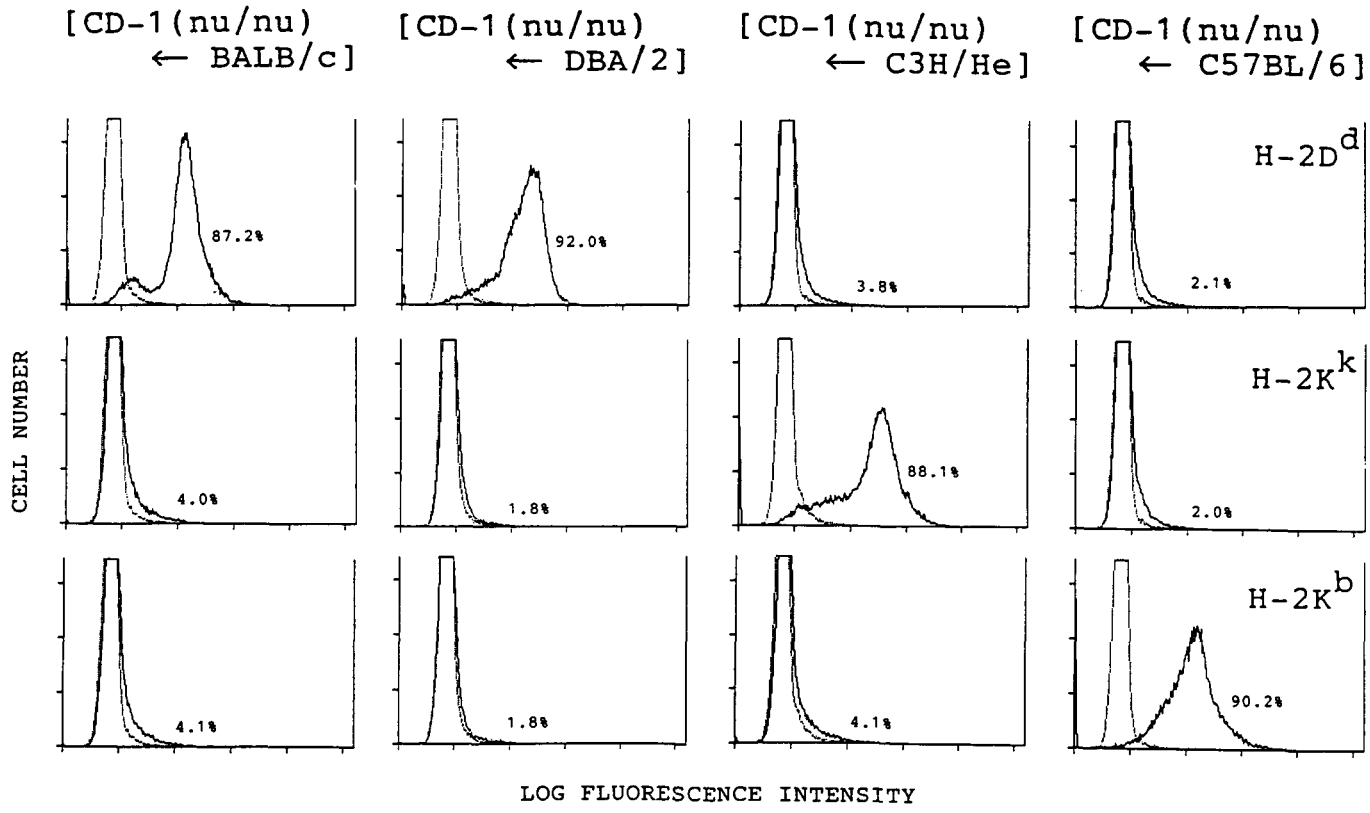

Figure 1. Fluorescence profiles of spleen cells from CD-1(nu/nu) chimera mice which were reconstituted with bone marrow cells of BALB/c(H-2^d), DBA/2(H-2^d), C3H/He(H-2^k) and C57BL/6(H-2^b) mice. Spleen cells were stained by indirect immunofluorescence with either monoclonal anti-H-2D^d, anti-H-2K^k, or anti-H-2K^b antibodies, followed by fluorescein isothiocyanate-anti-mouse IgG (Fc fragment, specific) antibody. (The percentages of positive cells are shown).

TABLE I

Tumor-neutralizing activity of Meth A-Im-SPL in the CD-1(nu/nu) chimeras

Expt.	Chimera Mice	Chimerism (%)[1]			Intradermal Inoculum[2]		Im-SPL Activity	
		H-2D^d	H-2K^k	H-2K^b	Meth A	Im-SPL	Tumor size[3]	No. Mice/Total[4]
A	CD-1(nu/nu)←BALB/c	87.2	<5	<5	+	−	16.7 ± 0.3	3/3
					+	+	0	0/8
	CD-1(nu/nu)←DBA/2	92.0	<5	<5	+	−	17.0 ± 1.9	3/3
					+	+	0	0/5
	CD-1(nu/nu)←C3H/He	<5	88.1	<5	+	−	18.8 ± 0.3	3/3
					+	+	12.0 ± 4.2	5/5
	CD-1(nu/nu)←C57BL/6	<5	<5	90.2	+	−	18.0 ± 2.1	3/3
					+	+	15.7 ± 1.4	6/6
B	CD-1(nu/nu)←B10	<5	<5	85.8	+	−	16.5 ± 0.9	5/5
					+	+	12.9 ± 1.4	5/5
	CD-1(nu/nu)←B10.D2	87.8	<5	<5	+	−	17.8 ± 0.8	5/5
					+	+	0	
	CD-1(nu/nu)←B10.A	88.4	91.8	<5	+	−	16.0 ± 1.9	5/5
					+	+	12.5 ± 4.2	5/5
	CD-1(nu/nu)←B10.BR	<5	92.8	<5	+	−	19.1 ± 1.2	5/5
					+	+	12.5 ± 1.7	5/5

[1] Mean percent positive cells in the spleen cells from one (experiment A) or two (experiment B) mice for each group. Spleen cells were stained with anti-H-2D^d, anti-H-2K^k or anti-H-2K^b mAb, followed by fluorescein isothiocyanate-conjugated anti-mouse IgG (Fc fragment-specific). The number of fluorescence-positive cells was determined with a FACS IV.

[2] Meth A (5×10^5/mouse) and Im-SPL (5×10^6/mouse) were inoculated intradermally on day 0.

[3] Tumor size is expressed as the square root of a × b mm (mean ± SD) on day 21.

[4] Number of tumor-bearing mice/total mice on day 21.

results showed that the effectors in Meth A-Im-SPL were mainly L3T4$^+$ T cells.

Tumor specificity of Meth A-Im-SPL/Meth A-Im-L3T4 in the "effector phase" in the [CD-1(nu/nu)←B10.D2] chimeras. We investigated the tumor specificity of Meth A-Im-SPL or Meth A-Im-L3T4 purified from Meth A-Im-SPL by the panning method (see *Materials and Methods*) in the [CD-1(nu/nu)←B10.D2] chimeras by means of the Winn assay. As shown in Table IV, Meth A-Im-SPL and Meth A-Im-L3T4 both neutralized Meth A tumors but they did not neutralize Meth 1 tumors (Table IV). This nonreactivity of the effectors with Meth 1 tumors was not affected by the coexistence of mitomycin C-pretreated Meth A tumors (Table IV). These results suggested that the tumor-neutralizing activity of Meth A-Im-SPL or Meth A-Im-L3T4 was tumor-specific in the "effector phase" as well as in the "induction phase."

Meth A-Im-L3T4 cooperated with the host H-2-identical or I-A region-identical bone marrow or bone marrow-derived cells. The tumor-neutralizing activity of

TABLE II

Tumor-neutralizing activity of Meth A-Im-SPL in the DDD/1(nu/nu) or BALB/c(nu/nu) chimeras

Expt.	Chimera Mice	Chimerism[a]	Intradermal Inoculum[b]		Im-SPL Activity	
		% donor	Meth A	Im-SPL	Tumor size[c]	No. mice/Total[d]
A	DDD/1(nu/nu)←B10	>85	+	−	22.3 ± 1.4	5/5
			+	+	18.8 ± 0.7	5/5
	DDD/1(nu/nu)←B10.D2	>85	+	−	21.6 ± 0.7	5/5
			+	+	0	0/5
	DDD/1(nu/nu)←B10.A	>85	+	−	20.3 ± 1.5	5/5
			+	+	15.3 ± 4.1	5/5
	DDD/1(nu/nu)←B10.BR	>85	+	−	21.0 ± 0.8	5/5
			+	+	18.0 ± 2.5	5/5
B	BALB/c(nu/nu)←B10	>90	+	−	19.0 ± 0.7	5/5
			+	+	12.6 ± 3.6	5/5
	BALB/c(nu/nu)←B10.D2	>90*	+	−	17.5 ± 0.6	5/5
			+	+	0	0/5
	BALB/c(nu/nu)←B10.A	>90	+	−	20.6 ± 1.9	5/5
			+	+	12.9 ± 1.7	5/5
	BALB/c(nu/nu)←B10.BR	>90	+	−	19.8 ± 1.8	5/5
			+	+	10.7 ± 4.4	5/5

[a] Mean positive cells of two mice (spleen cells) for each group. (* Anti-B10.D2 BALB/c serum was prepared by repeated i.p. inoculation of B10.D2 splenocytes. This antiserum could distinguish the cells of B10.D2 bone marrow cell origin in the BALB/c(nu/nu) chimera, on staining with fluorescein isothiocyanate-conjugated anti-mouse IgG (Fc fragment-specific) antibody with a FACS IV.)

[b] Meth A (5×10^5/mouse) and Im-SPL (5×10^6/mouse) were inoculated intradermally on day 0.

[c] Tumor size is expressed as square root of a × b mm (mean ± SD) on day 21.

[d] Number of tumor-bearing mice/total mice on day 21.

TABLE III

Cell surface markers of effector cells in Winn assay in the [CD-1(nu/nu)←BALB/c] chimera

Treatment of Effector Cells[a]	Tumor Size[b]	No. Mice/Total[c]
None	0	0/5
Complement	0	0/5
Anti-Thy-1.2 + complement	16.6 ± 1.7	5/5
Anti-L3T4 + complement	12.8 ± 6.1	4/5
Anti-Lyt-2.2 + complement	0	0/5
No effector cells transferred	18.4 ± 1.3	5/5

[a] Meth A (5×10^5/mouse) and effector cells (5×10^6/mouse) were inoculated intradermally into the [CD-1(nu/nu)←BALB/c] chimeras on day 0.

[b] Tumor size is expressed as square root of a × b mm (mean ± SD) on day 21.

[c] Number of tumor-bearing mice/total mice on day 21.

positively selected Meth A-Im-L3T4 cells was tested in the [CD-1(nu/nu)←B10 congenic mice] as recipients for the Winn assay (Table V, with the results summarized in Table VI). The tumor-neutralizing activity was not observed in the [CD-1(nu/nu)←B10], [CD-1(nu/nu)←B10.BR] or [CD-1(nu/nu)←B10.A] chimeras. On the other hand, in the [CD-1(nu/nu)←B10.D2] chimeras, Meth A-Im-L3T4 neutralized the tumors. These results showed that the full H-2 difference between the effector lymphocytes (Meth A-Im-L3T4) and donor bone marrow cells resulted in abolition of the neutralizing activity. Even though they showed partial matching in the H-2 subregion (i.e., S and D regions), tumor-neuturalizing activity was not observed. On the other hand, in the [CD-1(nu/nu)←B10.KEA2] chimeras, which were I-A region-matched, the tumor-neutralizing activity of Meth A-Im-L3T4 was observed. These results suggested that there was a requirement for genotypic identity between Meth A-Im-L3T4 and host bone marrow or bone marrow-derived cells in the tumor neutralizing process.

DISCUSSION

Through the use of chimera nude mice, we could investigate the in vivo genetic interaction between primed helper T cells (Meth A-Im-L3T4) and host bone marrow or bone marrow-derived cells. We, as well as others (2–4), previously showed (5) that antitumor effectors in immune mouse splenocytes were Lyt-1^+2^- T cells of the helper type lacking direct cytotoxicity against the tumor cells, and this makes it likely that they killed the tumors in collaboration with other immunocytes. Our results confirmed this hypothesis and showed that bone marrow

TABLE IV

Tumor specificity of Meth A-Im-SPL and Meth A-Im-L3T4 in the [CD-1(nu/nu)←BALB/c] chimeras

Mice	Intradermal Inoculum[a]	E_A:Meth A-Im-SPL		E_A:Meth A-Im-L3T4	
		Tumor size[b]	No. mice/total[c]	Tumor size[b]	No. mice/total[c]
[CD-1(nu/nu) ←BALB/c]	MMC-Meth A	0	0/3	0	0/3
	Meth 1	16.1 ± 0.7	5/5	18.3 ± 0.1	5/5
	Meth 1 + E_A	12.8 ± 0.5	5/5	15.3 ± 1.4	5/5
	Meth 1 + E_A + MMC-Meth A	11.4 ± 0.8	5/5	14.9 ± 0.5	5/5
	Meth A + E_A	0	0/5	0	0/5
	Meth A	ND[d]		21.1 ± 1.1	5/5
		E_1:Meth 1-Im-SPL			
BALB/c	Meth 1	16.9 ± 2.2	5/5		
	Meth A + E_1 + MMC-Meth 1	19.6 ± 3.3	5/5		
	Meth 1 + E_1	0	0/5		

[a] Mitomycin C (100 μg/ml, 37°C, 1 hr) pretreated Meth A (MMC-Meth A) or Meth 1 (MMC-Meth 1) (5×10^5/mouse), Meth A or Meth 1 (5×10^5/mouse), and effectors (E_A or E_1) (5×10^6/mouse) were inoculated intradermally on day 21. Meth A-Im-L3T4 were positively purified from Meth A-Im-SPL by the panning method.

[b] Tumor size is expressed as square root of a × b mm (mean ± SD) on day 21.

[c] Number of tumor-bearing mice/total mice on day 21.

[d] ND, not done.

TABLE V

Tumor-neutralizing activity of Meth A-Im-L3T4$^+$ T cells in CD-1(nu/nu) chimeras

Chimera Mice	Chimerism[a]	Intradermal Inoculum[b]		Im-L3T4 Activity	
	% donor	Meth A	Im-L3T4	Tumor size[c]	No. mice/total[d]
CD-1(nu/nu)←B10	>90	+	−	19.1 ± 2.4	5/5
		+	+	14.7 ± 1.9	5/5
CD-1(nu/nu)←B10.D2	>90	+	−	18.5 ± 2.3	5/5
		+	+	0	0/5
CD-1(nu/nu)←B10.A	>90	+	−	17.5 ± 2.0	5/5
		+	+	13.3 ± 2.7	5/5
CD-1(nu/nu)←B10.BR	>90	+	−	17.5 ± 1.1	5/5
		+	+	15.2 ± 0.8	5/5
CD-1(nu/nu)←B10.KEA2	*	+	−	20.8 ± 1.0	5/5
		+	+	0	0/5

[a] Mean % positive cells of two mice (spleen cells) for each group. (* I-A^d(+):49.2%; H-2K^d(+):<5%; H-2D^d(+):<5%, by FACS IV.)

[b] Meth A (5×10^5/mouse) and Im-L3T4 (5×10^6/mouse) were inoculated intradermally on day 0. Im-L3T4 cells were positively selected from Meth A-Im-SPL by the panning method.

[c] Tumor size is expressed as square root of a × b mm (mean ± SD) on day 21.

[d] Number of tumor-bearing mice/total mice on day 21.

TABLE VI

Summary of major histocompatibility complex phenotype of the mouse strains used as bone marrow sources

	H-2							Meth A-Im-L3T4 (H-2^d) Tumor-Neutralizing Activity
	K	A_β	A_α	E_β	E_α	S	D	
B10	b	b	b	b	b	b	b	−
B10.BR	k	k	k	k	k	k	k	−
B10.A	k	k	k	k	k	d	d	−
B10.D2	d	d	d	d	d	d	d	+
B10.KEA2	w9	d	d	d	7	w9	w9	+

cells or bone marrow-derived cells were involved in in vivo Meth A rejection due to Meth A-Im-SPL/Meth A-Im-L3T4 in an H-2 (I-A)-restricted fashion. Although the recruitment of the inoculated bone marrow cells to the tumor site was not detected in the present study, the H-2 restriction between Meth A-Im-SPL (Meth A-Im-L3T4) and the inoculated bone marrow cells suggests that bone marrow-derived cells come into contact with antitumor L3T4$^+$ cells and tumor cells, and then actively participate in suppression of the tumor growth. In this regard, it is important to determine the characteristics of bone marrow-derived cells recruited to the tumor site.

Among bone marrow-derived cells, Ia$^+$ macrophages (16) or epidermal Langerhans' cells (17) could be considered to be operating as antigen-presenting cells. However, this is unlikely because of the following reasons i) Meth A-Im-SPL include a sufficient number of Ia$^+$ accessory cells themselves, but they did not neutralize the tumors in H-2 unmatched chimeras. ii) Langerhans' cells were not detected in the epidermis of the chimeras, at least for 13 days after the chimerization (18). Nevertheless, tumor neutralization by Meth A-Im-SPL was observed in the chimeras 7 days after the inoculation of H-2 identical bone marrow cells (data not shown). These results suggest that Ia$^+$ accessory cells derived from the host bone marrow cells may not play an important role in tumor neutralization in the chimera nude mice.

The induction of cytotoxic T lymphocytes (CTL) in chimera nude mice can be ascribed to the effect of interleukin 2 secreted from "helper T cells" (19, 20). Even though CTL precursors were present in Meth A-Im-SPL, Meth A-Im-SPL could not neutralize Meth A tumor growth in H-2 unmatched chimeras. This minimized the CTL contribution in this situation. The induction of cytotoxic macrophages in the chimeras can also be ascribed to the factors secreted from the helper T cells (Meth A-Im-L3T4) upon stimulation with the antigen (Meth A). Macrophage activation may be mediated by such products as macrophage-activating factor (MAF) (21) and specific MAF (22). Because of tumor-specific eradication in the effector phase (Table IV), specific MAF seems more important than MAF, which induces the bystander effect (23). However, whether any of these cells (including mast cells (8)) are involved in tumor neutralization awaits further investigation for determination.

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