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Differences in Immune Responses to Tumor Induced in Syngeneic Hosts by Injection of Hybrid and Parental Tumor Cells

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KAMBE, M., ROU, K. and TACHIBANA, T. *Differences in Immune Responses to Tumor Induced in Syngeneic Hosts by Injection of Hybrid and Parental Tumor Cells.* Tohoku J. Exp. Med., 1994, **174** (1), 71-83 —— Immunization of C3H/He mice with L-FM3A#2 hybrid cells, made by fusion of ascitic mammary carcinoma FM3A#2 cells with 8-azaguanine resistant L_{AG} cells, both of C3H/He mouse origin, resulted in spleen T cell-dependent resistance to the parental FM3A/R cells. These spleen T cells, purified by passing through a nylon fiber column, could be demonstrated to have Thy-1.2 and Lyt-2.1 antigens, and not L3/T4 antigens. After immunizing with irradiated FM3A/R cells, cytotoxic cells other than cytotoxic T lymphocytes (CTL) appeared, these presumably being nonphagocytic macrophages or polymorphonuclear cells. In this case, anti MM antiserum was generated at an earlier stage than when mice were immunized with the L-FM3A#2 cells. The cytotoxic mechanism is discussed as to the significance of the surface antigen. ———— hybrid cell; MM antigen; CTL

Numerous investigations have revealed tumor cells to bear tumor specific transplantation antigens (TSTA) and, in general, that they elicit a cell-mediated anti-tumor response. For example, many tumor cells derived from spontaneous mammary carcinomas in C3H/He mice have a surface TSTA, named the MM antigen, which has never been detected in normal tissues or primary carcinoma cells (Nishioka et al. 1969a, b; Takeuchi et al. 1969; Irie 1971). It was found that the MM antigen on MM tumor cells is capable of inducing a predominantly humoral response in syngeneic C3H/He mice, while scarcely inducing any cellular response. Nishioka and his colleague first described that C3H/He mice which had been inoculated with a mixture of MM2 ascitic mammary tumor cells and rabbit anti-MM2 antiserum intraperitoneally (i.p.), acquired resistance to subsequent MM2 tumor cell challenge, and that the sera from these mice contained tumor specific antibodies capable of protecting the hosts against MM2 tumor growth (Nishioka et al. 1969a; Takeuchi et al. 1969).

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As described previously (Tachibana et al. 1974), we have selected a hybrid cell line (L-FM3A#2) after somatic cell hybridization of FM3A#2 tumor cells, an MM positive line, with 8-azaguanine-resistant L cells (L_{AG}) derived from a syngeneic C3H mouse, by UV-treated Sendai virus (HVJ). These hybrid cells express the MM antigen at a high level. When 1×10^7 L-FM3A#2 hybrid cells were inoculated into C3H/He mice i.p., no tumor growth was observed, but more than 80% of the animals demonstrated effective protection against subsequent challenge with 1×10^5 parental tumor cells two weeks later.

In the present study, the immune response elicited by inoculation of L-FM3A#2 hybrid cells was investigated and compared with that generated by γ-irradiated tumor cells. Immunization with the hybrid cells preferentially generated CTL specific for the MM antigen in the spleen of syngeneic hosts, while, in contrast, γ-irradiated tumor cells generated cytotoxic activity in the spleen mediated by non-T cells and non-phagocytic adherent cells, in addition to production of anti-MM antibodies at a high level.

Materials and Methods

Animals

Inbred 4–5 week-old C3H/HeSlc female mice, free from mouse mammary tumor viruses, were supplied by Shizuoka Agricultural Cooperative Association for Experimental Animals, Shizuoka.

Cells

Two transplantable ascitic tumor cell lines, FM3A#2 and FM3A/R were derived in 1970 from a cultured FM3A/B cell line, originating from a spontaneous mammary tumor arising in a C3H mouse. They have different number of chromosomes; the mordal numbers are 62 and 70, respectively (Tachibana et al. 1974). These cell lines have been maintained by serial transplantation in the peritoneal cavity of C3H/HeSlc mice. L_{AG} cells, an 8-azaguanine resistant subline of C3H mouse L cells, were cultured in Eagle's minimum essential medium supplemented with 10% fetal calf serum (FCS) (Gibco, Grand Island Biological Co., Grand Island, NY, USA) and 10 μg/ml of 8-azaguanine.

Fusion of FM3A#2 tumor cells with L_{AG} cells and selection of hybrid cell clones were performed as described by Murayama and Okada (1970). Three to 4 weeks after hybridization, the hybrid clone, L-FM3A#2 was selected and analyzed for its karyotype (Tachibana et al. 1974).

Antisera and complement

Anti-Thy-1.2 alloantiserum, rat IgG_{2b} anti-mouse L3/T4 monoclonal antibody, mouse IgG_3 anti-Lyt-2.1 monoclonal antibody and Low-toxic rabbit complement^R were supplied by Cedarlane Laboratories Limited (Hornby, Ontario, Canada). Anti-H-2K^k and anti-H-2D^k monoclonal antibodies were purchased

from Meiji Institute of Health Science (Tokyo).

Analysis of antigens on the various cells by EPICS^{TMV}

Ten million cells were incubated with 50 μl of various monoclonal antibodies against the concerned antigens for 30 min at 4°C, and then washed 3 times. They were indirectly stained by the addition of 50 μl of fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse IgG_1 (Biochemical Division, San Mateo, CA, USA) at a dilution of 1 : 50. One to 2×10^6 cells were analyzed on a EPICS^{TMV} (Coulter Electronics, Inc., Hialeah, FL, USA) for expression of the antigen.

Immunization and immune lymphoid cells

C3H/HeSlc mice were immunized by a single or three weekly i.p. injection(s) of 2×10^6 viable L-FM3A#2 cells or 1.5×10^6 γ-irradiated (10,000 R) FM3A/R tumor cells. The numbers of the two kinds of cells used for immunization were adjusted to give equal amounts of the MM antigen expressed on the cell surfaces. The immunized mice were killed two weeks after the last injection and lymphoid cells were harvested. Spleens and lymphonodes were made into single-cell suspensions and after removal of erythrocytes with 0.84% NH_4Cl buffered solution, cells were suspended in RPMI 1640 medium supplemented with 10% FCS, penicillin (100 IU/ml) and streptomycin (100 μg/ml) (Gibco). Peritoneal exudate (PE) cells were collected by washing the abdominal cavity with phosphate buffered saline (PBS).

Cell separation

Splenic T cells were prepared by passage through a nylon fiber column (Wako Pure Chemical Ind., Ltd., Osaka) according to the method of Julius et al. (1973). The effluent from the column contained highly purified T cells (>95% T-cell population as tested by dye exclusion assay with anti-Thy-1.2 and complement). Viability of the cells was usually greater than 95%.

To remove T cells, spleen cells were treated with anti-Thy-1.2 antiserum at room temperature for 20 min and washed three times. Then rabbit complement was added and incubated at 37°C for 30 min. This procedure brought the remaining Thy-1.2 positive cells to below 10%. To remove L3/T4-antigen-positive cells or Lyt-2.1positive cells, spleen cell preparations were treated with anti-L3/T4-antibody or anti-Lyt-2.1antibody, respectively, followed by rabbit complement, as described above.

To remove phagocytic cells, 2.5 mg of Silica particles (KAC^R; JIMRO, Japan Immuno Research Institute, Takasaki) suspended in PBS were intravenously (i.v.) injected into each mouse two days prior to killing. By this treatment, 70–80% of phagocytic cells were removed from the spleen cell preparations as judged by yeast ingestion testing.

To separate spleen cells into adherent and non-adherent cells, they were

seeded on Petri dishes and incubated at 37°C for 2 hr. Non-adherent cells were sucked off with pipettes, seeded again on other Petri dishes, and the same procedure repeated twice. Non-adherent cells consisted of lymphocytes (>80%) as judged by morphological criteria. Cells adhering to the dishes were collected with a cell scraper (Sumitomo Bakelite Co., Ltd., Tokyo) and found to comprise mainly macrophages (70-80%) (yeast ingestion test). Further removal of adherent cells was carried out according to the method of Ly and Mishell (1974); Spleen cells were applied to the Sephadex G-10 column and incubated at 37°C for 30 min. The effluent was a purified population of lymphocytes (>95%). The proportions of T- and B-lymphocytes were not significantly different before and after the treatment.

Cytotoxic assays

The Winn test was used as an in vivo cytotoxic assay; 5×10^4 FM3A/R cells were admixed with 1×10^7 effector cells and immediately injected into C3H/HeSlc mice i.p., then subsequent growth of tumor cells observed for one month.

In vitro assessment of cellular cytotoxicity was performed according to the method of Hashimoto and Sudo (1971); 4 ml of 1×10^6 FM3A/R cells/ml were mixed with 1 ml of 5 μCi/ml of ^{3}H-uridine, incubated at 37°C for two hr with occasionally shaking and washed three times. Labeled FM3A/R cells were then resuspended at the concentration of 5×10^5 cells/ml. Serial dilution of effector cells was made at concentrations of 5×10^7, 2.5×10^7 and 1.25×10^7 cells/ml. Then 0.1 ml each of both target and effector cell preparations were mixed in wells of type II microplates (Falcon #3040), and incubated at 37°C for various time periods. The cells were then collected with a multiple cell harvester (LABO MASH^R, Labo Science Co., Ltd., Tokyo) and CPM values of intact cells trapped on the filters were counted with a Beckman LK150^R liquid scintillation counter (Beckman Instruments, Inc., Fullerton, CA, USA). Specific lysis was calculated as follows:

$$\% \text{ specific lysis} = \left(1 - \frac{\text{cpm of cultures with added immune spleen cells}}{\text{cpm of cultures with added non-immune spleen cells}}\right)\times 100$$

Maximum % specific lysis was obtained after incubation for 12hr, and the most effective ratio of target cells to effector cells was 1 : 100. Therefore in this paper, the experiments were carried under these condition.

Results

Antigen expression of parental and hybrid cells

Since the hybrid cell line, L-FM3A#2 was selected and established in 1974 by one of us (Tachibana et al. 1974), it has been maintained in tissue culture. The hybrid cells share marker antigens of both parental cells, the L_{AG} cells expressing

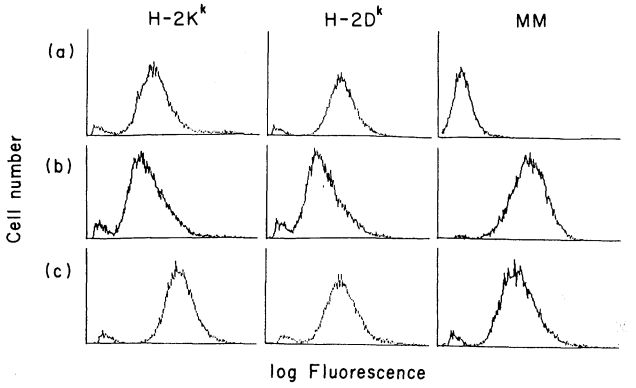

Fig. 1. Cytofluorographic analysis of cell surface H-2K^k, H-2D^k and MM antigen expression by L_{AG}, FM3A/R and L-FM3A#2 cells. Each line was incubated with anti-H-2K^k, anti-H-2D^k or anti-MM antibodies, and subsequently exposed to goat anti-mouse IgG conjugated to fluorescein isothiocyanate.
a, L_{AG} cells; b, FM3A/R cells; c, L-FM3A#2 cells.

the H-2^K antigen and the FM3A tumor cell line expressing both of the MM and the H-2^K antigen. Fig. 1 illustrates the results of EPICS analysis of the expression of H-2K^k, H-2D^k and MM antigens. The amounts of the three antigens on each cell were almost the same as in 1974. An inverse relationship between the expression of both H-2K^k and H-2D^k antigens and that of the MM antigen was apparent as previously observed in L_{AG} and FM3A/R cells (Tachibana et al. 1974). And the expressions of H-2 antigens and the MM antigen in L-FM3A#2 cells were as same as L_{AG} and FM3A/R cells, respectively.

Immunoprotection induced by immunization with hybrid cells

As previously reported (Tachibana et al. 1974), the L-FM3A#2 hybrid cells produced no tumors in normal syngenic mice while affording good specific immunoprotection against the challenge of parent tumor cells.

To examine the immune mechanism evoked by the injection of hybrid cells, C3H/HeSlc mice were immunized with L-FM3A#2 hybrid cells as in the Materials and Methods. Two weeks after the last injection, the spleen, lymph node and PE cell fractions and sera were prepared from the immunized mice and each of the preparations was examined for anti-tumor potency with the Winn test (Table 1).

Immune spleen cells completely suppressed the growth of admixed FM3A/R tumor cells, while protective effects of lymph node or PE cells from immune mice were only slight. Then immune spleen cells were fractionated to examine which were working as effectors. As seen in Table 2, the non-adherent cell fraction of the immune spleen cells collected from Petri dishes effectively suppressed the growth of admixed tumor cells. Immune spleen T-cells passing through a nylon fiber column similarly showed complete suppression of tumor growth in the Winn

TABLE 1. *Winn test results for FM3A/R cells mixed with lymphoid cells and antiserum*

Lymphoid cells or anti-MM-antiserum[a)]	Take incidence[b]	
	Exp. 1	Exp. 2
(−)	4/ 4	5/5
Anti-MM antiserum	3/ 3	4/4
Peritoneal exudate cells	7/10	3/5
Mesenterial lymph node cells	5/ 5	5/5
Other lymph node cells	4/ 6	4/5
Spleen cells	0/ 7**	0/5**

[a]Mixtures of FM3A/R cells with various lymphoid cells or anti-MM-antiserum from mice sensitized with L-FM3A#2 hybrid cells were inoculated into mice i.p., and emergence of tumors was observed.

[b]Number of mice tumors/number of mice tested

**$p<0.01$ Student's t-test.

TABLE 2. *Winn test results for FM3A/R cells mixed with various fractions of spleen cells*

Treatment of spleen cells[a]	Take incidence[b]	
	Exp. 1	Exp. 2
No spleen cells	4/4	5/5
Unfractionated normal spleen cells	3/3	4/4
Spleen cells sensitized with L-FM3A#2 cells		
Unfractionated	1/6*	0/5**
Non-adherent to plastic dish	0/5**	n.d.
Adherent to plastic dish	3/3	n.d.
Passed through nylon fiber column	0/9**	0/5**
Treated with anti Thy-1.2+complement	5/5	5/5
Treated with complement alone	0/5**	0/4**

[a]Spleen cells from each mouse were fractionated as indicated, and mixtures of FM3A/R cells and unfractionated or fractionated spleen cells were inoculated into mice i.p. and emergence of tumors were observed.

[b]Number of mice with tumors/number of mice tested.

n.d., not done.

*$0.01<p<0.05$ Student's t-test.

**$p<0.01$ Student's t-test.

test. Treatment of the immune spleen cells with anti-Thy-1.2 and complement abrogated their suppressive capacity and thus it was concluded that the suppressive capacity resides in T cells, indicating the generation of CTL by immunization with hybrid cells.

Character of the effector cells generated by immunization with hybrid cells

To examine the character of the CTL in the spleens immunized with the hybrid cells, cytotoxicity was measured in vitro by the ^{3}H-uridine method of Hashimoto and Sudo (1971). In preliminary experiments, the best condition to obtain maximal % specific lysis was the incubation for 12 hr, and 1 : 100 ratio of target cells to effector cells. The following experiments were therefore carried out under these conditions.

As shown in Table 3, when mice were sensitized with L-FM3A#2 cells or irradiated L-FM3A#2 cells, purified spleen T-cells passing through a nylon fiber column showed significantly greater cytotoxic activity against FM3A/R cells than that observed with whole spleen cells. In contrast, whole spleen preparations from mice immunized with irradiated FM3A/R tumor cells had high cytotoxic activity but the purified T-cells showed no cytotoxicity. To confirm this, spleen cells were treated with anti-Thy-1.2 and complement to remove T-cells. Table 4 shows that this treatment of spleen cells sensitized with hybrid cells reduced clearly cytotoxicity to about a half, while the same treatment of spleen cells sensitized with irradiated FM3A/R cells did not affect their activity. From these results, it is concluded that immunization with L-FM3A#2 hybrid cells, but not irradiated FM3A/R tumor cells, generates CTL in the spleen.

To further examine the character of the CTL, spleen cells were treated with

TABLE 3. *Specific lysis of FM3A/R cells by spleen cells sensitized with different antigenic cells*

Spleen cells		Cells used for immunization		
		% specific lysis[a]		
		L-FM3A#2	Irradiated L-FM3A#2	Irradiated FM3A/R
Exp. 1	Unfractionated	7.6±3.6	14.0±0.7	23.6±3.0
	T-enriched[b]	12.2±2.1*	18.5±1.8*	2.7±1.5**
Exp. 2	Unfractionated	11.1±2.1	13.6±1.2	15.9±3.2
	T-enriched	21.4±1.0**	17.1±0.8*	−5.3±0.7**
Exp. 3	Unfractionated	22.3±0.8	14.1±6.5	27.2±3.6
	T-enriched	34.6±4.0**	35.9±2.7**	−52.2±7.3**
Exp. 4	Unfractionated	12.9±4.4	23.5±2.1	31.3±5.6
	T-enriched	26.6±6.5**	36.5±6.0**	− 6.3±3.2**

[a] ^{3}H-UdR labeled FM3A/R cells were mixed with unfractionated spleen cells or T-enriched cell fractions.

[b] T-enriched fractions were obtained by passing through a nylon fiber column.

*$0.01 < p < 0.05$ Student's t-test: T-enriched as compared to unfractionated spleen cells.

**$p < 0.01$ Student's t-test.

TABLE 4. *The effect of treatment of spleen cells with anti-Thy-1.2 antibody and complement on specific lysis of FM3A/R cells*

	Treatment of spleen cells (% of specific lysis)					
	Exp. 1			Exp. 2		
	a	b	c	a	b	c
Immunization	—	α-Thy-1.2+C	C alone	—	α-Thy-1.2+C	C alone
L-FM3A#2	25.5 ±3.6	10.8* ±1.5	21.4 ±2.1	22.5 ±2.2	12.5* ±4.6	20.5 ±3.0
Irradiated L-FM3A#2	n.d.	n.d.	n.d.	18.6 ±1.1	10.6* ±3.6	19.1 ±1.8
Irradiated FM3A/R	23.3 ±2.4	20.1[ns] ±5.6	25.7 ±0.8	23.3 ±2.3	24.0[ns] ±0.9	21.4 ±4.7

[a]^{3}H-UdR labeled FM3A/R cells were mixed with spleen cells sensitized with L-FM3A#2, irradiated L-FM3A#2 or irradiated FM3A/R cells.

[b]Spleen cells were treated with anti-Thy-1.2 antibody and complement.

[c]Spleen cells were treated with complement alone.

n.d., not done.

[ns]Statistically not significant as compared to the lytic activity of spleen cells treated with complement alone.

*$0.01 < p < 0.05$ Student's t-test.

anti-Lyt-2.1 antibody or anti-L3/T4 antibody and complement (Table 5). CTL exposed to anti-Lyt-2.1 antibody and complement (>90% were L3/T4 positive cells) showed scarcely any cytotoxicity, whereas those treated with anti-L3/T4 antibody and complement (>90% were Lyt-2.1 positive) demonstrated effective cytotoxic activity. From these results, it is concluded that immunization with L-FM3A#2 hybrid cells generates Lyt-2.1 antigen positive and L3/T4 antigen

TABLE 5. *The effect of treatment of spleen cells with anti-Lyt-2.1 antibody or anti-L3/T4 antibody on specific lysis of FM3A/R cells*

Antibody	Treatment of spleen cells (% of specific lysis)								
	Exp. 1			Exp. 2			Exp. 3		
	(−)[a]	Ab+C[b]	C alone[c]	(−)	Ab+C	C alone	(−)	Ab+C	C alone
Anti-Lyt-2.1	19.8 ±3.4	7.5* ±1.1	18.3 ±2.7	13.8 ±2.1	4.0* ±0.9	14.5 ±0.5	20.3 ±2.2	8.5* ±1.3	18.4 ±0.1
Anti-L3/T4		22.5[ns] ±1.0	16.7 ±0.3		14.0[ns] ±0.6	16.9 ±0.7		25.0[ns] ±1.7	16.6 ±0.7

[a]C3H/He mice were sensitized with L-FM3A#2 cells three times.

[b]Spleen cells were treated with each antibody and complement.

[c]Spleen cells were treated with complement alone.

[ns]Statistically not significant as comparred to the lytic activity of spleen cells treated with complement alone.

*$0.01 < p < 0.05$ Studens's t-test.

negative CTL in the spleen which play an important role as the major effector cells in the tumoricidal mechanism.

Effector cells generated by immunization with irradiated tumor cells

To allow partial functional separation of cells, silica particles were administrated into the immune mice i.p., resulting in remarkably diminished populations of phagocytic cells in the spleen cell preparations. As shown in Table 6, in the case of immunization with irradiated FM3A/R cells, the cytotoxic activity of the cell fraction depleted of phagocytes somewhat increased, while immunization with

TABLE 6. *The effect of the injection of Silica in vivo on specific lysis of spleen cells*

Immunization[a]	Treatment of mine	% of specific lysis		
		Exp. 1	Exp. 2	Exp. 3
Irradiated FM3A/R	Untreated	12.2±4.2	16.7±5.3	12.4±2.3
	Silica[b]	20.1±2.3*	34.2±1.7*	14.6±3.6
L-FM3A#2	Untreated	n.d.	n.d.	23.5±2.7[ns]
	Silica	n.d.	n.d.	20.7±2.4[ns]

[a]C3H/He mice were sensitized with antigenic cells three times.

[b]Silica was injected into the C3H/He mice i.p. and the spleen cells used as effector cells.

n.d., not done.

[ns]Statistically not significant.

*$0.01 < p < 0.05$ Student's t-test.

TABLE 7. *Specific lysis by spleen cells after passage through a Sephadex G-10 column*

Immunization[a]	Treatment of spleen cells	% of specific lysis			
		Exp. 1	Exp. 2	Exp. 3	Exp. 4
Irradiated FM3A/R	Untreated	28.5 ±3.2	28.0 ±3.2	11.7 ±2.7	25.3 ±2.8
	Monocyte/neutrophil[b] depleted	−16.2* ±5.4	−3.9* ±3.3	−11.7* ±3.4	−1.6* ±2.4
L-FM3A#2	Untreated	n.d.	n.d.	18.1 ±4.9	13.3 ±2.1
	Monocyte/neutrophil depleted	n.d.	n.d.	20.5[ns] ±2.9	7.7[ns] ±2.5

[a]C3H/He mice mice were sensitized with each antigenic cells three times.

[b]Spleen cells were passed through a Sephadex G-10 column, and were used as effector cells.

n.d., not done

[ns]Statistically not significant.

*$p < 0.01$ Student's t-test.

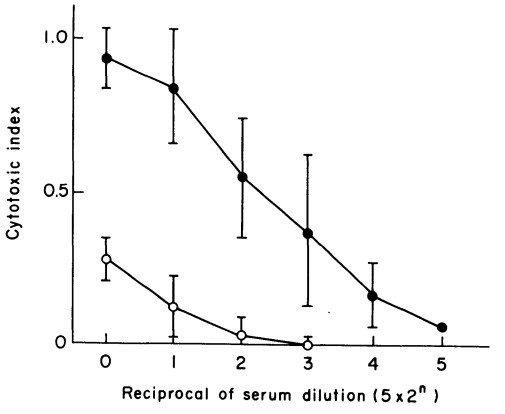

Fig. 2. Levels of anti-MM antibody in sera from C3H/He mice sensitized with irradiated FM3A/R or L-FM3A#2 cells.
Groups of eight C3H/He mice were i.p. inoculated with irradiated FM3A/R or L-FM3A#2 three times, and bled. The level of MM antibody in each individual serum was determined as in Materials and Methods. For immunization, irradiated FM3A/R (○—○), or irradiated L-FM3A#2 (●—●), were used. Cytotoxic index was represented as mean±S.D.

hybrid cell, the cytotoxicity remained unchanged.

Next, spleen cells were applied to a Sephadex G-10 column, on which monocytes and neutrophils selectively adhere, and the cytotoxic activity of the effluent cell fraction tested. As shown in Table 7, this treatment of spleen cells sensitized with irradiated FM3A/R cells completely abolished the activity, but no effect was observed for spleen cells sensitized with hybrid cells. The effluent cell fraction in both cases contained no phagocytes.

From the above experiments, it is suggested that non-phagocytic adherent cells are mainly concerned with the tumoricidal mechanism in mice immunized with irradiated FM3A/R tumor cells.

Levels of anti-MM antibody in sera after immunization with different antigenic cells

Immunized mice were bled and the levels of anti-MM antibody in sera measured by a complement-dependent microcytotoxic test. Two weeks after a single injection, anti-MM antibody could not be detected (data not shown). However, as shown in Fig. 2, level of antibody was high in mice sensitized three times with irradiated tumor cells, while it was very low in sera after injections with irradiated hybrid cells. Viable hybrid cells produced a little higher antibody than that made with the irradiated hydrid cells (data not shown).

Discussion

Recently, the idea that various kinds of T cell functions are associated with

surface structures encoded by the genes of the MHC has become widely recognized. For example, Doherty et al. (1976) pointed out that lymphocytic choriomeningitis virus (LCMV) infected cells generate CTL which recognize viral antigens associated with the H-2K or H-2D antigens in the MHC. In the present study, we could clearly demonstrate that C3H/He mice immunized with hybrid cells between L_{AG} cells and FM3A#2 ascitic mammary tumor cells (Tachibana et al. 1974), namely L-FM3A#2, produce splenic CTL, capable of lysing FM3A/R parent tumor cells.

Nishioka and his colleagues earier reported that C3H/He mice, inoculated with MM2 tumor cells, acquired resistance against such MM2 cells associated with production of cytotoxic antibodies in the serum (Nishioka et al. 1969a; Takeuchi et al. 1969). This antiserum was cytotoxic not only to MM2 cells, but also a series of ascitic mammary tumor cell lines including an Ehrlich ascites tumor, allogeneic leukemic cells and even allogeneic normal lymphocytes but not syngeneic lymphocytes (Chang et al. 1972), indicating that the antigen is different from the virion antigens of the mouse mammary tumor virus. The crossreacting antigen was detected as a fetal antigen appearing during the 7-11th days of the embryonal stage, and therefore, the MM antigen is considered as a fetal or alloantigen whose ectopic appearance is due to activation of a silent gene or an induced incapacitation of a regulator gene. The MM antigen could be extracted in deoxycholate solution, and has molecular weight of 20,000 (Irie et al. 1969).

Although there is an inverse relationship between the expression of the MM antigen and that of the $H\text{-}2K^k$ or $H\text{-}2D^k$ antigen on the surfaces of L_{AG} and FM3A/R cells, L-FM3A#2 hybrid cells are expressing both the $H\text{-}2K^k$, $H\text{-}2D^k$ antigen and MM antigens (Fig. 1). The hybrid cells are not capable of tumor growth in syngeneic C3H/He mice but do induce resistance against subsequent FM3A/R challenge.

The present comparative study of immune response elicited by hybrid cells clearly demonstrated that splenic T cells, i.e., non-adherent, Thy-1.2 positive, Lyt-2.1 positive and L3/T4 negative, were the effector cells for the supression of the growth of FM3A/R tumor cells. These CTL may recognize the MM antigens with restriction to MHC class I molecules interacting with Lyt-2.1 antigens which increase the avidity of the binding between the T cell receptors and MHC molecules (Dembic et al. 1987; Doyle and Strominger 1987; Gennings et al. 1988; Kuribayashi et al. 1989). The CTL are thought to act in the classical manner. But the cause for the residual cytotoxic activity after the treatment with anti-Thy-1 or anti-Lyt-2.1, and complement are not clear (Tables 4, 5). It might be due to the lack of complement. As in preliminaly experiments there were the cases in which the treatment of spleen cells with complement alone reduced the cytotoxic activity, the dilution of complement used was subtoxic and might be short of activity. But it can not be completely exclude the possibility of the existance of cytotoxic cells other than CTL having Thy-1 and Lyt-2.1 antigens. And natural killer activity was not detected in the spleen cells immunized with

hybrid cells in the ^{51}Cr relaese assay for 4hr, using Yac-1 cells as target cells (data was not shown). In addition, low levels of anti-MM antibodies did appear after several repeated injections of the cells. On the other hand, mice immunized with γ-irradiated FM3A/R cells produced antibodies at an earlier stage and in much higher quantities (Fig. 2). Therefore it is probable that while immunization with MM tumor cells induces an antibody response, immunization with hybrid cells provokes a cellular response, followed by delayed antibody production. It may be considered that co-expression of H-2^k antigen and MM antigen by irradiated parent tumor cells was not sufficient to elevate CTL activity in the present case (Fujiwara et al. 1976).

Since the relative expression of the H-2^k antigen on the hybrid cells was approximately twice that on FM3A/R cells (Fig. 1), this may be the determining factor. Immunization with γ-irradiated FM3A/R tumor cells generated non-T and non phagocytic adherent cells responsible for tumor cell toxicity in the spleen, presumably independent of the antibody production (Tables 6, 7). But the characters of these cells should be examined further.

From these results, it is now evident that hybrid cells have the MM antigen in a molecular from capable of inducing CTL generation, while the same antigen on tumor cells are present in a molecular form better capable of inducing antibody production. We speculate that changes of antigenic expression different from simple co-dominant expression of antigens can occur on somatic hybrid cells, especially in the case of cell fusion between two different cell lineages. These include both qualitative and quantitative changes, but no information on functional changes of cell surface antigens is available so far. The present findings indicate that a new avenue to immunological application of somatic cell hybridization may be open for cancer research and for analysis of antigen recognition mechanisms.

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