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Plant lectin, ATF1011, on the tumor cell surface augments tumor-specific immunity through activation of T cells specific for the lectin

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Summary. The possibility that a plant lectin as a carrier protein would specifically activate T cells, resulting in the augmentation of antitumor immunity was investigated. ATF1011, a nonmitogenic lectin for T cells purified from *Aloe arborescens Mill*, bound equally to normal and tumor cells. ATF1011 binding on the MM102 tumor cell surfaces augmented anti-trinitrophenyl (TNP) antibody production of murine splenocytes when the mice were primarily immunized with TNP-conjugated MM102 tumor cells. The alloreactive cytotoxic T cell response was also augmented by allostimulator cells binding ATF1011 on the cell surfaces. These augmented responses may be assumed to be mediated by the activation of helper T cells recognizing ATF1011 as a carrier protein. Killer T cells were induced against ATF1011 antigen in the H-2 restricted manner using syngeneic stimulator cells bearing ATF1011 on the cell surfaces. When this lectin was administered intralesionally into the tumors, induction of cytotoxic effector cells was demonstrated. These results suggest that intralesionally administered ATF1011 binds to the tumor cell membrane and activates T cells specific for this carrier lectin in situ, which results in the augmented induction of systemic antitumor immunity.

Introduction

In the field of tumor immunotherapy, there has been much effort to augment induction of various effectors to attack the tumors. Some are directed towards augmentation of antigen nonspecific immunity mediated by macrophages, natural killer cells (NK), lymphokine-activated killer cells and so forth [11, 17, 24], and others towards induction of antigen-specific cytotoxic effectors through activation of helper T (Th) cells. In the latter cases, limited antitumor immune responses observed have been mainly ascribed to the weak immunogenicity of tumor-associated transplantation antigens (TATA) and/or the presence of diverse suppressive mechanisms [4, 5]. Many studies have been pursued to augment anti-TATA immune responses by modifying tumor cell membranes with additional antigenic determinants and immunizing with such modified cells [8, 9, 10, 18]. Cytotoxic T cell (CTL) and B cell responses to TATA have been augmented under conditions in which Th cells are capable of fully manifesting their functions. If Th cells are activated by immunizing the host with modified cells bearing strong antigenic determinants on their cell surfaces, augmented anti-TATA immune responses may be introduced [21]. Plant lectins may be one of the candidates to couple new antigenic determinants on tumor cell surfaces, because they not only behave as an antigen to the host immune systems but have the capacity to bind in situ to the tumor cell surfaces [3, 28]. Accordingly lectins could be used as newly introduced antigenic determinants on tumor cells to induce a sufficient magnitude of activated Th cells in the host immune systems, which would result in augmentation of the TATA specific cellular immune response.

It is widely known that lectins have many immunological activities. For example, many of the known lectins share mitogenic activities with normal lymphocytes [23], and stimulate the release of various lymphokines and cytokines [22]. Further, it has been suggested that some lectins suppress the growth of transformed cells depending on the selective agglutinability of transformed cells over normal cells [27]. These activities are thought to be inappropriate for analyzing the functions of a lectin on the tumor cell surface as a carrier determinant in the host immune system. ATF1011, a lectin extracted and purified from *Aloe arborescens Mill*, has little agglutinability to erythrocytes of various species and no mitogenicity to mouse lmyphocytes (Ooki et al. 1987, paper submitted). To investigate the relation between newly introduced carrier determinants of the lectin on tumor cell surface and the augmentation of tumor-specific immunity, we have characterized the binding ability of the lectin to various kinds of normal and malignant cells, and thereafter analyzed the activation of Th cells specific for the carrier lectin. The present study provides the evidence that ATF1011 has the capacity to activate Th cells specific for the lectin and that intralesional (i. l.) injection of ATF1011 into the local tumor site results in the induction of tumor-specific CTLs acting systemically in the tumor-bearing host.

Materials and methods

Mice. Adult C3H/HeN and DBA/2 mice were purchased from Chales River Japan (Kanagawa, Japan) and maintained in the specific pathogen-free state. Normal females 7 to 10 weeks of age were used for the experiments.

Cells. MM46 and MM102 are mammary tumor cell lines originating from C3H/He. MH134 is a hepatoma cell line originating from C3H/He. Meth-A is a fibrosarcoma cell

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line originating from BALB/C. P815 is a mastocytoma cell line from DBA/2 and EL4 is a thymoma cell line from C57/BL6. These cell lines were maintained by weekly i. p. passage in syngeneic host mouse strains.

ATF1011. ATF1011 was kindly provided by Dr K. Toi (Ajinomoto C., Inc., Kanagawa, Japan). Details of the purification procedures are published elsewhere (Ooki et al. 1987, paper submitted) ATF1011 was extracted from *Aloe arborescens Mill* and purified to apparent homogeneity as detected on sodium dodecyl sulfatepolyacrylamide gel electrophoresis. The lectin was separable from Aloctin A and Aloctin B (lectins purified from the same origin on a Sephadex G-100 column). It was also distinguisable from Aloctin A and Aloctin B by its molecular weight and the lack of hemaggulutination activity towards human eryhtrocytes [28].

Binding experiment. ATF1011 was iodinated by the Iodogen method (Pierarce Chemical C., Ill, USA): 400 µl of a solution of ATF1011 (200 µg/ml) was added to Iodogen-coated glass tubes (40 µl/tube), followed by incubation on an ice bath for 40 min. The reaction was terminated by removing the sample mixture from the glass tube, and applying the mixture to a Sephadex G-25 column (Pharmacia Fine Chemicals, Uppsala, Sweden). The column was equilibrated and eluted with phosphate-buffered saline (PBS). Eluted fractions at the void volume were pooled and used as ^{125}I-labeled ATF1011. Radioactivity was found to be 460,000 cpm/g protein. For the binding experiments, cultured tumor cells, freshly prepared C3H/HeN splenocytes and thymocytes were washed with RPMI-1640 medium (GIBCO, Grand Island, NY, USA) 3 times and then medium was replaced with Dulbecco's modified Eagle's Medium (DMEM, GIBCO) containing 1.0% bovine serum albumin. The cells were incubated in Falcon 1058 tubes in a volume of 1 ml (2×10^6 cells/ml). To measure total binding, ^{125}I-labeled ATF1011 was added to one set of tubes. To determine nonspeicific binding, the same concentration of ^{125}I-labeled ATF1011 was added to another set of tubes in the presence of a large excess of unlabeled ATF1011. After 30 min, the tubes were centrifuged for 5 min at 400 *g*. The supernatants were removed and cell pellets washed 3 times with cold DMEM. After the final wash, the cell pellets were lysed with 0.1 *N* NaOH. The amount of radioactivity in the cell lysates was determined using a gamma counter (Packard 5260). Specific binding was determined by subtracting the amount of nonspecifically retained radioactivity from total radioactivity. Nonspecific binding constituted less than 20% of total binding.

Assay of direct cytotoxicity. Tumor cell lines were suspended in RPMI-1640 medium containing 10% fetal bovine serum (FBS) without antibiotics. Cell suspensions (1×10^5 cells/ml) were cultured in plastic dishes (Falcon 3002) in the presence or absence of lectins. At different time intervals after incubation in an atmosphere of 5% CO_2 in air at 37° C, cell suspensions were collected in plastic tubes and centrifuged. Cells were resuspended in RPMI-1640 and counted microscopically after eosin dye exclusion for determining cell number and viability.

Hapten conjugation. Chemical coating of cells with trinitrophenyl (TNP) hapten was carried out as previously described [9]. Cells ($1-10 \times 10^7$ were suspended in PBS (pH 7.2) containing 10 m*M* trinitrobenzenesulfonate (TNBS, Tokyo Kasei, Japan) and incubated at 37° C. After 10 min, cells were washed with PBS 3 times and suspended in the appropriate medium for the following experiments. For the modification of proteins, they were dissolved in PBS (pH 7.2) containing 10 m*M* TNBS and stirred for 12 h at room temperature. Reaction mixtures were dialysed against PBS 3 times and stored at −20° C. The numbers of hapten coupled to the protein molecules were 15 moles/mole ATF1011 and 11 moles/mole ovalbumin (OVA) respectively.

Coating of cells with ATF1011. For the coating of cells with ATF1011, $1-10 \times 10^7$ cells were incubated in 10 ml of PBS containing 50 mg/ml ATF1011 at 4° C for 1 h. After incubation, cells were washed with PBS 3 times to wash out the free ATF1011. This coating procedure resulted in the maximal binding of ATF1011 on the cell surfaces.

Plaque forming cell assay. The in vitro plaque forming cell (PFC) assay has been described in detail previously [20]. Briefly, splenocytes (3×10^6 cells/ml) were suspended in RPMI-1640 supplemented with 10 m*M* HEPES (GIBCO), 2.5×10^{-5} *M* 2-mercaptoethanol (2-ME, SERVA, Heidelberg, FRG), 100 units/ml penicillin, 100 µg/ml streptomycin and 10% FBS. These cells were cultured with antigens in 96-well culture plates (Corning 25860, NY, USA) at 37° C in a humidified atmosphere of 5% CO_2 in air. After 5 days, cells were collected, washed, and appropriately diluted in RPMI-1640 medium. PFC were counted according to the method previously described [1].

CTL assay. This assay was carried out as previously described [12]. Briefly, 4×10^6 responder splenocytes and X-ray-irradiated (2000 R) stimulator cells ($0.05-1.0 \times 10^6$) were collected in plastic tubes and pellet by centrifugation at 400 *g* for 5 min and the supernatants removed. Cells were resuspended in DMEM supplemented with 10 m*M* HEPES, 5×10^{-5} *M* 2-ME and 5% FBS at a concentration of $1-100 \times 10^4$ cells/ml in plastic tubes (Falcon 1058), and 1×10^4 ^{51}Cr-labeled target cells were added. After incubation for 3 h at 37 °C, tubes were centrifuged and the supernatants dissected from the pellets. Radioactivity was counted using a gammacounter. The CTL activity was calculated using the following formula: % specific lysis = (experimental ^{51}Cr release − control ^{51}Cr release)/(total ^{51}Cr incorporated − control ^{51}Cr release).

Induction of tumor-specific CTL by ATF1011 treatment. For this procedure, 1×10^6 MM46 or MM102 mammary tumor cells were implanted i. d. in C3H/HeN mice on day 0. ATF1011 was administered i. l. into the tumor sites on days 10 and 12 (50 mg/kg). The control mice received saline solution on the same schedule. On day 17, spelenocytes were collected from five tumor-bearing mice per group, and single cell suspensions were prepared for the next in vitro restimulation. These spleen cells were stimulated with 8000 R X-ray-irradiated tumor cells with or without ATF1011. Then 4 days after the cultivation, cytotoxicity to ATF1011-modified or unmodified tumor cells was determined using a ^{51}Cr release assay [16].

Table 1. Comparison of binding activity of ATF1011 with concanavalin A (ConA)

Cells	ATF1011		ConA	
	Ka[a] ($\times 10^6/M^-$)	Binding sites[b] ($\times 10^6$/cell)	Ka[a] ($\times 10^6/M^-$)	Binding sites[b] ($\times 10^6$/cell)
MM46	1.53	2.26	2.20	7.08
MM102	1.40	1.59	1.45	7.40
Meth-A	0.82	3.75	1.95	12.2
P815	0.62	7.30	0.99	19.5
Spleen	0.64	2.10	–	–
Thymus	2.12	1.38	–	–

[a] The affinity constant (Ka) was determined from the slope of straight line fitted to the data from Scatchard plot of binding experiment

[b] The maximum number of binding sites per cell was calculated by multiplying the moles of protein bound at saturation (abcissa intercept of Scatchard plot) by Avogadoro's number and dividing by the number of cells

Results

Binding of ATF1011 to normal and tumor cells.

Binding spectra of ATF1011 to various cells were investigated using ^{125}I-labeled ATF1011. As shown in Table 1, ^{125}I-ATF1011 bound to various kinds of murine tumor cells, as well as to murine thymocytes and splenocytes. Binding of radiolabeled ATF1011 to these cells was saturated and specifically inhibited by the addition of cold ATF1011 (data not shown). These results suggested the existence of specific binding sites for ATF1011 on the cell surfaces. The affinity constants and the numbers of binding sites on various cells were calculated and compared to those of concanavalin A (ConA).

Direct cytotoxicity of ATF1011 to the tumor cells

To investigate the presence of direct cytotoxicity of ATF1011, various cell lines were cultured in the presence of ATF1011 and the inhibition of growth was compared to that of ConA. As shown in Fig. 1, all the tumor cell lines showed almost the same growth in the presence of ATF1011 at concentrations ranging from 25 μg/ml to 100 μg/ml as in the absence. On the other hand, MM46, MH134, and EL4 did not grow in the presence of ConA (25 μg/ml). MM102 was resistant to low concentrations of ConA, but at high concentration (100 μg/ml), the growth was suppressed. These data indicated that the direct cytotoxicity of ATF1011 against tumor is subtle in contrast to ricin, abrin, and ConA [6, 27, 29]. From these results, ATF1011 is considered to be a lectin which binds to mammalian cells without direct toxicity.

Activation of Th cells in antibody production

ATF1011 on the tumor cell surface may behave as an antigenic determinant in the host immune system. To examine this possibility, an attempt was made to augment antibody production against the TNP hapten on the tumor cell surface through activiation of ATF1011-specific Th cells [25]. MM102 mammary tumor cells were chemically conjugated with TNP hapten and suspended in PBS containing ATF1011 for binding of the lectin onto the membranes

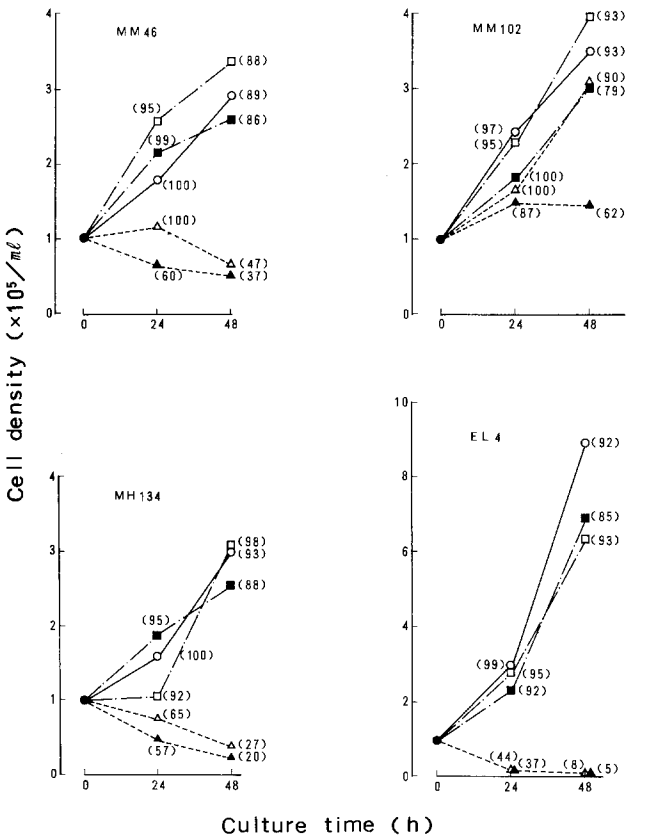

Fig. 1. Effect of AFT1011 on growth of various tumor cell lines in vitro. Viability is represented in parenthesises. Control (○), ATF1011 (□; 25 μg/ml, ■; 100 μg/ml), ConA (△; 25 μg/ml, ▲; 100 μg/ml)

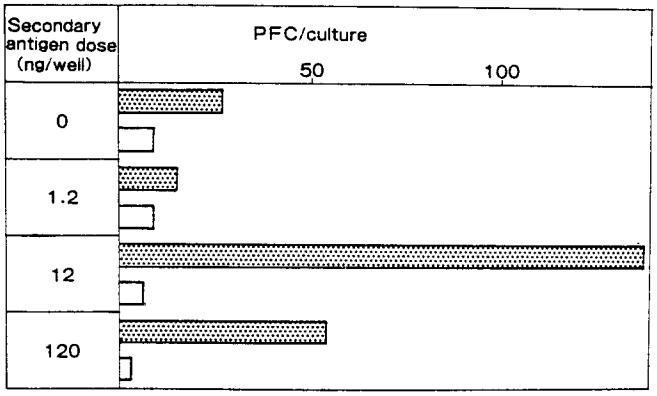

Fig. 2. ATF1011 on the cell surfaces acts as Th cell activator in T cell-dependent antibody production. MM102 cells were haptenized with trinitrophenyl (TNP) and suspended in 10 ml of ATF1011 solution (50 μg/ml phosphate-buffered saline PBS). After incubation at 4 °C for 1 h, cells were washed and injected in syngeneic C3H/HeN mice for primary immunization. After 5 days, splenocytes were collected and reactivated with secondary antigen (▨; TNP-ATF, □; TNP-ovalbumin). Then 4 days after cultivation, anti-TNP plaque forming cells (PFC) were determined

(TNP-MM102-ATF). A syngeneic C3H/HeN mouse was first immunized i. p. with TNP-MM102-ATF. After 5 days, spleen cells were collected and rechallenged in vitro with various doses of TNP conjugated ATF1011 (TNP-ATF) or TNP conjugated OVA (TNP-OVA) as a secondary antigen.

As shown in Fig. 2, anti-TNP PFC was specifically activated only in the case where TNP-ATF was used as the rechallenging antigen. These data suggested the possibility of recognition by Th cells of ATF1011 on the tumor cell membrane as a carrier protein.

Augmentation of CTL response by ATF1011

CTL have been reported as one of the relevant effector cells in tumor rejection [19]. For augmentation of the antitumor immune response, whether CTL induction is augmented by ATF1011 binding on the stimulator cell membrane was investigated. To this end, we tried to augment the in vitro alloreactive CTL response with ATF1011-treated allostimulator cells. As shown in Fig. 3, the response was markedly augmented in the induction phase by the presence of ATF1011 on the stimulator cells. On the other hand, in the effector phase, ATF1011 binding on the target cells did not change the target cell susceptibility. These data suggested that Th cells recognize ATF1011 on the stimulator alloantigen cells but induced effector CTLs do not. To investigate the effect of ATF1011 in the H-2-restricted antigen-specific CTL response C3H/HeN splenocytes were activated by ATF1011-modified syngeneic C3H/HeN splenocytes. As shown in Fig. 4, stimulator cells modified by ATF1011 induced CTLs specific for the ATF1011 antigen. When normal C3H/HeN splenocytes were used as stimulator cells, no detectable specific cytotoxicity was observed at the same effector : target ratio (data not shown). These results suggested that H-2-restricted CTLs are inducible by ATF1011 binding on the syngeneic stimulator cells and that ATF1011 on the cell surface can be recognized by CTLs as a compartment not of self or altered self.

Induction of antitumor CTLs in syngeneic tumor-bearing mice by i. l. administered ATF1011

To investigate the in vivo effects of ATF1011 on Th cells and/or CTLs, the potency of ATF1011 to induce a tumor-specific CTL response in the host immune cells was mea-

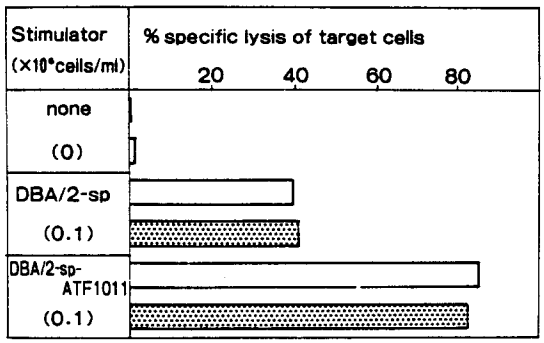

Fig. 3. Augmentation of alloreactive CTL by ATF1011-treated stimulator cells. DBA/2 splenocytes (1×10^7 2000 R X-ray-irradiated) were suspended in 10 ml of ATF1011 solution (50 μg/ml PBS) and incubated at 4 °C for 1 h. After washing, these cells were used as DBA/2-sp-ATF1011 stimulator cells. C3H/HeN splenocytes were used as responder cells and stimulated with X-ray-irradiated DBA/2 splenocytes (DBA/2-SP) or DBA/2-sp-ATF1011 by incubation at 37 °C for 5 days. After incubation, cytotoxicity against P815 (□) and P815-coated with ATF1011 (□) was measured. Effector : target = 50 : 1

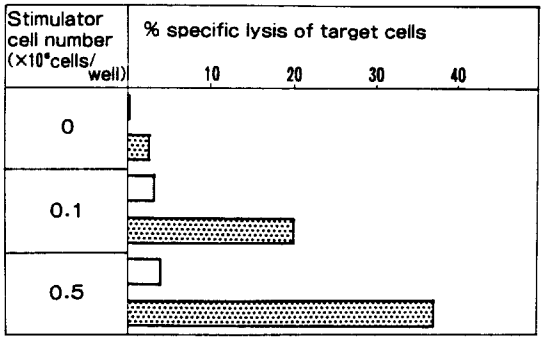

Fig. 4. Induction of H-2-restricted ATF1011-specific CTL. C3H/HeN splenocytes were stimulated with 2000 R X-ray-irradiated C3H/HeN splenocytes after coating with ATF1011. Cytotoxicity to ConA blast cells of C3H/HeN splenocytes (□) and ATF1011-coated ConA blast cells (▨) was measured. Effector : target = 30 : 1

Table 2. Intralesionally (i.l.) administered ATF1011 induces cytotoxic T cells (CTL) in syngeneic host-tumor systems

Host-tumor system	Secondary[b] stimulator	ATF1011[a] administration	% Lysis of[c]			
			MM46	MM46-ATF	MM102	MM102-ATF
C3H-MM46	None	−	1.8	5.9	0.1	0
		+	15.6	9.6	5.6	2.5
	MM46	−	17.8	0	0.2	0
		+	56.4	54.0	4.3	2.1
	MM46-ATF	−	6.7	0	0.3	0
		+	56.7	36.0	4.5	0.6
C3H-MM102	None	−	0.1	0	0	5.9
		+	1.6	5.0	4.4	6.7
	MM102	−	0	0	0	0
		+	1.8	1.5	12.4	15.1
	MM102-ATF	−	0	0	0	0
		+	3.7	0.1	14.3	14.4

[a] ATF1011 was administered i.l. into the tumor sites at a dose of 50 mg/kg on days 10 and 12 after tumor implantation
[b] On day 17, splenocytes were secondarily activated in vitro with 1×10^4 tumor cells (8000 R irradiated) in 24-well culture plate. Then 4 days after cultivation, induced cytotoxicity was determined
[c] Cytotoxicity was determined as indicated in *Materials and methods* at an effector: target ratio of 60 : 1

sured in experiments in which C3H-MM46 and C3HMM102 were used as syngeneic host tumor systems. As shown in Table 2, splenocytes from ATF1011-untreated host mice did not show in vitro cytotoxic activities against target tumor cells. On the other hand, splenocytes obtained from ATF1011-treated tumor-bearing mice were highly cytotoxic when secondarily stimulated by antigenic tumor cells and they specifically killed the same target tumor cells as antigen cells. The difference in killing activity between ATF1011-coated and noncoated secondary stimulator cells was not significant. Similarly the difference in susceptibility of target cells between ATF1011-treated and nontreated tumor cells was not significant. Thus, induction of CTLs in a syngeneic tumor-bearing host could be triggered by injection of ATF1011.

Discussion

Several mechanisms have been assumed on the antitumor activity of plant lectins [13, 14, 15], one of which is augmentation of host defence mechanisms. In this case, the target cells of lectin are hypothesized as T cell compartments [15], but the precise mechanisms of T cell activation are not clearly defined. In the present paper, we have presented the possibility that a plant lectin, ATF1011, augments antitumor immunity through activation of carrier lectin-specific T cells. Firstly, the binding nature of ATF1011 to various cells was analyzed. It became evident that ATF1011 binds to specific receptor sites on normal and malignant cells. The binding of ^{125}I-labeled ATF1011 showed saturation and was specifically inhibited by addition of cold ATF1011, suggesting the existence of receptor sites on these cells. It has been reported that ConA has direct cytotoxicity against tumor cells owing to specific binding sits on these cells, whereas most of such sites on normal cells are in a cryptic form [13, 14]. The binding sites of ATF1011 were thought to be expressed both on tumor cells and on normal cells. The number of binding sites and the affinity constants were not significantly different between normal and tumor cells. From these experiments, it was assumed that ATF1011 did not have tumor specific binding and cytotoxic activities like ConA [26]. In fact, growth of tumor cells was not affected by ATF1011 in the in vitro cytotoxic study. Further, pretreatment of Meth-A with ATF1011 did not reduce the ability of this cell to grow in the syngeneic host BALB/c mouse, whereas ConA completely diminished the transplantability (data not shown). From these data, it can be safely said that ATF1011 does not manifest direct cytotoxicity against tumor cells and normal lymphocytes.

Fujiwara et al. showed that virus-specific Th cells in the in vivo immunized host (with vaccinia virus) were reactive to viral antigens and they succeeded in augmentation of in vitro PFC responses [8]. Therefore we investigated the possibility that ATF1011 on the tumor cell has the potential to act as a carrier protein. To introduce antigenicity strong enough to be detected in the PFC test, MM102 tumor cells were chemically haptenized with TNP, and then coated with ATF1011. The TNP-MM102-ATF was injected i. p. in syngeneic C3H/HeN mice. After in vivo primary activation, splenocytes from these mice were secondarily activated in vitro using TNP-ATF or TNP-OVA as an antigen. As shown in Fig. 2, the activated Th cells were specific for ATF1011. The chemical covalent binding between the hapten molecule and the carrier protein was not necessarily required for activation of the carrier-specific Th cells and the coexistence of these two molecules on the same cell membrane was sufficient. The possibility of augmentation of CTL responses by activated Th cells specific for carrier ATF1011 was investigated, because not only Th cells in the antibody production but also Th cells in the CTL response were observed to be activated at the same time in the carrier-specific Th cell activation system [8]. Coating stimulator cells with ATF1011 manifested the potential to induce alloreactive CTL. In the effector phase, the presence or absence of ATF1011 on target cells did not change the susceptibility to effector CTL induced by ATF1011-treated alloantigen. These results indicated that ATF1011 augments the activation process of Th cells not only in B cell responses but also in alloreactive CTL responses. We could not detect ATF1011-specific CTLs in the alloreactive CTL response. The alloreactive CTL response may not be the desirable system for the investigation of CTL precursors specific for ATF1011, because, as reported by many investigators, hapten on the stimulator cell membrane may be recognized by CTL precursors in the H-2-restricted manner [26]. Our investigation supports the presence of ATF1011-specific CTLs in host immune system of tumor-bearing mice. When X-ray-irradiated syngeneic splenocytes coated with ATF1011 were used as stimulator cells, ATF1011-specific cytotoxicity was induced. Therefore, binding of ATF1011 on the tumor cell surface is though to be essential for the activation of these Th cells and CTLs. In the case of ATF1011 treatment, remarkably high tumor-specific cytotoxicity was induced by secondary activation of antigen-specific T cells. In the C3H-MM46 system, cytotoxicity to MM46 was induced when host splenocytes were reactivated with MM46 and cytotoxicity was detectable only by the ATF1011-treated splenocytes. This suggests that the injection of ATF1011 has the same carrier effect in the in vivo system as in vitro for the induction of cytotoxicity against MM46 tumor. In the case of MM102, cytotoxicity was also induced only in the ATF1011-treated host immune cells. The presence of ATF1011 on the target cells did not change the susceptibility of target cells to the effector CTLs in both MM46 and MM102 systems. Therefore it is presumed that induced CTLs are not specific for ATF1011 but specific for TATA expressed on the tumor cell surface.

In the in vivo application of ATF1011, antitumor immunity was induced effectively in syngeneic host-tumor systems. One of the mechanisms is thought to be activation of ATF1011-specific Th cells which results in the augmentation of antigen-specific and nonspecific immunity as was expected from in vitro augmentation of Th cells by ATF1011. Our hypothesis that binding of ATF1011 on the local tumor is essential for the activation of Th cells is not fully confirmed by these results of in vivo experiments. It is important, however, that local administration of ATF1011 is effective for the activation of systemic host antitumor immunity. In this case, several mechanisms other than local Th cell activation may be involved. One possibility is that contact of antigenic tumor cells with specific T cells is strengthened by ATF1011 as an agglutinin. This possibility is rather improbable, because the susceptibility of ATF1011-treated target cells to NK was not different from nontreated cells (data not shown). Moreover lectin-dependent cell-mediated cytotoxicity [2] was not de-

tected in the in vitro cytotoxic assay (data not shown). The second possibility is that ATF1011 injected locally into the tumor sites leaks out into the circulation and augments host immune responses systemically. But this possibility is also improbable, because i. v. or i. p. administration of ATF1011 was not effective (data not shown). The present paper proposed the possibility of tumor immunotherapy by activating Th cells specific for the plant lectin ATF1011. The possibility remains that these Th cells also produce other effectors which are effective in target tumor killing. The precise mechanisms and the optimum conditions for tumor immunotherapy by this lectin await further investigation.

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