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Antigen-presenting Cells Constitutively Bind Tumor Antigens in the Tumor-bearing State *in vivo* to Construct an Effective Immunogenic Unit

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Antigen-presenting cells (APC) constitutively process endogenous (self) proteins to bind the processed peptides to Ia molecules. In the present study, we investigated whether the same associative recognition also holds true for tumor-associated antigens (TAA) that are regarded as "self" molecules in tumor-bearing hosts. The following results were obtained: (i) an APC-depleted splenic T cell population from CSA1M tumor-immunized hosts was stimulated to produce interleukin 2 *in vitro* when co-cultured with APC from CSA1M-bearing mice, but not from normal mice; and (ii) a Thy-1$^+$ cell-depleted APC population from CSA1M-bearing mice could produce CSA1M tumor-specific protection *in vivo* when inoculated into naive syngeneic mice. These results provide evidence for the functional binding *in vivo* of TAA to APC in the tumor-bearing state. The results are discussed in the context of the paradox that tumor-bearers fail to reject their own malignancy despite the formation on APC of an effective immunogenic unit which is capable of stimulating tumor-specific T cells.

Key words: Tumor antigen — Antigen-presenting cell — CD4$^+$ T cell — Tumor-bearing state

In general, CD4$^+$ helper T cells (Th)[2] recognize processed antigen in association with class II major histocompatibility complex (MHC)-encoded molecules (Ia antigens) expressed on antigen-presenting cells (APC).[1-3] Thus, the role of APC in the activation of Th has been well established in the context of interaction between antigen and Ia on APC.[4-6] Ia antigens on APC have the capacity to bind to many different peptides that are generated by antigen processing.[7-9] Recent studies have also revealed that APC do not distinguish between self and non-self proteins and that Ia antigens on APC bind peptides derived from endogenous as well as exogenous proteins.[4-6, 10] Thus, it is increasingly evident that self proteins are picked up and processed by self APC and that self protein-derived autologous peptides constitutively occupy the antigen-binding sites of Ia molecules.[11, 12] We have addressed the question of whether APC are functioning to bind and present endogenously generated tumor-associated antigens (TAA) *in vivo* in the tumor-bearing state.

An APC-containing population was prepared by treating spleen cells from normal or CSA1M tumor-bearing BALB/c mice with anti-Thy-1.2 plus complement (C). Splenic T cells depleted of APC were also obtained by passing CSA1M tumor-immunized BALB/c spleen cells over a nylon wool column. CSA1M-immune T cells were cultured alone or together with the above two sources of APC-containing splenic fractions. Culture supernatants (SN) were examined for the production of interleukin 2 (IL-2). The results of Table I demonstrate that the anti-CSA1M purified T cell population can produce IL-2 without addition of CSA1M TAA to cultures when cultured with splenic APC from CSA1M-bearing mice, but not from normal mice.

To investigate whether the APC-containing population from CSA1M-bearing mice can stimulate tumor-specific T cells *in vivo*, normal BALB/c mice were given 5 rounds of inoculation with the APC-containing population at the dose of 4×10^6 cells/time, and challenged with viable CSA1M tumor cells. Fig. 1 shows the growth of CSA1M tumor cells that were inoculated for challenge one week after the final administration of the APC-containing population. The results (Fig. 1B) illustrate that an APC-containing population prepared from CSA1M-bearing mice is capable of producing anti-CSA1M protection without the requirement of pulsing *in vitro* with the relevant CSA1M TAA.

We next asked whether the above-described immune protection can be ascribed to APC contained in spleen cells. Thus, the effect of APC-depletion of spleen cell suspensions on *in vivo* protection was investigated. APC-depletion was performed by passing the spleen cells over

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[2] Abbreviations used in this paper: Th, helper T cells; APC, antigen-presenting cells; TAA, tumor-associated antigens; IL-2, interleukin 2; MHC, major histocompatibility complex; C, complement.

Table I. Capacity of APC from CSA1M-bearing Mice to Stimulate CSA1M-immune T Cells for the Production of IL-2

Responding spleen cells[a]	APC from mice[b]	IL-2 production (cpm)[c] Exp. 1	Exp. 2
Normal T	—	168 (1.06)	75 (1.13)
Normal T	Normal	139 (1.17)	972 (1.20)
Normal T	Tumor-bearing	162 (1.15)	1330 (1.01)
Immune T	—	652 (1.14)	225 (1.09)
Immune T	Normal	621 (1.40)	2299 (1.03)
Immune T	Tumor-bearing	9324 (1.01)	12760 (1.01)
—	Normal	67 (1.21)	ND
—	Tumor-bearing	81 (1.11)	ND

a) CSA1M-immune mice were prepared by resecting a tumor of CSA1M-bearing mice followed by viable CSA1M cell challenge, and used 10 days after the challenge. T cell population from normal or CSA1M-immune mice was prepared by passing spleen cells over a nylon wool column as described.[13]

b) To obtain an APC-containing population, spleen cells from normal or 7- to 8-wk CSA1M-bearing BALB/c mice were treated with anti-Thy-1.2 antibody plus complement (C) twice and 2000 R X-irradiated before use.

c) The T cell fraction (10^7/well) of normal or immune spleen cells was cultured alone or with APC-containing populations (10^5/well for Exp. 1 or 10^6/well for Exp. 2) in the absence of exogenous tumor antigens for 2 days in a 2-ml volume in 24-well culture plates. Culture supernatants were assessed for the ability to support the growth of IL-2-dependent CTLL-2 cell line as described.[17] ND, not detected.

Table II. Tumor-specific Protection Induced by Administration of APC-containing Population from Tumor-bearing Mice

APC from mice[a]	Challenged with tumor[b]	Incidence of tumor take	Mean tumor diameter (mm)
Normal		10/10	10.2 (1.03)
CSA1M-bearing	CSA1M	2/10	<3.0
Meth A-bearing		10/10	9.8 (1.07)
Normal		10/10	11.3 (1.04)
CSA1M-bearing	Meth A	10/10	10.4 (1.05)
Meth A-bearing		1/10	<3.0

a) BALB/c mice were inoculated s.c. with T cell-depleted, APC-containing splenic fraction (4×10^6 cells/mouse/time) from normal or indicated tumor-bearing BALB/c mice 5 times at 1 wk intervals.

b) One week after the final APC-inoculation, the mice were challenged s.c. with viable CSA1M (1×10^5) or Meth A (1×10^4) tumor cells. Incidence of tumor take and mean tumor diameter±SE were determined 40 days after the tumor cell challenge.

either a nylon wool column or two rounds of G-10 columns. The results indicate that protective immunity was provided only when the transferred cell population contained APC (compare Fig. 1 Panel B with Panels C and D). These results also confirm that this protection can not be ascribed to tumor-sensitized T cells that may be contaminating the spleen cells from tumor-bearing mice, because anti-tumor protection was not produced even in the T cell-enriched fraction obtained by passing over a nylon wool column.

To examine the tumor specificity of protective immunity induced by APC from tumor-bearing mice, APC-containing populations were prepared from CSA1M- and Meth A-bearing BALB/c mice. Normal BALB/c mice were immunized with either type of these APC-populations and challenged with homologous or heterologous tumor cells. The results (Table II) show that APC from CSA1M-bearing mice were capable of inducing protection against the challenge with CSA1M tumor cells but failed to produce any significant protection against the challenge with Meth A tumor cells. Likewise, inoculation of APC from Meth A-bearing mice resulted in selective induction of anti-Meth A immune protection, indicating that anti-tumor protective immunity induced with the use of APC from tumor-bearing mice is tumor-specific.

It has been documented that various suppressive mechanisms are generated and various types of immune dysfunction are induced during the tumor-bearing state. Recent studies from our laboratory have demonstrated selective immune dysfunction of the self Ia-restricted $CD4^+$ Th pathway.[13] Although this pathway involves participation of both $CD4^+$ Th and APC, our results revealed that such immune dysfunction was attributable to an intrinsic dysfunction at the level of $CD4^+$ Th rather than APC.[13] In addition to our previous observation of unimpaired APC function for processing of exogenous antigens, the present study demonstrated that APC in tumor-bearing hosts constitutively bind TAA and present them in native or processed forms to produce tumor-specific immune protection. Although the anti-tumor effect of APC would be obscured due to the immune dysfunction of $CD4^+$ Th in the tumor-bearing state, such an APC capacity was clearly illustrated when they were transferred into normal syngeneic mice free of the deleterious effects of the tumor-bearing state. This implies the importance of removal of the suppressive potential induced by the tumor-bearing state for the expression of the above APC effects on tumor immunity. Studies will also be required to investigate whether the binding of TAA to APC takes place in primary and clinical tumor systems as well as transplantable tumor models as used in this study.

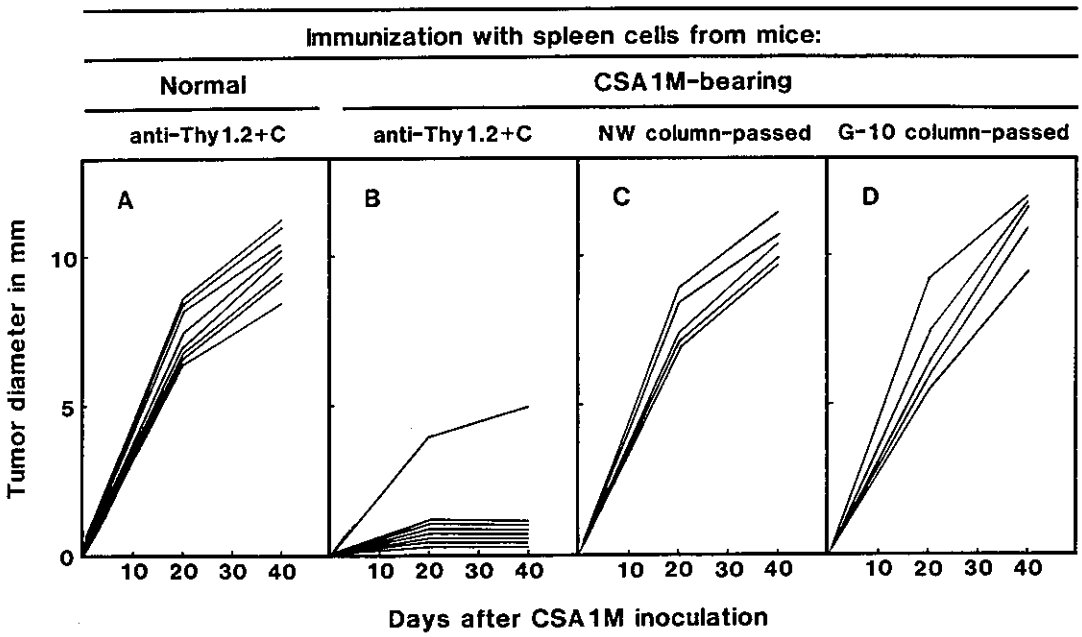

Fig. 1. Induction of anti-CSA1M immune protection by inoculation of a T cell-depleted, APC-containing splenic population from CSA1M-bearing mice. BALB/c mice were inoculated 5 times at one week intervals with the following splenic populations (4×10^6 cells/mouse): A, normal BALB/c spleen cells treated with monoclonal anti-Thy-1.2 antibody plus C twice (a Thy-1^+cell-depleted, APC-containing population); B, a Thy-1^+ cell-depleted, APC-containing population prepared from spleen cells of 7- to 8-wk CSA1M tumor-bearing BALB/c mice; C, spleen cells from CSA1M-bearing mice that were depleted of APC by passing over a nylon wool (NW) column[13]; D, spleen cells from CSA1M-bearing mice that were depleted of APC by two rounds of passages over Sephadex G-10 columns.[14] APC-depletion in populations C and D was confirmed as previously described.[17] Each line represents the data from one mouse.

We have recently established CSA1M-specific $CD4^+$ T cell lines that produce IL-2 and interferon-γ upon stimulation with CSA1M TAA. It was found that these T cell lines were stimulated with either CSA1M membrane in the presence of normal APC, APC pulsed *in vitro* with CSA1M membrane[14] or APC prepared from CSA1M-bearing mice and such stimulation was inhibited by addition to cultures of anti-CD4 or anti-$I\text{-}A^d$ antibody (J. Shimizu *et al.*, manuscript in preparation). These results are compatible with the notion that a form of TAA exists in association with MHC molecules on APC. Considerable progress has been made in analyzing the molecules that are bound to class II MHC antigens. Peptides constitutively bound to Ia molecules remain bound during the affinity purification of Ia, and such peptides (self proteins from normal APC or processed antigens from antigen-pulsed APC) can be separated on gel filtration and/or chromatography following an acid dissociation procedure.[11, 15, 16] By comparing Ia-binding molecules from normal APC and APC from tumor-bearing mice, it should be possible to isolate molecules specifically bound to Ia from tumor-bearing APC. Such an approach could contribute to the characterization of TAA or processed form(s) of TAA that are effective for priming tumor-specific T cells *in vivo*.

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