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Mediation of *in vivo* Tumor-neutralizing Activity by Lyt-2^+ as Well as L3T4^+ T Cell Subsets [*1]

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The present study reexamines the cell surface nature of T cells mediating *in vivo* protective tumor immunity with the use of anti-L3T4 and -Lyt-2 antibodies. C3H/HeN mice hyperimmune against syngeneic MH134 hepatoma or MCH-1-A1 fibrosarcoma were prepared by intradermal (id) inoculation of viable tumor cells followed by surgical resection of the tumor and by repeated challenges with viable tumor cells. Spleen cells from these mice were fractionated into L3T4^+ or Lyt-2^+ T cell subset by treatment with anti-Lyt-2 or -L3T4 antibody plus complement (C). Winn assays performed by utilizing such fractionated T cells have revealed that both L3T4^+ and Lyt-2^+ T cell subsets from hyperimmune mice produced complete tumor protection. Flow microfluorometry study illustrated that the treatment with anti-L3T4 or -Lyt-2 antibody plus C resulted in the complete isolation of L3T4^- Lyt-2^+ (Lyt-2^+) or L3T4^+ Lyt-2^- (L3T4^+) T cell subset, respectively. This contrasted with the failure of treatment with anti-Lyt-1 antibody plus C to isolate all T cells expressing Lyt-2 marker. It was further demonstrated that each subset of T cells exerted its anti-tumor effect in a tumor-specific way and without a requirement for the other alternative subpopulation of unprimed T cells. These results indicate that Lyt-2^+ T cell subset can be successfully isolated by treatment with anti-L3T4 but not with anti-Lyt-1 antibody plus C, and that each single subset of Lyt-2^+ and L3T4^+ T cells can function as *in vivo* effector T cells.

Key words: L3T4^+ T cell — Lyt-2^+ T cell — Anti-tumor effect

Several animal models have been developed in which the adoptive transfer of tumor-specific immune T cells results in the rejection of a subsequent challenge with tumor cells or the eradication of growing tumor cells pre-inoculated. These models provided insights into how appropriately selected and amplified T cells from the tumor-preimmunized host might be utilized to promote tumor eradication.

Analyses of the nature of effector cells acting on sarcomas and leukemias have suggested that cytotoxic T lymphocytes (CTL)[*4] are critically important for therapy.[1-3] Moreover, recent studies along this line have demonstrated that infusion of cloned CTL can mediate a therapeutic effect.[4-6] However, the administration of purified noncytolytic Lyt-1^+2^- T cells in the mouse, or their analog in the rat, has also been shown to be effective in specific therapy or prevention of chemically induced and virally induced sarcomas,[7] a spontaneous plasmacytoma,[8] chemically induced hepatoma,[9] and a virally induced leukemia,[10] and recent studies have demonstrated that these noncytolytic T cells mediate the anti-tumor effect without the participation of CTL.[8, 11] Thus, studies to determine the nature of the T cell subsets responsible for the observed anti-tumor effect of adoptively transferred T cells in animal models and the mechanisms by which the tumor is eliminated *in vivo* have yielded somewhat conflicting results.

Since anti-Lyt-1 antibody reacts with a large portion of Lyt-2^+ subset as well as most of the Lyt-2^- T cells, utilization of anti-Lyt-1

[*1] This constitutes Part I of a series entitled "Role of tumor-specific Lyt-2^+ T cells in tumor growth inhibition *in vivo*."
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[*4] Abbreviations used in this paper: CTL, cytotoxic T lymphocytes; id, intradermal or intradermally; C, complement; FITC, fluorescein isothiocyanate; FMF, flow microfluorometry.

antibody may fail to isolate all Lyt-2^+ T cells as a single population. In this context, the development of anti-L3T4 monoclonal antibody has made it possible to isolate the Lyt-2^+ T cell subset.[12,13] Therefore, with the use of anti-L3T4 and -Lyt-2 antibodies, we have reexamined the phenotype of T cells which function to initiate or mediate *in vivo* tumor growth-inhibition. The results demonstrated that the L3T4^+ T cell subset which was isolated by treatment with anti-Lyt-2 antibody plus complement and had been described as the Lyt-1^+2^- subset again produced potent tumor-protecting potential. Moreover, Lyt-2^+ T cell subset isolated by treatment with anti-L3T4 antibody plus complement, particularly from spleen cells of anti-tumor hyperimmune mice, also produced tumor growth inhibition. Since the efficacy of the Lyt-2^+ T cell subset was demonstrated in the MH134 tumor model in which tumor cells are not lysed by anti-tumor Lyt-2^+ T cells,[9] the results are discussed in the context of mechanisms by which each T cell subset exerts its *in vivo* anti-tumor effect.

MATERIALS AND METHODS

Mice and Tumors Female C3H/HeN mice, obtained from Charles River Laboratory, were used at 6–9 weeks of age. MH134 hepatoma and X5563 plasmacytoma, both derived from C3H/He strain and maintained by serial intraperitoneal transplantation, and MCH-1-A1 fibrosarcoma, which was recently induced in C3H/HeN strain in our laboratory by using methylcholanthrene, were utilized.

Immunization to MH134 Hepatoma or MCH-1-A1 Fibrosarcoma Cells C3H/HeN mice (7–8 weeks old) were inoculated intradermally (id) with 10^6 viable tumor cells, followed by the surgical resection of the tumor 7 days thereafter. Seven days after the tumor resection, mice were challenged id with 10^5 viable MH134 tumor cells. The mice which did not develop a tumor mass 3 weeks after the tumor challenge were regarded as "immune," and the mice which resisted two additional challenges with 10^5 and 10^6 viable MH134 tumor cells were used as "hyperimmune" mice.

Monoclonal Antibodies Monoclonal antibodies to Lyt-1.1, L3T4 or Lyt-2 antigens were used. Anti-Lyt-1.1 was purchased from New England Nuclear, Boston, MA. The GK1.5 monoclonal antibody-producing hybridoma line, specific for the L3T4 molecule, was a gift of Dr. F. Fitch, University of Chicago, IL, and the 3.155 monoclonal antibody-producing hybridoma line, specific for the Lyt-2 molecule, was obtained from ATCC, Rockville, MD.

The GK1.5 hybridoma cells were inoculated into pristane-primed BALB/c *nu/nu* mice intraperitoneally and ascitic fluids were collected. Gamma-globulin fractions of cell-free ascites were obtained by precipitation at 40% saturation with ammonium sulfate. Fluorescein isothiocyanate (FITC) was conjugated to GK1.5 monoclonal antibody by regular methods, followed by DE-52 (Whatman Biochemicals Ltd., England) ion-exchange chromatography. The F/P ratio of FITC/GK1.5 was 1.6. FITC-conjugated anti-Lyt-2 and anti-Thy1.2 antibodies were the products of Becton Dickinson Immunocytometry Systems, Mountain View, CA and of Bio Yeda Ltd., Rehovot, Israel, respectively.

Treatment of Immune Spleen Cells with Antibody plus Complement Spleen cells (10^8) from immunized mice were incubated at room temperature for 30 min with ascitic form of anti-Lyt-1.1, -L3T4 or -Lyt-2 monoclonal antibody at a dilution of 1:100 for anti-Lyt-1.1 or 1:50 for anti-L3T4 and -Lyt-2. Cells were washed and incubated at 37° for 45 min with rabbit complement (C) preabsorbed with syngeneic mouse spleen cells at a final dilution of 1/20.

Tumor-neutralization Test (Winn Assay) Spleen cells ($1–2\times10^7$) from normal or tumor-immunized mice were admixed with 10^5 viable tumor cells. The mixture was inoculated id in a volume of 0.1 ml into syngeneic C3H/HeN recipient mice, and tumor growth was expressed as the mean tumor diameter$\pm$SE of five mice/group.

Immunofluorescence Staining and Flow Microfluorometry (FMF) The preparation and staining procedures were essentially the same as described previously.[14] Briefly, 1×10^6 spleen cells were incubated at 4° for 30 min with FITC-conjugated antibodies, washed twice, resuspended and analyzed for fluorescence. These procedures were performed in Hanks' balanced salt solution (without phenol red) containing 0.1% bovine serum albumin (BSA) and 0.1% sodium azide. FMF analysis was performed by using a Spectrum III (Ortho Diagnostic Systems Inc., Raritan, NJ). All data were collected by using log amplification, and dead cells were rejected from analysis on the basis of forward light scattering.

Preparation of T Cell-depleted Mice Adult thymectomy of C3H/HeN mice was performed at 6 weeks of age, followed 2 days later by an ip inoculation of 0.25 ml of rabbit anti-thymocyte serum. Then 3 weeks later, the mice received whole body X-irradiation of 850 R and immediate iv reconstitution with 3×10^6 syngeneic adult bone marrow cells pretreated with anti-Thy-1.2 plus rabbit C.[8] These mice were used after an additional 3 weeks.

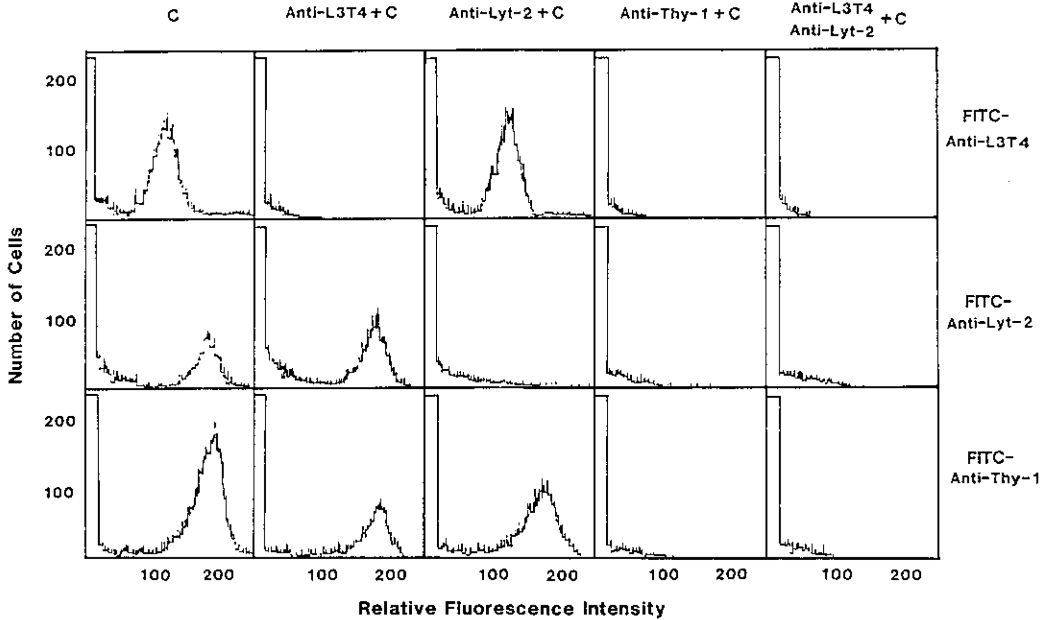

Fig. 1. Flow microfluorometric (FMF) study of spleen cells after treatment with anti-L3T4 or anti-Lyt-2 antibody plus complement (C). MH134-primed spleen cells were treated with various monoclonal antibodies (shown) plus C. Cells were stained with either anti-L3T4, anti-Lyt-2 or anti-Thy-1.2 monoclonal antibody conjugated with FITC.

T cell depletion in these mice was confirmed by fluorescence-activated cell sorter analysis and functional studies of lymphoid cells from these mice using T cell and B cell mitogens as previously described.[8, 9]

Results

We first examined the efficacy of GK1.5 (anti-L3T4) and 3.155 (anti-Lyt-2) monoclonal antibodies to separate the respective Lyt-2^+ and L3T4^+ T cell subsets and investigated the effect of treatment with anti-Lyt-1.1 antibody thus far utilized on these two T cell subsets. Spleen cells from MH134-hyperimmune mice were treated with either of the above monoclonal antibodies plus complement (C) and submitted to flow microfluorometric (FMF) analyses. The results of Fig. 1 demonstrate that a) L3T4^+ T cell subset as detected by FITC-conjugated anti-L3T4 antibody was isolated by treatment with anti-Lyt-2 plus C; b) likewise, Lyt-2^+ T cell subset was obtained by treatment with anti-L3T4 plus C and c) both subpopulations were eliminated

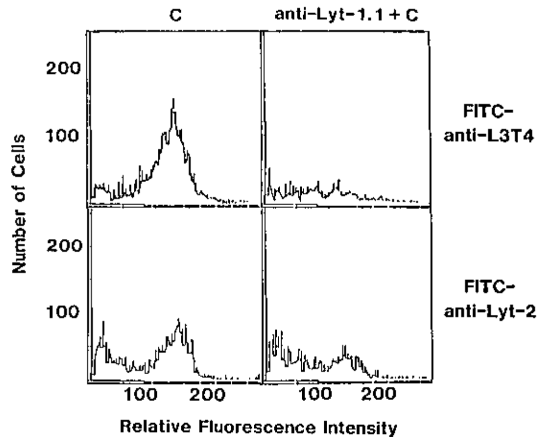

Fig. 2. FMF study of spleen cells after treatment with anti-Lyt-1.1 antibody plus C.

by treatment with either anti-Thy-1.2 or a mixture of anti-L3T4 and -Lyt-2 (cocktail killing) plus C. It should be noted that the treatment with anti-L3T4 or Lyt-2 antibody

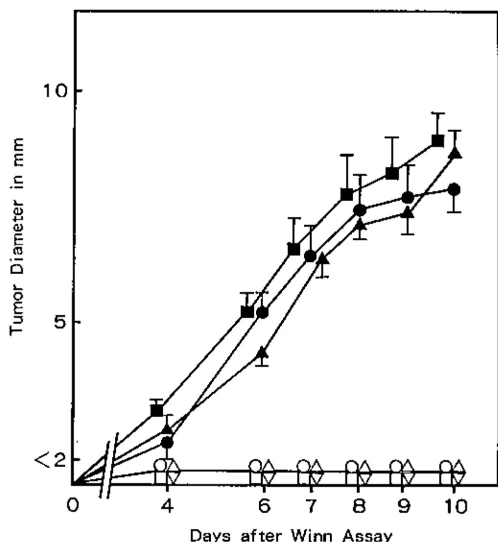

Fig. 3. Tumor-neutralizing activity of both L3T4⁺ and Lyt-2⁺ T cell subsets from MH134-hyperimmune mice. Spleen cells from MH134-hyperimmune mice were untreated (○) or treated with either C (△), anti-Lyt-2 +C (▽), anti-L3T4 +C (□), anti-Lyt-2 and -L3T4+C (■) or anti-Thy-1.2+C (▲). These fractionated spleen cells (10^7) or normal untreated spleen cells (●) were mixed with 10^5 viable MH134 tumor cells.

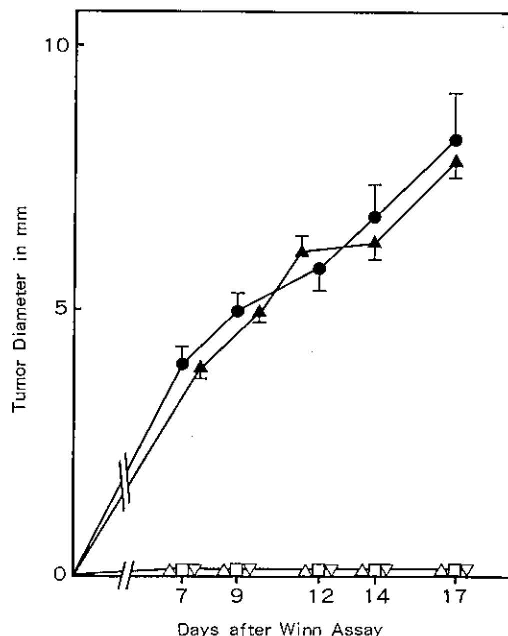

Fig. 4. Tumor-neutralizing activity of both L3T4⁺ and Lyt-2⁺ T cell subsets from MCH-1-A1-hyperimmune mice. Spleen cells from MCH-1-A1-hyperimmune mice were treated with either C (△), anti-Lyt-2+C (▽), anti-L3T4+C (□), or anti-Thy-1.2+C (▲). These fractionated spleen cells (10^7) or normal untreated spleen cells (●) were mixed with 10^5 viable MCH-1-A1 tumor cells.

results in only marginal damage to the respective Lyt-2⁺ or L3T4⁺ T cell subset.

In contrast, it was revealed that treatment with anti-Lyt-1.1 plus C not only resulted in almost complete elimination of L3T4⁺ T cells, but also elicited appreciable elimination of Lyt-2⁺ T cells (Fig. 2). Although L3T4⁻ Lyt-2⁺ T cells can be obtained, utilization of anti-Lyt-1.1 antibody failed to isolate all Lyt-2⁺ T cells as a single subpopulation. In the following experiments, anti-L3T4 and anti-Lyt-2 antibodies were, therefore, used to determine the nature of effector T cell mediating tumor cell eradication *in vivo.*

Portions of the same L3T4⁺ or Lyt-2⁺ T cells as used in Fig. 1 were tested for ability to neutralize the growth of viable MH134 tumor cells in Winn assays (Fig. 3). The results demonstrate that tumor protection was mediated by Lyt-2⁺ as well as L3T4⁺ T cell subsets from MH 134-hyperimmune mice. This was also the case in another syngeneic tumor model. Thus, both Lyt-2⁺ and L3T4⁺ T cell subsets from MCH-1-Al-hyperimmune mice produced complete tumor protection (Fig. 4). In addition, the results of Table I demonstrate that tumor-neutralizing activity mediated by either subset of T cells is tumor-specific.

In the preceding experiments, tumor-neutralizing activity of donor T cells was measured in normal recipient mice. Thus, whether participation of unprimed L3T4⁺ or Lyt-2⁺ T cells from hosts is required for the mediation of tumor rejection responses by donor type of immune T cells has not been determined. This was examined by preparing T cell-depleted mice and by utilizing these mice as recipient mice in Winn assay. As shown in Fig. 5, both L3T4⁺ and Lyt-2⁺ subsets from hyperimmune mice produced tumor protection in T cell-depleted recipient mice as completely as in normal recipient mice. Thus, these results indicate that either

Table I. Specificity of Tumor Neutralization Mediated by L3T4+ or Lyt-2+ T Cells from Hyperimmune Mice

Spleen cells[a] (subset)	Tumor cells	Tumor diameter[b] (mm)	
		day 7	day 11
Normal	MH134	6.6±0.8	11.3±0.6
MH134-hyperimmune (L3T4+)		3.0	3.0
MH134-hyperimmune (Lyt-2+)		3.0	3.0
Normal	X5563[c]	7.3±0.5	13.3±0.7
MH134-hyperimmune (L3T4+)		7.5±0.6	13.5±0.6
MH134-hyperimmune (Lyt-2+)		6.6±0.8	11.9±0.7

a) L3T4+ or Lyt-2+ T cell subset was prepared from MH134-hyperimmune spleen cells by treatment with anti-Lyt-2 or anti-L3T4 antibody plus C.
b) Spleen cells (10^7) were mixed with 10^5 viable tumor cells and the mixture was inoculated id into syngeneic recipient mice. Tumor growth was expressed as the mean tumor diameter±SE of 5 mice/group.
c) The growth of X5563 tumor cells was completely inhibited by anti-X5563 immunized spleen cells at the spleen: tumor cell ratio of 100:1.

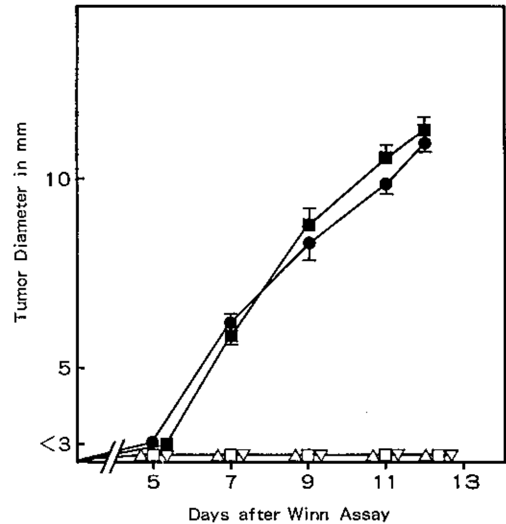

Fig. 5. Tumor-neutralizing activity of MH134-hyperimmune L3T4+ or Lyt-2+ T cells in T cell-depleted mice. A total of 10^7 normal spleen cells (●) or hyperimmune spleen cells treated with either C alone (△), anti-Lyt-2+C (▽), anti-L3T4+C (□) or anti-Lyt-2 and -L3T4+C (■) were mixed with 10^5 viable MH134 tumor cells and the mixture was inoculated into T cell-depleted recipient mice.

subset of T cells exerts its tumor-neutralizing effect under conditions in which the other alternative subset of unprimed T cells can not be recruited to participate in rejection responses.

Discussion

Despite ample evidence for the quintessential role of T lymphocytes in rejecting tumor cells as well as allografts,[15, 16] the effector mechanisms responsible for the graft rejection have been poorly understood. In fact, studies to determine the nature of the T cell subsets responsible for the mediation of *in vivo* antitumor effect in various animal tumor models have yielded somewhat conflicting results. Thus, many results have been presented to implicate CTL in the phenomenon of tumor or allograft rejection.[16, 17] On the other hand, it has become increasingly apparent that transfer of tumor immunity can be achieved with T cells bearing the markers of non-CTL, helper/delayed-type hypersensitivity (DTH) T cells (Lyt-1+2− T cells in the mouse).[7-10] Such findings in tumor rejection parallel the results of similar experiments in histoincompatible allograft rejection systems.[18-21] Although the authors of these studies have placed strong emphasis on the role of helper/DTH T cell subset in rejection responses, analysis of the specificity of monoclonal antibodies used for fractionation of T cell subsets has caused a reevaluation of the relative importance of this T cell subset[22] and a reexamination of the role of T cells bearing the classical CTL phenotype in the graft rejection.[23]

Treatment of lymphocytes with anti-Lyt-2 antibody plus C results in the isolation of Lyt-2− (Lyt-1+2−) T cell subset, whereas treatment with anti-Lyt-1 plus C fails to isolate all Lyt-2+ T cells since the anti-Lyt-1 antibody reacts with not only almost all Lyt-2− T cells but also an appreciable portion of Lyt-2+ T cells. However, the utilization of anti-L3T4 monoclonal antibody, recently developed,[12, 13] made it possible to obtain the whole of the

Lyt-2$^+$ T cell subset. This was also confirmed in the present study (Figs. 1 and 2), and these results thus prompted us to re-investigate the potential role of the Lyt-2$^+$ T cell subset obtained with the use of anti-L3T4 antibody.

The present study demonstrated that when anti-tumor Lyt-2$^+$ and L3T4$^+$ T cell subsets were obtained from splenic T cells of MH134 or MCH-1-Al-hyperimmune mice, both L3T 4$^+$ and Lyt-2$^+$ T cell subsets produced complete tumor protection. Thus, these observations confirm the efficacy of Lyt-2$^-$ T cell subset, which has been described as Lyt-1$^+$2$^-$ T cells [8-10] and is now designated as L3T4$^+$ Lyt-2$^-$ T cell subpopulation. In addition, the present data have revealed the ability of the Lyt-2$^+$ T cell subset to mediate tumor-neutralization as well.

Although the present analysis of the effector T cell nature has revealed the efficacy of both L3T4$^+$ and Lyt-2$^+$ T cell subsets as *in vivo* effector T cells in the present tumor models, the important issue would be to elucidate the cellular and molecular mechanisms by which each T cell subset exerts its anti-tumor effect *in vivo*. In this context, it would be of value to discuss such potential mechanisms based on our recent observations as well as previous results.

In most syngeneic tumor models, tumor-specific CTL activity can be detected in spleen or lymph node cells from tumor-immunized or tumor-regressed mice. Since the generation of CTL responses requires the participation of helper T cells expressing Lyt-1$^+$2$^-$ (L3T4$^+$ Lyt-2$^-$) T cell surface marker, tumor-specific L3T4$^+$ T cell subset could function as helper T cells assisting CTL responses against tumor cells.[24-27] Such an Lyt-1$^+$2$^-$ T cell-participating pathway leading to CTL generation might be visualized as operating for tumor protection in tumor models in which tumor antigens stimulate CTL responses. However, the operation of such a mechanism may be unlikely especially in the MH134 tumor model, since this tumor model is one in which anti-tumor CTL response is not detected.[9,26] In addition, the existence of the pathway distinct from that leading to CTL generation was substantiated by demonstrating the anti-tumor effect of this L3T4$^+$ T cell subset in T cell-depleted recipient mice possessing no CTL precursors.[8,9,28] This has been illustrated in several tumor models and it has been established that L3T4$^+$ Lyt-2$^-$ T cells exert their effect by producing various lymphokines such as macrophage activating factor (MAF) and by activating non-T cell effectors which function as ultimate tumoricidal effector cells.[29-31]

Concerning the function of Lyt-2$^+$ T cells, Lyt-2 antigen has originally been described to represent CTL phenotype. However, the ability of Lyt-2$^+$ T cells to function as helper cells in immune responses was also shown by Swain *et al.*[32,33] They demonstrated the helper activity of Lyt-2$^+$ T cells for histoincompatible B cells. Subsequently, it was shown by Singer and his colleagues that a subset of Lyt-2$^+$ T cells functions as helper cells for the activation of histocompatible CTL precursors in conventional class I allospecific CTL responses.[34] Moreover, the helper activity of these Lyt-2$^+$ T cells was confirmed by their ability to produce IL2.[35] Evidence has also emerged that portions of tumor-specific Lyt-2$^+$ T cells are capable of producing lymphokine(s).[36] In the FBL leukemia model, stimulation with FBL leukemia cells of tumor-immune T cells results in production of γ-interferon (γ-IFN), which renders macrophages capable of lysing FBL.

Thus, it is increasingly evident that in addition to the direct cytolytic function of Lyt-2$^+$ T cells as CTL, at least a part of Lyt-2$^+$ T cells is responsible for an anti-tumor mechanism similar to that observed for the Lyt-1$^+$2$^-$ (L3T4$^+$) T cell subset. This emphasizes the importance of understanding the mechanism by which each effector T cell subset exerts its anti-tumor effect, as well as mere identification of effector T cell phenotypes. Studies are in progress to determine whether the anti-tumor Lyt-2$^+$ T cell subset mediates its anti-tumor effect through the production of lymphokines capable of activating non-T cell anti-tumor effectors.

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REFERENCES

1) Mills, C. D. and North, R. J. Expression of passively transferred immunity against an established tumor depends on generation of cytolytic T cells in recipients. Inhibition by suppressor T cells. *J. Exp. Med.*, **157**, 1448–1460 (1983).
2) Rosenstein, M., Eberlein, T. J. and Rosenberg, S. A. Adoptive immunotherapy of established syngeneic solid tumors: role of T lymphoid subpopulations. *J. Immunol.*, **132**, 2117–2122 (1984).
3) Evans, R. Phenotypes associated with tumor rejection mediated by cyclophosphamide and syngeneic tumor-sensitized T lymphocytes: potential mechanisms of action. *Int. J. Cancer*, **33**, 381–388 (1984).
4) Dailey, M. O., Pillemer, E. and Weissman, I. L. Protection against syngeneic lymphoma by a long-lived cytotoxic T-cell clone. *Proc. Natl. Acad. Sci. USA*, **79**, 5384–5387 (1984).
5) Rosenstein, M. and Rosenberg, S. A. Generation of lytic and proliferative lymphoid clones to syngeneic tumor: *in vitro* and *in vivo* studies. *J. Natl. Cancer Inst.*, **72**, 1161–1165 (1984).
6) Yamasaki, T., Handa, H., Yamashita, J., Watanabe, Y., Namba, Y. and Hanaoka, M. Specific adoptive immunotherapy with tumor-specific cytotoxic T-lymphocyte clone for murine malignant gliomas. *Cancer Res.*, **44**, 1776–1783 (1984).
7) Fernandes-Cruz, E., Woda, B. A. and Feldman, J. D. Elimination of syngeneic sarcomas in rats by a subset of T lymphocytes. *J. Exp. Med.*, **152**, 823–841 (1980).
8) Fujiwara, H., Fukuzawa, M., Yoshioka, T., Nakajima, H. and Hamaoka, T. The role of tumor-specific Lyt-1^+2^- T cells in eradicating tumor cells *in vivo*. I. Lyt-1^+2^- T cells do not necessarily require recruitment of host's cytotoxic T cell precursors for implementation of *in vivo* immunity. *J. Immunol.*, **133**, 1671–1676 (1984).
9) Fukuzawa, M., Fujiwara, H., Yoshioka, T., Itoh, K. and Hamaoka, T. Tumor-specific Lyt-1^+2^- T cells can reject tumor cells *in vivo* without inducing cytotoxic T lymphocyte responses. *Transplant. Proc.*, **17**, 599–605 (1985).
10) Greenberg, P. D., Cheever, M. A. and Fefer, A. Eradication of disseminated murine leukemia by chemoimmunotherapy with cyclophosphamide and adoptively transferred immune syngeneic Lyt-1^+2^- lymphocytes. *J. Exp. Med.*, **154**, 952–963 (1982).
11) Greenberg, P. H., Kern, D. E. and Cheever, M. A. Therapy of disseminated murine leukemia with cyclophosphamide and immune Lyt-1^+2^- T cells. Tumor eradication does not require participation of cytotoxic T cells. *J. Exp. Med.*, **161**, 1122–1134 (1985).
12) Dialynas, D. P., Quan, Z. S., Wall, K. A., Pierres, A., Quintans, J., Loken, M. R., Pierres, M. and Fitch, F. W. Characterization of the murine T cell surface molecule, designated L3T4, identified by monoclonal antibody GK-1.5: similarity of L3T4 to the human Leu-3/T4 molecule. *J. Immunol.*, **131**, 2445–2451 (1983).
13) Dialynas, D. P., Wilde, D. B., Marrack, P., Pierres, A., Wall, K. A., Havran, W., Otten, G., Loken, M. R., Pierres, M., Kapler, J. and Fitch, F. W. Characterization of the murine antigenic determinant, designated L3T4a, recognized by monoclonal antibody GK1.5: expression of L3T4a by functional T cell clones appears to correlate primarily with class II MHC antigen-reactivity. *Immunol. Rev.*, **74**, 29–56 (1983).
14) Ogata, M., Shimizu, J., Tsuchida, T., Takai, Y., Fujiwara, H. and Hamaoka, T. Non-H-linked genetic regulation of cytotoxic responses to hapten-modified syngeneic cells. I. Non-H-2-linked Ir gene defect expressed on T cells is not predetermined at the stage of bone marrow cells. *J. Immunol.*, **136**, 1178–1185 (1986).
15) Cerottini, J. C. and Brunner, K. T. Cell-mediated cytotoxicity, allograft rejection and tumor immunity. *Adv. Immunol.*, **18**, 67–132 (1974).
16) Herberman, R. B., Holden, H. T., Varesio, L., Taniyama, T., Pucetti, P., Kirchner, H., Gerson, J., White, S., Keisari, Y. and Haskill, J. L. Immunologic reactivity of lymphoid cells in tumors. *Contemp. Top. Immunobiol.*, **10**, 61–78 (1980).
17) Wagner, H., Hardt, C., Heeg, K., Pfissenmaier, K., Solback, W., Barlett, R., Stoskinger, H. and Rollinghoff, M. T-T cell interactions during cytotoxic T lymphocyte (CTL) responses: T cell derived helper factor (interleukin 2) as a probe to analyze CTL responsiveness and thymic maturation of CTL progenitors. *Immunol. Rev.*, **51**, 215–225 (1980).
18) Leveland, B. E., Hogarth, P. M., Geredig, R. H. and Mckenzie, I. F. C. Cells mediating graft rejection in the mouse. I. Lyt-1 cells mediate skin graft rejection. *J. Exp. Med.*, **153**, 1044–1057 (1981).
19) Dallman, M. J. and Mason, D. W. Cellular mechanisms of skin allograft rejection in the

rat. *Transplant. Proc.*, **15**, 335–338 (1983).

20) Lowry, R. P., Gurley, K. E., Blackburn, J. and Forbes, R. D. C. Delayed type hypersensitivity and lymphocytotoxicity in cardiac allograft rejection. *Transplant. Proc.*, **15**, 343–346 (1983).

21) Tilney, N. L., Kupiec-Weglinshi, J. N., Heidecke, C. D., Lear, P. A. and Strom, T. B. Mechanisms of rejection and prolongation of vascularized organ allografts. *Immunol. Rev.*, **77**, 185–216 (1984).

22) Mason, D. W. and Morris, P. J. Effector mechanisms in allograft rejection. *Ann. Rev. Immunol.*, **4**, 119–145 (1986).

23) LeFrancois, L. and Bevan, M. J. A reexamination of the role of Lyt-2-positive T cells in murine skin graft rejection. *J. Exp. Med.*, **159**, 57–67 (1984).

24) Fujiwara, H., Yoshioka, T., Shima, J., Kosugi, A., Itoh, K. and Hamaoka, T. Helper T cells against tumor-associated antigens (TAA): preferential induction of helper T cell activities involved in anti-TAA cytotoxic and antibody responses. *J. Immunol.*, **136**, 2715–2719 (1986).

25) Kern, D. E., Klarnet, J. P., Jensen, M. C. V. and Greenberg, P. D. Requirement for recognition of class II molecules and processed tumor antigen for optimal generation of syngeneic tumor-specific class I-restricted CTL. *J. Immunol.*, **136**, 4303–4310 (1986).

26) Kosugi, A., Yoshioka, T., Suda, T., Sano, H., Takahama, Y., Fujiwara, H. and Hamaoka, T. The activation of L3T4 helper T cells assisting the generation of anti-tumor Lyt-2 cytotoxic T lymphocytes: requirement of Ia-positive antigen-presenting cells for processing and presentation of tumor antigens. *J. Leucocyte Biol.*, **42**, 632–641 (1987).

27) Yamamoto, H., Takata, M. and Fujimoto, S. The role of L3T4-positive T lymphocytes in the generation of anti-tumor immunity in the mouse. *Jpn. J. Cancer Res. (Gann)*, **78**, 176–184 (1987).

28) Shimizu, Y., Fujiwara, H., Ueda, S., Wakamiya, N., Kato, S. and Hamaoka, T. The augmentation of tumor-specific immunity by virus-help. II. Enhanced induction of cytotoxic T lymphocyte and antibody responses to tumor antigens by vaccinia-reactive helper T cells. *Eur. J. Immunol.*, **14**, 839–843 (1984).

29) Fujiwara, H., Takai, Y., Sakamoto, K. and Hamaoka, T. The mechanism of tumor growth inhibition by tumor-specific Lyt-1^+2^- T cells. I. Anti-tumor effect of Lyt-1^+2^- T cells depends on the existence of adherent cells. *J. Immunol.*, **135**, 2187–2191 (1985).

30) Sakamoto, K., Fujiwara, H., Nakajima, H., Yoshioka, T., Takai, Y. and Hamaoka, T. The mechanism of tumor growth inhibition of tunor-specific Lyt-1^+2^- T cells. II. Requirements of adherent cells for activating Lyt-1^+2^- T cells as well as for functioning as antitumor effectors activated by factor(s) from Lyt-1^+2^- T cells. *Jpn. J. Cancer Res. (Gann)*, **77**, 1142–1152 (1986).

31) Nakajima, J., Fujiwara, H., Takai, Y., Izumi, Y., Sano, S., Tsuchida, T. and Hamaoka, T. Studies on macrophage-activating factor (MAF) in anti-tumor immune responses. I. Tumor specific Lyt-1^+2^- T cells are required for producing MAF able to generate cytolytic as well as cytostatic macrophages. *J. Immunol.*, **135**, 2199–2205 (1985).

32) Swain, S. L. and Panfili, P. R. Helper cells activated by allogeneic H-2K or H-2D differences have Ly phenotype distinct from those responsive to I differences. *J. Immunol.*, **122**, 383–391 (1979).

33) Swain, S. L., Bakke, A., English, M. and Dutton, R. W. Ly phenotype and MHC recognition: the allohelper that recognizes K or D is a mature Ly123 cell. *J. Immunol.*, **123**, 2716–2724 (1979).

34) Mizuochi, T., Golding, H., Rosenberg, A. S., Glimcher, L. H., Malek, T. R. and Singer, A. Both L3T4$^+$ and Lyt-2$^+$ helper T cells initiate cytotoxic T lymphocyte responses against allogeneic major histocompatibility antigens but not against trinitrophenyl-modified self. *J. Exp. Med.*, **162**, 427–443 (1985).

35) Mizuochi, T., Ono, S., Malek, T. R. and Singer, A. Characterization of two distinct primary T cell populations that secrete interleukin 2 upon recognition of class I or class II major histocompatibility antigens. *J. Exp. Med.*, **163**, 603–619 (1986).

36) Greenberg, P. D. Effector mechanisms by which adoptively transferred T cells promote tumor eradication. *In* "Cellular Immunotherapy of Cancer," ed. R. P. Gale, R. L. Truitt and M. M. Bortin (1987), in press. Alan R Liss Inc., New York.