Cutting Edge: CD4\(^+\) T Cell Control of CD8\(^+\) T Cell Reactivity to a Model Tumor Antigen

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Neoantigens resulting from the inherent genomic instability of tumor cells generally do not trigger immune recognition. Similarly, transfection of tumors with model Ags often fails to elicit CD8$^+$ T cell responses or alter a tumor’s growth rate or lethality. We report here that the adoptive transfer of activated Th1-type CD4$^+$ T cells specific for a model tumor Ag results in the de novo generation of CD8$^+$ T cells with specificity to that Ag and concomitant tumor destruction. The anti-tumor effects of the CD4$^+$ T cells required the presence of both MHC class I and class II on host cells, as evidenced by experiments in knockout mice, suggesting that CD4$^+$ T cells enhanced the ability of host APC to activate endogenous CD8$^+$ T cells. These results indicate that the apparent inability of tumor cells expressing highly immunogenic epitopes to activate tumor-specific CD8$^+$ T cells can be altered by activated CD4$^+$ T cells. The Journal of Immunology, 2000, 164: 562–565.

Most tumor cells are characterized by genetic instability, which results in deletions and additions to the tumor genome (1) and leads to the generation of new tumor Ags. These neoantigens often fail to elicit immune recognition and tumor destruction. In mouse models, tumor cells have been transfected with genes encoding a variety of highly immunogenic Ags with no change in the tumor’s growth rate or lethality. These Ags include OVA (2), glycoprotein from lymphocytic choriomeningitis virus (3), nucleoprotein (4), and hemagglutinin (5) from influenza A virus and β-galactosidase (β-gal)$^2$ from Escherichia coli (6).

CD8$^+$ T cells have long been thought to play a central role as immune effectors in the tumor setting. However, while LacZ-transfected tumor cell lines can be recognized by β-gal-specific CD8$^+$ T cells, they do not elicit β-gal-specific CD8$^+$ T cells. Why is this the case? β-Gal is large (1023 aa) and contains many potential immunogenic epitopes. Ag-specific cytolytic responses are efficiently elicited after immunization of mice with a variety of different recombinant viruses encoding LacZ (7).

CD4$^+$ T cells may serve to control the activation and persistence of the CD8$^+$ T cell response, because they play a major role in the induction of autoimmune disease and in the orchestration of antiviral immunity (8–11). CD8$^+$ T cells specific for viral Ags have been found to be inhibited under conditions in which CD4$^+$ T cell help was lacking (12). To explore the impact of CD4$^+$ T cells on Ag-specific CD8$^+$ T cells in the tumor setting, we generated a β-gal-specific, IL-2-secreting, Th1-type CD4$^+$ T cell clone, B12, and used a LacZ-transduced tumor, WP4.β-gal, as a target. Using this experimental system, we address the question: What are the effects of Ag-specific CD4$^+$ T cells on the function of endogenous, Ag-specific CD8$^+$ T cells?

Materials and Methods
Generation of CD4$^+$ T cell clones
C57BL/6 mice (National Cancer Institute, Frederick, MD) were immunized with a vaccinia virus expressing β-gal (13). Three weeks later, spleens were harvested and CD4$^+$ cells were isolated by passage over a negative selection column (R&D Systems, Minneapolis, MN). CD4$^+$ T cells were plated at 8 × 10$^5$ cells/well in 24-well plates in complete media (6). Irradiated syngeneic splenocytes pulsed with 100 µg/ml of β-gal protein (Sigma, St. Louis, MO) were added at 2 × 10$^5$ cells/well every 2 wk. Recombinant IL-2 (Chiron, Emeryville, CA) was added on day 3 of culture at 5 CU/ml. Reactivity after limiting dilution cloning was assessed by incubation of 2 × 10$^5$ CD4$^+$ T cells with 2 × 10$^3$ splenocytes pulsed with 100 µg/ml β-gal protein. Supernatants were evaluated for the presence of IL-4 and IL-2 by ELISA (Endogen, Woburn, MA). Control CD4$^+$ T cell clones were similarly generated from mice immunized with OVA protein (Sigma) in IFA. FACS analysis was performed on the T cell clone using anti-CD4 (L3T4) and anti-CD8 (Ly-2) Abs (PharMingen, San Diego, CA). Stained cells were analyzed on a FACScan (Becton Dickinson, Mountain View, CA).

For Ab blocking experiments, 10$^5$ effectors were incubated with 1 × 10$^3$ splenocytes in the presence of 20 µg/ml of Abs specific for I-A$^b$ (M5/114.15.2), CD4 (L3T4), or MHC class I (28-6-8) (PharMingen). After 30 min, 100 µg/ml of OVA or β-gal protein was added. Supernatants were assayed for the presence of IL-2 by ELISA (Endogen).

Tumors
The weakly immunogenic, methylcholanthrene-induced MCA-205 fibrosarcoma and a clone of this line, WP4.WT, have been described previously (14). WP4.WT was retrovirally transduced with an LSXN-based retrovirus encoding the LacZ gene driven by a long terminal repeat to produce the WP4.β-gal line, which was cloned by limiting dilution and selected for high expression of β-gal as assessed by 5-bromo-4-chloro-3 indolyl β-galactoside (X-Gal) staining.

In vivo experiments
A total of 5 × 10$^3$ tumor cells were injected i.v., and CD4$^+$ T cells were transferred i.v. 3 days later. Mice were sacrificed on day 14–16 after tumor.
I-A\textsuperscript{b} restricted as evidenced by block -

B12 clone was CD4 dependent and

confirmed to be CD4

ing experiments.

Clones obtained by lim-

iting dilution of a \( \beta \)-gal-specific, CD4\textsuperscript{+}, Th1-type clone. A. Clones obtained by limit-

ing dilution of a \( \beta \)-gal-specific, CD4\textsuperscript{+} bulk line were assayed for re-

lease of IL-4 (top) or IL-2 (bottom) when cocultured in the presence of splenocytes pulsed with OVA or \( \beta \)-gal protein. B. The B12 clone was con-

firmed to be CD4\textsuperscript{+} by FACS analysis. C. \( \beta \)-gal-specific IL-2 secretion by the

B12 clone was CD4\textsuperscript{+} dependent and I-A\textsuperscript{b} restricted as evidenced by blocking

experiments.

Results and Discussion

Generation of an experimental tumor system using \( \beta \)-gal as a model Ag

To study the effects of Ag-specific CD4\textsuperscript{+} T cells on the function of endogenous, Ag-specific CD8\textsuperscript{+} T cells, we generated a \( \beta \)-gal specific, CD4\textsuperscript{+} T cell population from mice immunized with a vaccinia virus containing the LacZ gene. After enrichment for CD4\textsuperscript{+} T cells, splenocytes were restimulated in vitro with \( \beta \)-gal protein. The \( \beta \)-gal-specific T cell line was cloned by limiting di-

lution, yielding two types of specific clones: those that secreted pre-

dominantly Th2-type cytokines (Fig. 1A).

One Th1-type CD4\textsuperscript{+} (Fig. 1B) clone, B12, produced IL-2 and IFN-\( \gamma \), but not IL-4 or IL-10, and grew continuously for over 9 mo, allowing for further study. Ab-blocking experiments showed that the \( \beta \)-gal reactivity was class II restricted and CD4\textsuperscript{+} protein.

The \( \beta \)-gal-specific T cell line was cloned by limiting di-

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dominantly Th1-type cytokines, and those that secreted pre-

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One Th1-type CD4\textsuperscript{+} (Fig. 1B) clone, B12, produced IL-2 and IFN-\( \gamma \), but not IL-4 or IL-10, and grew continuously for over 9 mo, allowing for further study. Ab-blocking experiments showed that the \( \beta \)-gal reactivity was class II restricted and CD4\textsuperscript{+} protein.

CD4\textsuperscript{+} T cell clone mediates regression of \( \beta \)-gal-expressing tumor in vivo: requirement for host MHC class I and class II molecules

To evaluate if the B12 \( \beta \)-gal-specific, CD4\textsuperscript{+} T cell clone could mediate tumor destruction, the WP4,\( \beta \)-gal tumor was injected i.v. and allowed to establish for 3 days. Groups treated with the B12 clone had significantly fewer pulmonary nodules than untreated controls at doses as low as 1 \( \times \) 10\textsuperscript{5} cells in titering experiments (data not shown). Treatment of WP4,\( \beta \)-gal with 3 \( \times \) 10\textsuperscript{5} B12 clone significantly reduced the average number of lung nodules in repeated experiments (for example from 85 to 17 in Fig. 2A or 174 to 6 in Fig. 2D). The presence of host CD8\textsuperscript{+} cells was required for this effect (Fig. 2B). To determine the specificity of the observed tumor treatment, the parental WP4.WT was included in the same experiment. No therapy was observed in mice bearing the parental tumor, indicating that tumor expression of \( \beta \)-gal was required (Fig. 2C). Adoptive transfer of a control Th1-type CD4\textsuperscript{+} T cell clone specific for OVA had no effect on the \( \beta \)-gal expressing tumor (not shown).

No specific release of IL-2 was observed upon coculture of the CD4\textsuperscript{+} B12 clone with tumor. As is characteristic of mouse sarco-

mas, the WP4.WT and WP4,\( \beta \)-gal tumor cell lines stained positive for MHC class I but negative for MHC class II (not shown), even after treatment with IFN-\( \gamma \). In contrast, the expression of I-A\textsuperscript{b} was up-regulated on the B16 melanoma control cell line after incubation with IFN-\( \gamma \), as previously shown (16).

CD4\textsuperscript{+} T cells are known to interact with MHC class II-express-

ing host cells, especially dendritic cells (DC), altering their ability to activate other components of the immune system. To assess whether the presence of host MHC class I and MHC class II was required for tumor eradication after adoptive transfer of the CD4\textsuperscript{+} B12 clone, we performed experiments using the WP4,\( \beta \)-gal tumor in KO mice with homozygous deficiencies in MHC class I but negative for MHC class II (not shown).

Injection, lungs were removed and stained with india ink, and lung nodules were counted in a blinded fashion. In Ab-depletion experiments, mice were given the mAb 2.43 as described (2). Depletion was confirmed by FACS analysis. Class II knockout (KO) and \( \beta_2 \)-microglobulin (\( \beta_2\)-m) KO mice (C57BL/6TacBR[KO]AP\textsuperscript{N5} and C57BL/6GhpTacBR[KO]\( \beta_2\)-m\textsuperscript{N5}) and their respective controls are on a B6 background and were obtained from Taconic (Germantown, NY).

To generate CD8\textsuperscript{+} cultures, mice were inoculated with tumor cells then treated with 3 \( \times \) 10\textsuperscript{5} CD4\textsuperscript{+} T cells 3 days later. Fifteen days later, spleen lymphocytes were harvested and cultured in the presence of tumor (not shown).

The B12 clone was con-
a requirement for both MHC class I and MHC class II expression by host cells.

Antitumor effect of CD4\(^+\) T cells is through an indirect mechanism involving the recruitment of host Ag-specific CD8\(^+\) T cells

APC, such as DC, can cross-present shed \(\beta\)-gal tumor Ag in the context of both MHC class I and class II molecules. The interaction of a host APC with Ag-specific CD4\(^+\) T cells can make it capable of stimulating naive, endogenous CD8\(^+\) cytotoxic T cells, which may then function as specific effector cells capable of lysing tumor. CD8\(^+\) T cells were important effector cells in our model because depletion of CD8\(^+\) cells (B). Ag specificity was determined by treatment of mice bearing either the WP4.WT (C) or the WP4.\(\beta\)-gal tumor (D–F). Identically treated \(\beta_2m\) KO mice and MHC class II KO mice are shown in E and F, respectively (\(p_1 = 0.007\) in A and \(p_2 = 0.008\) in D, no treatment vs treatment with B12, as assessed by the Kruskal-Wallis test).

FIGURE 2. Adoptive transfer of the B12 clone results in eradication of pulmonary nodules established for 3 days. Mice were given \(3 \times 10^5\) B12 clone 3 days after i.v. injection of tumor. Pulmonary nodules were enumerated in a coded, blinded fashion 14–16 days later in repeated experiments. Representative experiments are shown. The ability of the B12 clone to mediate tumor regression was assessed in wild-type mice (A) and in mice that were depleted of CD8\(^+\) cells (B). Ag specificity was determined by treatment of mice bearing either the WP4.WT (C) or the WP4.\(\beta\)-gal tumor (D–F). Identically treated \(\beta_2m\) KO mice and MHC class II KO mice are shown in E and F, respectively (\(p_1 = 0.007\) in A and \(p_2 = 0.008\) in D, no treatment vs treatment with B12, as assessed by the Kruskal-Wallis test).

FIGURE 3. De novo generation of \(\beta\)-gal-reactive CD8\(^+\) T cells occurs only in mice treated by adoptive transfer of B12 clone. A. Spleens from mice bearing WP4.WT or WP4.\(\beta\)-gal tumor and treated with \(3 \times 10^5\) B12 clone or with nothing were removed 12 days after treatment and restimulated for 6 days with the MHC class I-restricted \(\beta\)-gal\(_{96-103}\) peptide or control peptide. Splenocytes were then cultured for 24 h with \(\beta\)-gal\(_{96-103}\) or control peptide. Stimulation index was calculated by dividing the amount of Ag-specific IFN-\(\gamma\) released by nonspecific release. B. Mice receiving WP4.\(\beta\)-gal tumor were treated with B12 clone or an OVA-reactive clone. Twelve days later, spleens were removed, restimulated, and assayed as above. C. Class II KO or wild-type mice bearing WP4.\(\beta\)-gal were treated with B12 clone or with nothing. Spleens were removed, restimulated, and assayed as above. The results shown are the averages from three independently performed experiments in A and C and two independently performed experiments in B.
β-gal-specific CD8+ T cells (Fig. 3B). These findings are consisten-
tent with experiments described above in which we failed to ob-
serve a reduction of WP4.β-gal nodules in mice treated with OVA-
specific CD4+ T cells. Finally, no specific CD8+ T cells were
induced after transfer of the B12 clone to MHC class II KO mice
(Fig. 3C). This finding correlated with the failure of the B12 clone
to eradicate tumor in MHC class II KO mice (see Fig. 2).

Implications for immune activation of anti-tumor CD8+ T cells

The data presented here point to an active cooperation between
CD4+ and CD8+ T cells in the eradication of tumor cells. The
adoptive transfer of CD4+ T cells has been reported to treat estab-
lished tumor (17–19); however, CD4+ T cells in these systems
were hypothesized to act through NK or macrophage effector cells
or by direct lysis of a MHC class II-positive tumor. Ossendorp et
al. have found that generation of specific CD4+ T cells through
immunization with a helper epitope resulted in increased anti-
tumor activity that is mediated by CD8+ effector cells, even when
the tumor cells used are MHC class II negative (20). The present
manuscript is the first in which the transfer of CD4+ T cells spe-
cific for a model tumor Ag have been found to elicit the de novo
generation of CD8+ T cells specific for that same Ag.

CD8+ T cells have been widely reported to transfer tumor im-
munity and to treat established tumors upon adoptive transfer (21,
22). They are thought to work by directly destroying tumor cells in
the environment of the tumor, where help is limiting and inflamma-
tory cytokine interactions. In contrast, adoptive transfer of CD4+
T cells to treat established tumors in this model system suggests
that these DC are then unable to activate naive CD8+ T cells, important mediators
of tumor destruction. Provision of Ag-specific CD4+ T cells by adop-
tive transfer or by activation in vivo after vaccination may result in
the de novo induction of CD8+ T cell function in patients with
cancer.

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References
1994. Insertion signal sequence fused to minimal peptides elicits specific CD8+
T-cell responses and prolongs survival of thymoma-bearing mice. Cancer Res.
54:4155.
3. Speiser, D. E., R. Miranda, A. Zakarian, M. F. Bachmann, K. McCall-Faienza,
tigens expressed by solid tumors do not efficiently stimulate naive or activated
expression confer antigen-presenting cell capacity to tumors in vivo? J. Exp.
Med. 183:769.
5. Staveley-O'Carroll, K., E. Sotomayor, J. Montgomery, I. Borrell, L. Hwang,
USA 95:1178.
and N. P. Restifo. 1995. Active immunotherapy of cancer with a nonreplicating re-
combinant fowlpox virus encoding a model tumor-associated antigen. J. Immu-
noI. 154:4685.
8. Mattoullis, M., R. J. Conception, and R. Ahmed. 1994. CD4+ T cells are
required to sustain CD8+ cytotoxic T-cell responses during chronic viral infec-
10. Overwijk, W. W., D. S. Lee, D. R. Surman, K. R. Irvine, C. E. Touloukian,
Vaccination with a recombinant vaccinia virus encoding a “self” antigen induces
autimmune vitiligo and tumor cell destruction in mice: requirement for CD4+ T
11. Kurts, C., F. R. Carbone, M. Barnard, E. Blaney, R. W. Heath, and
J. F. Miller. 1997. CD4+ T cell help impairs CD8+ T cell deletion induced by
cross-presentation of self-antigens and favors autoimmune. J. Exp. Med. 186:
2057.
D. T. Man, and R. Ahmed. 1998. Viral immune evasion due to persistence of
activated T cells without effector function. J. Exp. Med. 188:2205.
transduced with the gene for tumor necrosis factor α: evidence for paracrine
of a Kb-restricted CTL epitope of beta-galactosidase: potential use in develop-
17. Kalm, M., H. Sugawara, P. McGowan, K. Okuno, S. Nagoya, K. E. Hellstrom,
I. Hellstrom, and P. Greenberg. 1994. CD4+ T cell clones specific for the human
p97 melanoma-associated antigen can eradicate pulmonary metastases from a
adoptive transfer of CD4+ anti-tumor T cells requires tumor expression of cell
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.
T helper cell requirement for optimal induction of cytotoxic T lymphocytes
against major histocompatibility complex class II negative tumors. J. Exp.
Med. 188:317.
transfer of cytotoxic T lymphocytes and by vaccination with minimal essential
22. Overwijk, W. W., A. Tsung, K. R. Irvine, T. J. Goletz, K. Tsung, M. W. Carroll,
C. Liu, B. Moss, S. A. Rosenberg, and N. P. Restifo. 1998. gp100/pmel 17 is a murine tumor rejection antigen: induction of “self”-reactive,
tumoridical T cells using high-affinity, altered peptide ligand. J. Exp. Med.
188:2277.
1998. T-cell help for cytotoxic T lymphocytes is mediated by CD40-CD40L
be a temporal bridge between a CD4+ T-helper and a T-killer cell. Nature 393:
474.
25. Celli, M., D. Scheidegger, K. Palmer-Lehmann, P. Lane, A. Lanavecchia,
and G. Alber. 1996. Ligation of CD40 on dendritic cells triggers production of high
levels of interleukin-12 and enhances T cell stimulatory capacity: T-T help via
and G. Schuler. 1996. High level IL-12 production by murine dendritic cells:
upregulation via MHC class II and CD40 molecules and downregulation by IL-4
Med. 184:741.