

CUTTING EDGE

Cutting Edge: CD8⁺ Effector T Cells Reject Tumors by Direct Antigen Recognition but Indirect Action on Host Cells¹Thomas Schüler^{2*} and Thomas Blankenstein^{*†}

CD8⁺ effector T cells recognize malignant cells by monitoring their surface for the presence of tumor-derived peptides bound to MHC class I molecules. In addition, tumor-derived Ags can be cross-presented to CD8⁺ effector T cells by APCs. IFN- γ production by CD8⁺ T cells is often critical for tumor rejection. However, it remained unclear whether 1) CD8⁺ T cells secrete IFN- γ in response to Ag recognition on tumor cells or APCs and 2) whether IFN- γ mediates its antitumor effect by acting on host or tumor cells. We show in this study that CD8⁺ effector T cells can reject tumors in bone marrow-chimeric mice incapable of cross-presenting Ag by bone marrow-derived APCs and that tumor rejection required host cells to express IFN- γ R. Together, CD8⁺ effector T cells recognize Ag directly on tumor cells, and this recognition is sufficient to reject tumors by IFN- γ acting on host cells. *The Journal of Immunology*, 2003, 170: 4427–4431.

The generation of T cell-dependent tumor immunity is impaired in IFN- γ -deficient (IFN- γ ^{-/-}) (1, 2) and IFN- γ R-deficient (IFN- γ R^{-/-}) mice (3, 4), suggesting that T cells have to secrete IFN- γ to allow subsequent tumor rejection. To secrete IFN- γ , CD8⁺, and CD4⁺ T cells must recognize peptides bound to MHC class I or class II molecules, respectively. MHC class II-negative tumors can be rejected by CD4⁺ T cells in an IFN- γ -dependent fashion (1, 3, 5) suggesting that, in the effector phase, MHC class II⁺ APCs present tumor-derived Ags to CD4⁺ T cells that subsequently induce tumor rejection. If exogenous Ags are presented via MHC class I, this process is termed cross-presentation (6). Cross-presentation is mediated by bone marrow (BM)³-derived APCs and is often critical during the priming phase of an antitumor immune response when naive, tumor-specific CD8⁺ T cells are activated (7). Despite numerous reports supporting the requirement for cross-presentation during antitumor CD8⁺ T cell priming, it is unknown whether cross-presentation is also required in the effector phase. Tumor Ags are cross-presented

by APCs within tumors (8, 9) and tumor-draining lymph nodes (10–12). Because tumors sometimes express low levels of MHC class I, it appeared likely that cross-presentation, in addition to the priming phase, might also be required in the effector phase to provide CD8⁺ T effector cells with sufficient peptide-MHC class I complexes to activate them.

In addition to the question of whether Ag cross-presentation is necessary for tumor rejection in the effector phase, it is unclear whether IFN- γ mediates its antitumor effect directly via tumor cells or indirectly via host cells. To answer these questions, we injected B16-OVA melanoma cells, which express chicken OVA as surrogate tumor Ag, into IFN- γ R^{-/-}, IFN- γ ^{-/-} mice and BM-chimeric mice, the latter being unable to cross-present the OVA-derived peptide OVA_{257–264} (SIINFEKL) via BM-derived APCs. B16 cells were chosen, because they express a very low amount of MHC class I molecules and should reveal the necessity of Ag cross-presentation for tumor rejection in the effector phase. Three days after tumor cell inoculation, in vitro-activated OVA_{257–264}-specific CD8⁺ T cells from RAG1-deficient OT-I mice (OT-I \times RAG1^{-/-}) were transferred to tumor-bearing mice to measure tumor rejection. We show in this study that CD8⁺ T cell-mediated tumor rejection required IFN- γ R expression on host cells and was independent of host IFN- γ . Additionally, tumor rejection did not require cross-presentation of OVA by BM-derived APCs. Our data indicate that CD8⁺ T cells, although recognizing Ag on tumor cells, mediate tumor rejection, at least in part, by IFN- γ acting on host cells.

Materials and Methods

Mice

C57BL/6J (B6), IFN- γ ^{-/-} (B6.129S7-Ifng^{tm1TS}), IFN- γ R^{-/-} (B6.129S7-IfngR^{tm1Ag}), and bm1 mice (B6.C-H2^{bm1}/ByJ) (all on the C57BL/6 background) were purchased from The Jackson Laboratory (Bar Harbor, ME). C57BL/6 OT-I mice (13) express a transgenic TCR (α 2V β 5.1) specific for the H2-K^b-restricted peptide OVA_{257–264} (SIINFEKL) derived from chicken OVA and were provided by M. Zenke (Max Delbrück Center for Molecular Medicine) with kind permission of F. Carbone and W. Heath (The Walter and Eliza Hall Institute, Melbourne, Australia). OT-I mice were crossed to

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³ Abbreviations used in this paper: BM, bone marrow; B16p, parental B16; DC, dendritic cell.

RAG1^{-/-} (B6.129S7-Rag1^{tm1 Mom}) mice obtained from The Jackson Laboratory. RAG1-deficient OT-I mice (OT-I × RAG1^{-/-}) were used for the experiments.

Cell lines, tumor cell injection, and adoptive T cell transfer

The OVA-transfected, B6-derived melanoma cell line B16-OVA (14, 15) was kindly provided by Dr. R. Dutton (Trudeau Institute, Saranac Lake, NY). B16-OVA and parental B16 (B16p) cells were cultured in DMEM plus 10% FCS and penicillin/streptomycin. G418 (0.5 mg/ml) was added to the culture medium for B16-OVA cells. Before injection into mice, the cells were trypsinized and washed twice in PBS. B16-OVA cells (1×10^5) were injected s.c. in the abdominal region. Mice that developed a tumor of 1 cm in diameter were scored as tumor positive. The tumor diameter was determined as the mean of the largest diameter and the diameter at right angle. Mice that are shown as tumor free at the end of the experiments (day 60) had completely rejected the tumor.

To generate activated CD8⁺ OT-I cells, spleens from OT-I × RAG1^{-/-} mice were obtained, and single-cell suspensions were prepared. After removal of RBCs by NH₄Cl treatment, 2×10^6 /ml spleen cells were cultured for 3 days in RPMI 1640 plus 10% FCS, penicillin/streptomycin, MEM, and 2-ME (50 μM) (complete RPMI) containing 1 μg/ml peptide OVA₂₅₇₋₂₆₄. Before the i.v. injection of 1×10^7 CD8⁺Vα2⁺ OT-I cells into the tail vein of the indicated mice, the cells were washed twice with PBS, and the percentage of transgenic OT-I cells was determined with a FACSCalibur flow cytometer (BD Biosciences, Mountain View, CA) using mAbs for CD8α (53-6.7), TCR Vβ5.1/5.2 (MR9-4), and/or TCR Vα2 chain (B20.1) (all BD Pharmingen, Hamburg, Germany).

For intracellular cytokine staining, the intracellular staining kit from BD Pharmingen was used. The cells were stained with mAbs for CD8α, Vα2, and IFN-γ (XMG1.2) and analyzed with a FACSCalibur flow cytometer (BD Biosciences).

For the generation of BM chimeras, BM donors were injected i.p. with 2 mg of rat mAb GK1.5 (anti-CD4) and 2.43 (anti-CD8) 5 and 2 days before BM isolation. BM cells were isolated from the femurs and resuspended in PBS, and 1×10^7 cells were injected i.v. into the tail vein of lethally irradiated (9 Gy) B6 mice. The mice were maintained on antibiotic water for 6 wk after reconstitution.

Results and Discussion

The rejection of B16-OVA tumors by OT-I cells requires IFN-γR expression on host cells and is independent of host IFN-γ

At first, we asked whether in vitro-activated CD8⁺ OT-I cells mediated the rejection of B16-OVA tumors in vivo. To generate tumor-specific effector cells, spleen cells from OT-I × RAG1^{-/-} mice were activated in vitro with the specific peptide OVA₂₅₇₋₂₆₄. After 72 h of culture, the majority of the nonadherent cells showed expression of CD8 and the TCR Vα2 chain (Fig. 1). OT-I cells (1×10^7) were injected i.v. into the tail vein of B6 mice that had been injected with 1×10^5 B16-OVA cells 3 days before. As a control, B6 mice bearing B16-OVA tumors were left untreated. As shown in Fig. 2A, all control mice rap-

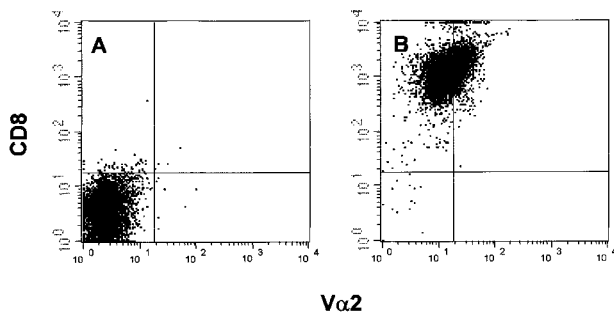


FIGURE 1. After in vitro stimulation, the majority of RAG1^{-/-} × OT-I spleen cells are transgenic CD8⁺ OT-I cells. *A* and *B*, Spleen cells from RAG1^{-/-} × OT-I mice were stimulated for 72 h with 1 μg/ml OVA₂₅₇₋₂₆₄. *B*, The percentage of transgenic CD8⁺ T cells was determined by FACS analysis using mAbs for CD8α and the TCR Vα2 chain. *A*, The specificity of the staining was verified with isotype-matched control Abs.

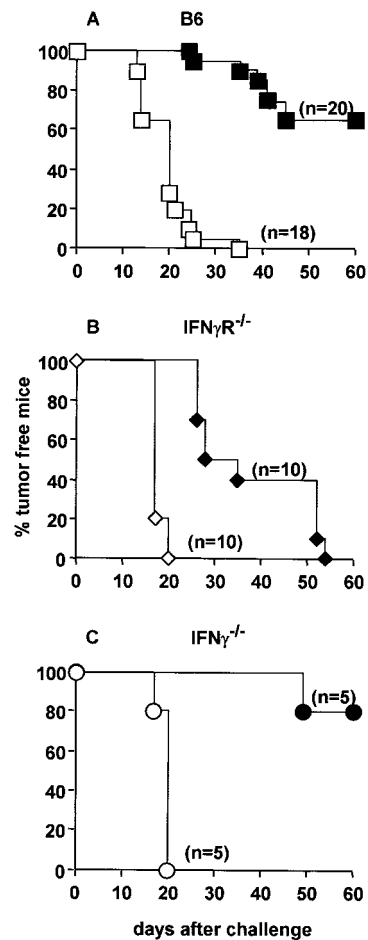


FIGURE 2. The rejection of B16-OVA requires IFN-γ from CD8⁺ OT-I cells and the expression of IFN-γR on host cells. B6 (*A*), IFN-γR^{-/-} (*B*), and IFN-γ^{-/-} (*C*) mice were injected s.c. with 1×10^5 B16-OVA cells. Three days later, the mice received 1×10^7 activated CD8⁺ OT-I cells i.v. (■, ◆, and ●) or were left untreated (□, ◇, and ○). Shown is the percentage of tumor-free mice after tumor cell injection. Results from four (*A*), two (*B*), and one (*C*) experiment(s) are shown. The numbers indicate the total numbers of mice analyzed. Mice that had not developed tumors until day 60 remained tumor free. Mice of one experimental group were sacrificed 7 mo after tumor cell inoculation and showed no signs of tumor growth.

idly developed tumors, whereas the administration of OT-I cells strongly delayed tumor development in 35% and led to complete tumor rejection in 65% of B6 mice. Although it is usually believed that CD8⁺ T cells directly kill tumor cells via perforin and CD95 ligand, it was shown that the efficacy of CD8⁺ T cells to reject tumors in vivo correlated best with their ability to secrete IFN-γ rather than with their in vitro cytotoxicity (16, 17). It was proposed that IFN-γ contributes to CD8⁺ T cell-mediated tumor rejection, because it up-regulates MHC class I on tumor cells, thereby increasing their susceptibility to direct killing (18). In agreement with this interpretation, some IFN-γ-unresponsive tumor cells are less tumorigenic than their IFN-γ-responsive counterparts (19), even though the difference in other tumor models appears to be marginal (3). In any case, this suggested that the absence of IFN-γ signaling in tumor cells allows them to escape CD8⁺ T cell attack. In apparent contrast, tumor rejection by CD4⁺ T cell effectors required IFN-γR expression on host but not tumor cells (3, 5). Therefore, it remained unclear whether tumor rejection by CD8⁺ T

cells resulted from the action of IFN- γ on tumor (direct pathway) or host cells (indirect pathway). To answer this question, IFN- γ R^{-/-} mice were injected s.c. with 1×10^5 B16-OVA cells and 3 days later i.v. with 1×10^7 OT-I cells. As a control, tumor-bearing IFN- γ R^{-/-} mice were left untreated. As shown in Fig. 2B, all IFN- γ R^{-/-} control mice developed tumors with similar kinetics as B6 control mice (Fig. 2A) showing that the lack of host IFN- γ R did not alter tumor growth (Fig. 2B). Surprisingly, all IFN- γ R^{-/-} mice reconstituted with OT-I cells also developed tumors. This defect was not due to the deletion of OT-I cells, because they could be detected in tumor-bearing IFN- γ R^{-/-} mice (Fig. 3). Thus, for CD8⁺ T cell-mediated tumor rejection, IFN- γ R expression by tumor cells was not sufficient but required IFN- γ R expression on host cells (Fig. 2B).

Because cells of the host could secrete IFN- γ in addition to the transferred CD8⁺ T cells, we next asked whether IFN- γ derived from OT-I cells was sufficient for tumor rejection or whether other cell types were required as a source of IFN- γ . For this purpose, IFN- γ R^{-/-} mice were injected with B16-OVA cells and reconstituted with OT-I cells 3 days later. Tumor-bearing control mice were left untreated. As shown in Fig. 2C, all control mice developed tumors, whereas 80% of the CD8⁺ T cell-reconstituted IFN- γ R^{-/-} mice rejected the tumor. This experiment showed that IFN- γ from OT-I cells was sufficient for the rejection of s.c. tumors, although we do not want to exclude that endogenous cells might contribute IFN- γ for the rejection of tumors in other tissues (2).

The data shown so far suggested that OT-I cells had to produce IFN- γ to allow tumor rejection. However, it remained unclear whether this rejection required Ag encounter in vivo or whether in vitro stimulation was sufficient for OT-I cells to reject tumors. To answer this question, B6 mice were injected with B16p cells and received activated OT-I cells 3 days later or

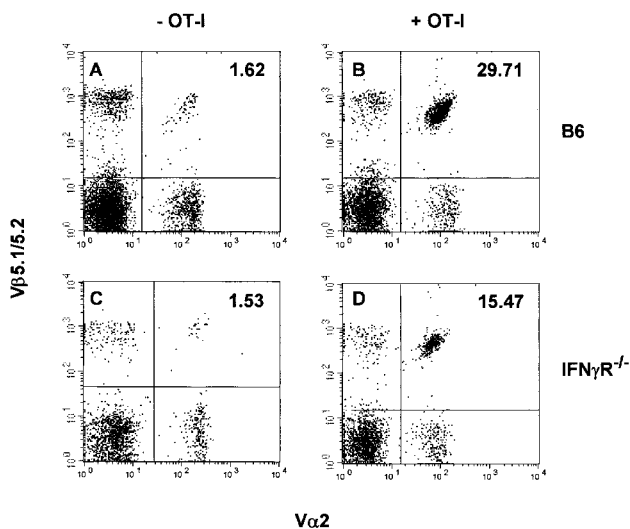


FIGURE 3. CD8⁺ OT-I cells survive in tumor-bearing IFN- γ R^{-/-} mice. Spleen cells from individual untreated B6 mice (A), B16-OVA-free, OT-I-reconstituted B6 recipients (day 97 after tumor cell injection) (B), B16-OVA-bearing IFN- γ R^{-/-} mice (day 18 after tumor cell injection) (C), and B16-OVA-bearing, OT-I-reconstituted IFN- γ R^{-/-} mice (day 26 after tumor cell injection) (D) were analyzed for the expression of CD8 α , V α 2, and V β 5.1 by FACS. Shown is the V α 2 and V β 5.1 expression on CD8⁺ T cells. In spleens of six tumor-bearing IFN- γ R^{-/-} mice, increased numbers of CD8 α ⁺ V α 2⁺V β 5.1⁺ cells could be detected after adoptive T cell transfer but not in its absence.

were left untreated. As shown in Fig. 4A, 90% of the parental tumors grew after T cell transfer, similar to nonreconstituted mice, which all developed tumors. This was in contrast to mice bearing B16-OVA tumors, which efficiently rejected tumors after OT-I transfer (Fig. 2A). The lack of tumor rejection in B6 mice bearing B16p tumors was not due to the deletion or functional inactivation of OT-I cells, because they were present in the spleens of B16p-bearing mice (Fig. 4B) and produced IFN- γ in response to OVA₂₅₇₋₂₆₄ (Fig. 4D). These data demonstrated that OT-I cells required Ag contact to produce IFN- γ and reject B16 tumors.

Tumor rejection does not require BM-derived APCs to cross-present tumor-derived OVA to OT-I cells

The secretion of IFN- γ by CD8⁺ T cells can be turned on or off within minutes after the recognition of MHC class I-peptide complexes or their withdrawal, respectively (20). In the effector

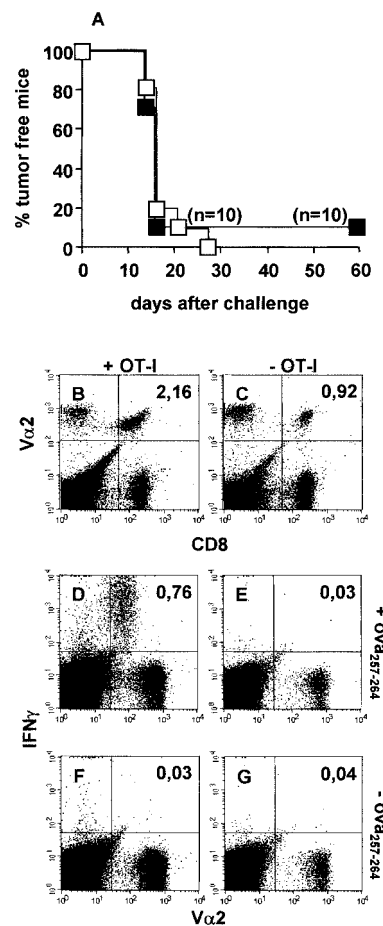
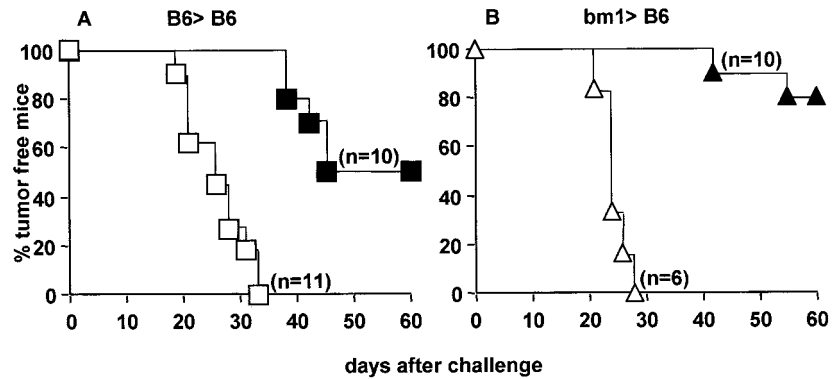


FIGURE 4. In the absence of OVA expression, tumors cannot be rejected, although CD8⁺ OT-I cells survive and retain their ability to produce IFN- γ . A, B6 mice were injected s.c. with 1×10^5 B16p cells. Three days later, the mice received 1×10^7 activated CD8⁺ OT-I cells i.v. or were left untreated. Shown is the percentage of tumor-free mice after tumor cell injection. Combined results from two independent experiments are shown. The numbers indicate the total number of mice analyzed. When the tumors had reached a size of 1 cm in diameter (day 17 after tumor-cell inoculation), spleen cells from OT-I-reconstituted (B, D, and F) and nonreconstituted (C, E, and G) B6 mice were analyzed for CD8 and V α 2 expression (B and C) or incubated for 6 h with or without 10 μ g/ml OVA₂₅₇₋₂₆₄ (D–G). Shown is the expression of V α 2 and IFN- γ by CD8⁺ cells after stimulation with OVA₂₅₇₋₂₆₄ (D and E) or in its absence (F and G). Similar results were obtained from four mice per group.

FIGURE 5. CD8⁺ T cell-mediated rejection of B16-OVA does not require cross-presentation of OVA by BM-derived APCs. B6 mice were irradiated lethally and reconstituted with BM from B6 mice (B6>B6) (A) or bm1 mice (bm1>B6) (B). Ten weeks after BM transplantation, mice were injected s.c. with 1×10^5 B16-OVA cells. Three days later, the mice received 1×10^7 activated CD8⁺ OT-I cells i.v. (■ and ▲) or were left untreated (□ and △). Shown is the percentage of tumor-free mice after tumor cell injection. Combined results from two independent experiments are shown. The numbers indicate the total number of mice analyzed.



phase of an antitumor immune response, MHC class I-peptide complexes could be presented to tumor-specific CD8⁺ T cells by tumor cells but also by BM-derived APCs, such as dendritic cells (DCs), which take up cell-derived Ags to present them via the alternative MHC class I pathway (21). The induction of antitumor CD8⁺ T cells responses, similar to those against bacteria, viruses, and self-Ags, depends on cross-presentation by BM-derived APCs (22–25). The fact that 1) the rejection of B16 tumors by OT-I cells depended on Ag recognition in vivo (Figs. 2A and 4A), 2) B16-OVA tumors are rejected by OT-I cells despite the very low level of MHC class I expression (data not shown), and 3) tumor Ags are cross-presented by APCs within tumors (8, 9, 26) and tumor-draining lymph nodes (10–12) suggested that cross-presentation of tumor Ags by BM-derived APCs in the effector phase might be required for tumor rejection. To test this hypothesis, we generated BM-chimeric mice that were incapable of presenting OVA_{257–264} by BM-derived cells. For this purpose, lethally irradiated B6 mice were reconstituted with BM from bm1 mice (bm1>B6 chimeras) that express mutated H-2K^b molecules and cannot present OVA_{257–264} to OT-I cells (27). Additionally, B6 mice were reconstituted with B6 BM (B6>B6 chimeras). Ten weeks after BM reconstitution, chimeric mice were injected with 1×10^5 B16-OVA cells and 3 days later with in vitro-activated OT-I cells. Tumor-bearing control mice were left untreated. As shown in Fig. 5A, 50% of the B6>B6 OT-I cell-recipients rejected B16-OVA tumors, whereas all control mice developed a tumor. Similarly, 80% of the bm1>B6 mice remained tumor free after T cell transfer, whereas all control mice developed tumors (Fig. 5B). This experiment demonstrated that OT-I cells mediate tumor rejection in the absence of cross-presentation of OVA_{257–265} by BM-derived cells. Although this suggested that OT-I cells recognized their Ag on tumor cells, we cannot formally exclude that non-BM-derived cells, e.g. endothelial cells, cross-presented OVA. However, we think that this is unlikely, because the depletion of DCs completely abolishes cross-priming of CTLs in vivo (28).

It is important to note that tumor rejection in B6>B6 chimeric mice was less efficient than in bm1>B6 chimeric mice. Recently, it was shown that tumor-infiltrating DCs cross-presenting tumor-derived Ags are functionally impaired and cannot stimulate T cells properly (26). Because B16-OVA tumors were infiltrated by DCs (data not shown), it is possible that Ag-specific inactivation of OT-I cells by BM-derived APCs impaired tumor rejection in B6>B6 but not bm1>B6 chimeric mice, because the latter could not cross-present OVA to OT-I CTLs. However, this point requires further investigation.

The data presented in this study suggest that OT-I cells secreted IFN- γ in response to Ag recognition on tumor cells. Surprisingly, tumor rejection required IFN- γ R expression on host cells indicating that direct Ag recognition on tumor cells allowed CD8⁺ effector T cells to reject tumors by IFN- γ action on host cells. It is important to note that tumor growth was delayed in OT-I-recipient IFN- γ R^{-/-} mice compared with nonreconstituted mice (Fig. 2B). This delay may reflect tumor cell killing by OT-I effector cells that, however, cannot fully prevent tumor growth in the absence of IFN- γ R expression on host cells (Fig. 2B).

Recently, it was shown that CD8⁺ T cells rejected histo-incompatible skin grafts in an IFN- γ -dependent fashion (29). This observation leads to the questions of whether the same host cells have to respond to IFN- γ for tumor or skin graft rejection. Given that IFN- γ exerts its antitumor effect locally, the tumor stroma cells should contain the cells that have to be responsive for IFN- γ . Rejection of MHC class II⁻ tumors by CD4⁺ T cells involves angiostasis that requires IFN- γ responsiveness of non-BM-derived cells in the effector phase (3). Parallel studies showed that IFN- γ /CD8⁺ T cell-dependent tumor rejection similarly involved angiostasis,⁴ indicating that the target cells of IFN- γ in our model were tumor stroma cells such as endothelial cells or fibroblasts, although other cells such as macrophages cannot be excluded. The data presented in this study suggest a three cell-type interaction in which CD8⁺ effector T cells recognize Ag on tumor cells and secrete IFN- γ that has to act on host cells to induce tumor rejection. This mechanism is in contrast to the current view suggesting that tumor rejection by CD8⁺ T cells is primarily mediated by direct killing. We hypothesize that the presented three-cell-type interaction during tumor rejection is also operative in certain autoimmune diseases and during immune responses against pathogens.

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References

- Hung, K., R. Hayashi, A. Lafond-Walker, C. Lowenstein, D. Pardoll, and H. Levitsky. 1998. The central role of CD4⁺ T cells in the antitumor immune response. *J. Exp. Med.* 188:2357.
- Prevost-Blondel, A., M. Neuenhahn, M. Rawiel, and H. Pircher. 2000. Differential requirement of perforin and IFN- γ in CD8 T cell-mediated immune responses against B16.F10 melanoma cells expressing a viral antigen. *Eur. J. Immunol.* 30:2507.

⁴ Z. Qin, J. Schwartzkopf, F. Pradera, T. Kammertöns, B. Seliger, H. Pircher, and T. Blankenstein. A crucial requirement of interferon- γ -mediated angiostasis for tumor rejection by CD8⁺ T cells. *Submitted for publication.*

3. Qin, Z., and T. Blankenstein. 2000. CD4⁺ T cell-mediated tumor rejection involves inhibition of angiogenesis that is dependent on IFN- γ receptor expression by nonhematopoietic cells. *Immunity* 12:677.
4. Ibe, S., Z. Qin, T. Schüler, S. Preiss, and T. Blankenstein. 2001. Tumor rejection by disturbing tumor stroma cell interactions. *J. Exp. Med.* 194:1549.
5. Mumberg, D., P. A. Monach, S. Wanderling, M. Philip, A. Y. Toledano, R. D. Schreiber, and H. Schreiber. 1999. CD4⁺ T cells eliminate MHC class II-negative cancer cells in vivo by indirect effects of IFN- γ . *Proc. Natl. Acad. Sci. USA* 96:8633.
6. Bevan, M. J. 1976. Cross-priming for a secondary cytotoxic response to minor H antigens with H-2 congenic cells which do not cross-react in the cytotoxic assay. *J. Exp. Med.* 143:1283.
7. Huang, A. Y., P. Golumbek, M. Ahmadzadeh, E. Jaffee, D. Pardoll, and H. Levitsky. 1994. Role of bone marrow-derived cells in presenting MHC class I-restricted tumor antigens. *Science* 264:961.
8. Pulaski, B. A., K. Y. Yeh, N. Shastri, K. M. Maltby, D. P. Penney, E. M. Lord, and J. G. Frelinger. 1996. Interleukin 3 enhances cytotoxic T lymphocyte development and class I major histocompatibility complex "re-presentation" of exogenous antigen by tumor-infiltrating antigen-presenting cells. *Proc. Natl. Acad. Sci. USA* 93:3669.
9. Chiodoni, C., P. Paglia, A. Stoppacciaro, M. Rodolfo, M. Parenza, and M. P. Colombo. 1999. Dendritic cells infiltrating tumors cotransduced with granulocyte/macrophage colony-stimulating factor (GM-CSF) and CD40 ligand genes take up and present endogenous tumor-associated antigens, and prime naive mice for a cytotoxic T lymphocyte response. *J. Exp. Med.* 190:125.
10. Marzo, A. L., R. A. Lake, D. Lo, L. Sherman, A. McWilliam, D. Nelson, B. W. Robinson, and B. Scott. 1999. Tumor antigens are constitutively presented in the draining lymph nodes. *J. Immunol.* 162:5838.
11. Nelson, D. J., S. Mukherjee, C. Bundell, S. Fisher, D. van Hagen, and B. Robinson. 2001. Tumor progression despite efficient tumor antigen cross-presentation and effective "arming" of tumor antigen-specific CTL. *J. Immunol.* 166:5557.
12. Nguyen, L. T., A. R. Elford, K. Murakami, K. M. Garza, S. P. Schoenberger, B. Odermatt, D. E. Speiser, and P. S. Ohashi. 2002. Tumor growth enhances cross-presentation leading to limited T cell activation without tolerance. *J. Exp. Med.* 195:423.
13. Hogquist, K. A., S. C. Jameson, W. R. Heath, J. L. Howard, M. J. Bevan, and F. R. Carbone. 1994. T cell receptor antagonist peptides induce positive selection. *Cell* 76:17.
14. Brown, D. M., T. L. Fisher, C. Wei, J. G. Frelinger, and E. M. Lord. 2001. Tumours can act as adjuvants for humoral immunity. *Immunology* 102:486.
15. Dobrzanski, M. J., J. B. Reome, and R. W. Dutton. 1999. Therapeutic effects of tumor-reactive type 1 and type 2 CD8⁺ T cell subpopulations in established pulmonary metastases. *J. Immunol.* 162:6671.
16. Barth, R. J., Jr., J. J. Mule, P. J. Spiess, and S. A. Rosenberg. 1991. Interferon- γ and tumor necrosis factor have a role in tumor regressions mediated by murine CD8⁺ tumor-infiltrating lymphocytes. *J. Exp. Med.* 173:647.
17. Becker, C., H. Pohla, B. Frankenberger, T. Schüler, M. Assenmacher, D. J. Schendel, and T. Blankenstein. 2001. Adoptive tumor therapy with T lymphocytes enriched through an IFN- γ capture assay. *Nat. Med.* 7:1159.
18. Bohm, W., S. Thoma, F. Leithauer, P. Moller, R. Schirmbeck, and J. Reimann. 1998. T cell-mediated, IFN- γ -facilitated rejection of murine B16 melanomas. *J. Immunol.* 161:897.
19. Dighe, A. S., E. Richards, L. J. Old, and R. D. Schreiber. 1994. Enhanced in vivo growth and resistance to rejection of tumor cells expressing dominant negative IFN- γ receptors. *Immunity* 1:447.
20. Slifka, M. K., F. Rodriguez, and J. L. Whitton. 1999. Rapid on/off cycling of cytokine production by virus-specific CD8⁺ T cells. *Nature* 401:76.
21. Heath, W. R., and F. R. Carbone. 2001. Cross-presentation, dendritic cells, tolerance and immunity. *Annu. Rev. Immunol.* 19:47.
22. Lenz, L. L., E. A. Butz, and M. J. Bevan. 2000. Requirements for bone marrow-derived antigen-presenting cells in priming cytotoxic T cell responses to intracellular pathogens. *J. Exp. Med.* 192:1135.
23. Sigal, L. J., S. Crotty, R. Andino, and K. L. Rock. 1999. Cytotoxic T-cell immunity to virus-infected non-haematopoietic cells requires presentation of exogenous antigen. *Nature* 398:77.
24. Sigal, L. J., and K. L. Rock. 2000. Bone marrow-derived antigen-presenting cells are required for the generation of cytotoxic T lymphocyte responses to viruses and use transporter associated with antigen presentation (TAP)-dependent and -independent pathways of antigen presentation. *J. Exp. Med.* 192:1143.
25. Kurts, C., W. R. Heath, F. R. Carbone, J. Allison, J. F. Miller, and H. Kosaka. 1996. Constitutive class I-restricted exogenous presentation of self antigens in vivo. *J. Exp. Med.* 184:923.
26. Vicari, A. P., C. Chiodoni, C. Vaure, S. Ait-Yahia, C. Dercamp, F. Matsos, O. Reynard, C. Taverne, P. Merle, M. P. Colombo, et al. 2002. Reversal of tumor-induced dendritic cell paralysis by CpG immunostimulatory oligonucleotide and anti-interleukin 10 receptor antibody. *J. Exp. Med.* 196:541.
27. Nikolic-Zugic, J., and F. R. Carbone. 1990. The effect of mutations in the MHC class I peptide binding groove on the cytotoxic T lymphocyte recognition of the K^b-restricted ovalbumin determinant. *Eur. J. Immunol.* 20:2431.
28. Jung, S., D. Unutmaz, P. Wong, G. Sano, K. De los Santos, T. Sparwasser, S. Wu, S. Vuthoori, K. Ko, F. Zavala, et al. 2002. In vivo depletion of CD11c⁺ dendritic cells abrogates priming of CD8⁺ T cells by exogenous cell-associated antigens. *Immunity* 17:211.
29. Valujskikh, A., O. Lantz, S. Celli, P. Matzinger, and P. S. Heeger. 2002. Cross-primed CD8⁺ T cells mediate graft rejection via a distinct effector pathway. *Nat. Immunol.* 3:844.