Activation-Induced Cell Death Limits Effector Function of CD4 Tumor-Specific T Cells

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A number of studies have documented a critical role for tumor-specific CD4+ cells in the augmentation of immunotherapeutic effector mechanisms. However, in the context of an extensive tumor burden, chronic stimulation of such CD4+ T cells often leads to the up-regulation of both Fas and Fas ligand, and coexpression of these molecules can potentially result in activation-induced cell death and the subsequent loss of effector activity. To evaluate the importance of T cell persistence in an experimental model of immunotherapy, we used DO11 Th1 cells from wild-type, Fas-deficient, and Fas ligand-deficient mice as effector populations specific for a model tumor Ag consisting of an OVA-derived transmembrane fusion protein. We found that the prolonged survival of Fas-deficient DO11 Th1 cells led to a more sustained tumor-specific response both in vitro and in vivo. Importantly, both Fas- and Fas ligand-deficient Th1 cells delayed tumor growth and cause regression of established tumors more effectively than wild-type Th1 cells, indicating that resistance to activation-induced cell death significantly enhances T cell effector activity. The Journal of Immunology, 2004, 172: 6598–6606.

The rapid clonal expansion of pathogen-specific lymphocytes is an essential feature of the adaptive immune response. However, the regulatory mechanisms that limit cell survival and excessive cytokine production in response to both foreign and self Ags are equally important. This is particularly relevant when an expanded or primed population is reactivated. Thus, the stimulation of primed T cells frequently leads to a burst of cytokine production, but is then followed by extensive apoptosis of the reactive cells (1–3). This phenomenon of activation-induced cell death (AICD)3 is mediated by receptor/ligand interactions of the TNF/TNFR family. In the case of Th1 cells, Fas and Fas ligand (FasL) appear to be the principal mediators of AICD.

FasL is a proapoptotic cell surface molecule that can induce apoptosis in target cells expressing the cell surface receptor Fas. Fas is expressed on a variety of cell types throughout the body and is up-regulated in T cells upon activation. The expression of FasL is more tightly regulated, as overexpression can lead to excessive cell death and/or chemokine-driven inflammatory responses (4–8). FasL can be expressed by T cells, but only upon activation through the TCR; naive T cells do not express FasL. Chronic stimulation has been found to further up-regulate FasL expression (9). FasL plays an essential role in T cell regulation, helping to maintain the homeostasis of lymphocyte populations through the elimination of activated and autoreactive cells. Mice that lack Fas (lpr) or FasL (gld) have an abundance of activated autoreactive cells and develop severe autoimmune disease, suggesting that Fas/FasL interactions serve to effectively limit reactions to self Ags.

Although traditionally CD8 CTLs have been the focus of tumor immunotherapy, numerous studies have demonstrated a critical contribution by CD4 T cells in the development of effective anti-tumor responses (10–13). In some cases, CD4 cells appear to function independently of CD8 T cells. In other studies, CD4 cells initiate and maintain CD8 responses by activating APCs and/or by secreting cytokines such as IL-2 that directly promote CD8 T cell expansion (14–19). Thus, enhancement of CD4 tumor-specific T cells may have far-reaching effects on many of the mechanisms that contribute to tumor rejection.

Immunotherapy is most effective against a small tumor burden (20). With larger tumors, a limited number of effector cells is constantly challenged by an overwhelming amount of tumor Ag. As seen with autoreactive T cells, this chronic stimulation may cause the activated T cells to up-regulate Fas and FasL and undergo AICD. In fact, Restifo and colleagues (21) demonstrated that the ability of certain tumor cells to induce tumor-specific T cells to undergo apoptosis was blocked by anti-Fas Abs. We reasoned that if tumor-specific T cells did not express Fas (or FasL), then they would be unable to undergo AICD, and their prolonged survival would render them a more persistent tumor effector population.

To test this premise, we established an experimental model that involved wild-type (wt), Fas-deficient, and Fas-ligand-deficient T cells from OVA-specific DO11 TCR transgenic mice together with a syngeneic tumor line expressing a membrane-associated OVA fusion protein. We found that the increased survival of Fas- or FasL-deficient T cells dramatically enhanced their in vivo efficacy, as assessed by their capacity to limit tumor progression as well as their capacity to reject established tumors.

Materials and Methods

Mice

BALB/c-D011, BALB/c-D011/lpr, and BALB/c-D011/gld mice were bred and maintained at the Laboratory Animal Science Center, Boston University Medical Center. Six- to 8-wk-old male or female BALB/cJ mice were purchased from The Jackson Laboratory (Bar Harbor, ME).
Reagents

Polynuclear goat anti-OVA antisera was obtained from ICN Pharmaceuti- cals (Irvine, CA). Mouse monoclonal OVA-specific IgG PE was obtained from Jackson Immunoresearch Laboratories (West Grove, PA). Abs specific for the DO11 TCR, derived from the hybridoma KJ2-26, were affinity purified and biotinylated by standard methods. Anti-IL-4 was obtained from the cell line 11B11, kindly provided by A. Abbas (University of California, San Francisco, CA). Murine IL-12 was obtained from BD Pharmingen (San Diego, CA). OVA peptide (OVA 323–339) was synthesized by Research Genetics (Huntsville, AL).

Derivation of the tGO construct

The tGO construct was created from pJR033, an OVA-green fluorescent protein (GFP) fusion protein construct in a mammalian expression plasmid derived from the kanamycin-resistant Vical pVR1012 (kindly provided by J. Richmond, Whitehead Institute, Cambridge, MA). It includes 230–359 of OVA and incorporates the stimulatory peptide for DO11. The yeast GFP fusion protein was isolated by BglII/EcoRI restriction digest and ligated in frame with the transmembrane domain of the transferrin receptor (aa 1–118) (kindly provided by R. Maki and A. Abbas, University of California, California). The transmembrane domain of the transferrin receptor-GFP-OVA fusion protein (tGO) was then inserted into the mammalian expression vector pEBB for transfection.

Cell lines

The BALB/c B lymphoma cell line A20 was obtained from the American Tissue Culture Collection (Manassas, VA) and grown in 10% FCS RPMI 1640 supplemented with 1× penicillin/streptomycin/glutamine and 50 μM 2-ME (Life Technologies, Grand Island, NY). The A20 line is Fas positive and FasL negative. The original A20 tumor line did not consistently form s.c. tumors when injected s.c. into syngeneic mice. To isolate cells that routinely grew as solid tumors, BALB/cJ mice were sublethally irradiated and injected s.c. with 2×106 A20 cells. A tumor that developed in one of these mice was excised, and the new line (A20irr) was cotransfected by electroporation with the tGO expression vector and pDNA3-His (Invitro- gen, San Diego, CA) using the Gene Pulser II (BioRad, Hercules, CA) with 320 V and 950 μF in OptiMem medium (Life Technologies). G418-resistant transfectants were selected with 0.9 mg/ml G418 for 14 days. Following G418 selection, GFP+ and GFP− clones were isolated twice by limiting dilution to ensure clonality. Transfected clones expressing GFP were screened by function (stimulation of DO11 T cells) and cell surface expression of both GFP and OVA (FACS staining with a goat polyclonal antisera to OVA and a PE-conjugated donkey anti-goat). A20-GO clones expressing comparable levels of GFP and OVA, as determined by flow cytometry, were independently isolated. A representative clone was chosen for additional experiments, and designated A20-tGO. GFP+ OVA− A20irr cells were used as control cells throughout the study. Cell lines were maintained in RPMI 1640 with 0.9 mg/ml G418 (Life Technologies).

Generation of Th1 cells

DO11 Th1/wt, Th1/lpr, and Th1/gld T cells were generated from lymph nodes of 4- to 5-wk-old BALB/c DO11 wt, lpr, or gld mice. Lymph node cell suspensions were C68 depleted by Ab and complement and skewed to a Th1 phenotype by culture with pOVA (323–339) (20 μg/ml) in the presence of IL-12 (2.5 ng/ml) (BD Pharmingen) and anti-IL-4 mAb (11B11) (22). Each set of Th1 cells was generated from the lymph nodes obtained from three to five mice. Consistency in multiple experiments was ensured by expanding cells in IL-2 for 2 days and freezing stock vials of cells on day 5. A small number of cells was retained and restimulated with plate-bound anti-CD3 for 24 h to confirm Th1 phenotype and sensitivity to AICD. Culture supernatants were tested for cytokine production by ELISA with mAbs and standards obtained from BD Pharmingen. They were rou- tinely found to produce IL-2, IFN-γ, and TNF-α and not to produce IL-4.

In vitro assays

DO11 Th1 cells were thawed and expanded in IL-2 for 4–6 days. IL-2 was then withdrawn for 2 days to allow the cells to revert to a resting pheno- type. On the day of the assay, viable cells were isolated by density gradient centrifugation (Lymphocyte-M; Cedarslane Laboratories, Hornby, Ontario, Canada). Cells were then plated in 96-well flat-bottom plates at concen- trations ranging from 1.8×105 to 6×107 cells/well. Stimulator A20 and A20-tGO cells were fixed by a 30-min incubation at 25°C with freshly prepared 0.5% paraformaldehyde, washed twice in PBS, and plated at 6×106 cells/well. Cells were cultured for 24 or 72 h. For proliferation assays, [3H]thymidine (New England Nuclear, Boston, MA) was added for an additional 16 h of culture at a final concentration of 25 μCi/ml. DNA was isolated with a PHD Harvester (Cambridge Technology, Cambridge, MA), and [3H]thymidine incorporation was measured with an LKB Wallac 1212 Rackbeta counter (PerkinElmer, Wellesley, MA). For cytotoxic assays, 6×104 51Cr-labeled A20 or A20-GO cells were added to each well. Culture supernatants were collected 16 h later, and percent specific lysis was determined, as previously described (7).

In vivo tumor studies

Wild-type BALB/cJ mice were sublethally irradiated (450 rad). Three to 5 h later, 2×106 A20 or A20-tGO tumor cells were s.c. injected into the right flank. DO11 Th1 cells were injected i.v. either immediately before when the tumor cells were injected or 10 days after the tumor cells had been injected when tumors were palpable (6–10 mm). Previously skewed DO11 Th1 cells came from frozen stock vials and were thawed and ex- panded in IL-2 for 4–5 days. Cells were then expanded by a second round of stimulation in vitro with peptide-pulsed, mitomycin-treated spleocytes. Cells were subsequently expanded in IL-2 and were injected either on day 4 postactivation (activated) or on day 8–10 postactivation following 2 days with no additional IL-2 (rested). Mice were monitored for tumor growth by measurement of tumor diameter for >100 days.

Th1 persistence following A20-tGO rejection

Spleens were removed from mice at 42 or >100 days following tumor inoculation. Th1 persistence was determined by both functional and phe- notypic criteria. To determine functional activity, 5×106 spleen cells were stimulated with 1×106 fixed A20 or A20-GO cells for 40 h, and prolif- eration was assessed by [3H]thymidine incorporation. For evaluation by FACS, 1×106 spleocytes were incubated with 2.4G2 to block nonspe- cific binding by FcγRs and then stained with FITC-conjugated anti-CD4 and PE-conjugated J11-D16 (anti-clonotype). Samples were analyzed on a FACSScan flow cytometer. Acquired data were plotted using Flowjo soft- ware (Tree Star, San Carlos, CA) with contour plot settings of 10% probability, smoothing factor of 5, and a 0.2% threshold.

Results

A20-GO cells are potent stimulators of DO11 Th1 cells

To provide an appropriate tumor target for DO11 effector cells, the H2b, class II-positive, Fas-positive B cell lymphoma line A20 was transfected with a construct encoding an OVA-GFP fusion protein linked to the transmembrane domain of the transferrin receptor (Fig. 1A). The transferrin receptor targets the early endosome, where it releases iron and is recycled back to the surface of the cell. Transferrin has been found to facilitate delivery of Ag into the class II-processing pathway, and transferrin-peptide conjugates have been shown to increase presentation 100-fold compared with free peptide (23–26). Although the mechanism is not entirely under- stood, targeting the early endosome may lead to efficient Ag digestion and entry into the processing pathway. A20 cells previously selected for their ability to progress as s.c. tumors in BALB/c mice were transfected by electroporation, and stable transfectants were isolated by resistance to G418. Expression of the fusion protein was confirmed by flow cytometry using as criteria the coex- pression of GFP and OVA (Fig. 1B). It was further shown that the A20-tGO cells effectively pre- sented the pOVA in the context of class II. DO11/lpr cells were stimulated with A20 cells, A20 cells pulsed with pOVA, or A20- tGO cells, and proliferation was assessed by [3H]thymidine incor- poration. A20-GO cells stimulated the DO11/lpr cells much more effectively than the standard method of peptide-pulsed APCs (Fig. 1C). These results clearly demonstrate that the OVA fusion protein is efficiently processed and presented by MHC class II.

Increased DO11/lpr survival results in increased proliferation and effector activity in response to OVA

Once a tumor line expressing a defined model tumor Ag was es- tablished, the role of Fas in the survival and efficacy of tumor-specific T cells could be examined. DO11 Th1 cells were gener- ated from Fas-sufficient (DO11/wt), Fas-deficient (DO11/lpr), and FasL-deficient (DO11/gld) BALB/c mice by standard procedures.
DO11/lpr cells were stimulated with fixed A20 or A20-tGO cells for either 24 or 72 h, and proliferation was assessed by \[^{3}H\]thymidine incorporation. At both time points, the response of the DO11/lpr cells was at least 10-fold higher than DO11/wt, consistent with the premise that a sustained proliferative response is best maintained in the absence of AICD (Fig. 2, A and B).

It was important to confirm that DO11/lpr cells still retained their capacity to express FasL and kill Fas\(^-\) target cells after this prolonged proliferative response. \(^{51}\)Cr-labeled A20 or A20-tGO targets were added to cultures activated as above at 24 and 72 h poststimulation, and cytotoxicity was assessed by chromium release. The lytic activity of the DO11/lpr cultures was significantly greater than the DO11/wt cultures at all time points (Fig. 2, C and D). Target cell lysis was FasL dependent, as the DO11/gld cells lacked any detectable cytotoxic activity. These results establish that in vitro the lack of Fas expression allows T cells to survive longer, and the increased survival translates into both increased proliferation and effector activity in response to stimulation with Ag.

DO11/lpr cells prevent A20-tGO tumor growth more effectively than DO11/wt cells

Having established that expression of OVA and GFP does not alter the growth of s.c. tumors (data not shown), and that in vitro Fas-deficient DO11 Th1/lpr cells are better effector cells than Fas-sufficient DO11 Th1/wt cells, the efficacy of Fas-deficient T cells was evaluated in vivo. Sublethally irradiated BALB/cJ mice were injected i.v. with \(1 \times 10^6\) rested DO11/wt, DO11/lpr, or DO11/gld cells. On the same day, \(2 \times 10^6\) A20 or A20-tGO cells were injected s.c., and mice were monitored for tumor growth over the next 100 days. A dose titration demonstrated that all mice constituted with high numbers (\(5 \times 10^6\)) of either wt or mutant Th1 cells were protected from tumor challenge (data not shown). At a lower dose of T cells (\(1 \times 10^6\)), Fas-deficient DO11/lpr cells prevented tumor growth in a significantly higher percentage of mice than DO11/wt cells. Thus, the lack of Fas led to increased effector activity in vivo, as had been seen in vitro (Fig. 3). Surprisingly, although FasL-deficient DO11/gld cells were not able to kill A20-tGO cells in vitro, in vivo the DO11/gld cells were able to prevent the outgrowth of the A20-tGO tumors more effectively than DO11 wt cells and almost as well as DO11/lpr cells (Fig. 3). DO11/gld T cells cannot undergo AICD due to their lack of functional FasL, and their enhanced effector activity, in comparison with DO11/wt, is most likely due to their prolonged survival in vivo.

Increased frequency of DO11/lpr cells after A20-tGO rejection correlates with a stronger proliferative response to OVA

To directly compare the in vivo persistence of DO11 wt, lpr, and gld cells, we analyzed mice originally injected with \(5 \times 10^6\) DO11 Th1 cells in which Th1 cells from all three strains uniformly blocked A20-tGO progression (and all mice survived indefinitely). Six weeks after the original tumor challenge, spleen cell suspensions from the DO11 wt, gld, and lpr mice were examined by flow cytometry, using the KJ1-26 anti-clonotype Ab. DO11/lpr cells constituted a significantly higher percentage of the splenic CD4 population than did DO11/wt cells (4.17% compared with 0.75%). Consistent with their capacity to reject the original A20-tGO cells, the DO11/gld cells also persisted to a greater extent than the DO11/wt cells (2.75% compared with 0.75%) (Fig. 4A).

The selected survival of the Fas- and FasL-deficient cells was confirmed in a functional assay. Spleen cells from the different groups of mice were stimulated in vitro with A20 or A20-tGO cells, and proliferation was assessed by \[^{3}H\]thymidine incorporation. Avidin-dextran labeled A20 or A20-tGO cells were added to cultures activated as above at 24 and 72 h poststimulation, and cytotoxicity was assessed by chromium release. The lytic activity of the DO11/lpr cultures was significantly greater than the DO11/wt cultures at all time points (Fig. 2, C and D). Target cell lysis was FasL dependent, as the DO11/gld cells lacked any detectable cytotoxic activity. These results establish that in vivo the lack of Fas expression allows T cells to survive longer, and the increased survival translates into both increased proliferation and effector activity in response to stimulation with Ag.

Activated DO11/lpr cells are more effective than rested DO11/lpr or activated DO11/wt cells in preventing A20-tGO tumor growth in vivo

Activated T cells have been shown to express different chemokine receptors and home to different locations in the body than rested T cells (27, 28). The migration differences between rested and activated T cell effector cells can influence their effector capacity in vivo. To compare the antitumor activity of activated and rested
DO11 Th1 cells, sublethally irradiated BALB/cJ mice were injected i.v. with $1 \times 10^6$ activated or rested DO11/wt or DO11/lpr cells on the same day that $2 \times 10^6$ A20 or A20-tGO cells were injected s.c. Mice injected with rested DO11/wt cells exhibited delayed tumor growth in comparison with mice that received no DO11 cells (average of 38 vs 25 days until the development of a 15-mm tumor), but all mice developed 15-mm tumors by day 67. However, the injection of activated DO11/wt cells significantly increased survival with an average of 58 days until the development of a 15-mm tumor. Most importantly, activated DO11/lpr cells completely prevented tumor growth in eight of nine mice, and the one tumor that developed did not reach 15 mm until day 92. In comparison, 64% of mice injected with rested DO11/lpr developed 15-mm tumors with an average of 59 days (Fig. 5A). Effector activity was specific, as neither rested nor activated DO11 cells could prevent the outgrowth of the original A20 cells (data not shown). These results demonstrate that DO11/lpr cells can eliminate tumor cells more effectively than DO11/wt cells regardless of their activation status upon injection; however, activated DO11 cells are significantly more effective than rested cells in vivo.

To determine whether the activation status also affects persistence, spleens from tumor-free mice were harvested at day 137, and the persistence of DO11 cells was determined in a functional assay. Spleen cells from mice injected with rested or activated DO11/lpr cells were stimulated with fixed A20-tGO cells for 24 (A) or 72 (B) h, and proliferation was assessed by $[^{3}H]$thymidine incorporation during additional 16 h of culture. Data are presented as the mean cpm of triplicate wells ± SD. To evaluate cytotoxic activity, DO11/wt, DO11/lpr, or DO11/gld T cells were stimulated with fixed A20-tGO cells for 24 (C) or 72 (D) h. $[^{51}Cr]$-labeled A20 or A20-tGO targets were then added to the same wells for an additional 16 h, and cytotoxicity was assessed by chromium release. Error is negligible, except where shown (<5000 cpm for $[^{3}H]$ incorporation or <2% specific lysis for chromium release). The results shown are representative of at least three independent experiments.

**FIGURE 2.** Increased DO11/lpr survival results in increased proliferation and effector activity in response to OVA. DO11/wt, DO11/lpr, or DO11/gld T cells were stimulated with fixed A20-tGO cells for 24 (A) or 72 (B) h, and proliferation was assessed by $[^{3}H]$thymidine incorporation during additional 16 h of culture. Data are presented as the mean cpm of triplicate wells ± SD. To evaluate cytotoxic activity, DO11/wt, DO11/lpr, or DO11/gld T cells were stimulated with fixed A20-tGO cells for 24 (C) or 72 (D) h. $[^{51}Cr]$-labeled A20 or A20-tGO targets were then added to the same wells for an additional 16 h, and cytotoxicity was assessed by chromium release. Error is negligible, except where shown (<5000 cpm for $[^{3}H]$ incorporation or <2% specific lysis for chromium release). The results shown are representative of at least three independent experiments.
DO11/lpr cells. In the majority of mice injected with DO11/wt cells, the original tumor continued to grow with only slightly delayed kinetics compared with mice that did not receive T cells (33.3 days compared with 20.0 days until the development of a 15-mm tumor) (Fig. 6A). However, 3 of 10 mice had s.c. tumors that initially regressed, but later developed secondary tumors. In contrast, tumors in all mice injected with DO11/lpr cells developed overt evidence of necrosis, with large scabs seen at the tumor site, that was invariably associated with tumor rejection. At day 39, the average tumor size was 1.7 mm compared with 12.3 mm for mice injected with DO11/wt cells (Fig. 6D). Mice injected with DO11/lpr cells had an intermediate phenotype in which scab formation was less apparent, although tumors still regressed in 7 of 10 mice (Fig. 6C). DO11/lpr cells were clearly the most effective population for destroying the original s.c. tumor.

Recurring secondary A20-tGO tumors have lost expression of the tGO construct

Although tumors in all the mice injected with DO11/lpr cells regressed, the majority of these mice later developed secondary tumors, typically in the draining lymph nodes and not at the original site (Fig. 6, A–C). As these secondary tumors developed, the mice showed increasing signs of morbidity and often had to be sacrificed before tumors at the s.c. site reached 15 mm. The consistent emergence of secondary tumors in the latter studies indicated that the tumors could not longer be contained by the DO11 population, either because they were resistant to cytotoxic effector molecules or because they were no longer recognized by the DO11 receptor. We found that the secondary tumors were consistently Fas positive by both FACS analysis and FasL-dependent cytotoxic assays (data not shown), so they at least remained sensitive to FasL.

To evaluate OVA expression, both primary and secondary tumors from at least four mice were tested for their ability to stimulate proliferation of DO11 Th1 cells. Tumors that grew s.c. and reached 15 mm between days 20–30 were considered primary tumors. Tumors that developed in the inguinal lymph nodes at later time points (>day 35) were considered secondary tumors. Primary tumors were able to stimulate DO11/lpr cell proliferation and could also be killed by DO11 cells (Fig. 7A, and data not shown). No proliferation was detected in response to cells from secondary tumors (Fig. 7B). The outgrowth of secondary tumors in all cases was therefore associated with the loss of expression of the tGO construct.

Discussion

We have developed an experimental system using wt, Fas-deficient, and FasL-deficient Th1 cells with a syngeneic tumor line that expresses a model cell surface Ag. In vitro, Fas-deficient DO11 Th1 cells demonstrated a more sustained proliferative response to the OVA + tumor line than Fas-sufficient DO11 Th1 cells. This extended proliferative response translated into prolonged effector function, as demonstrated by cytotoxic activity. To test the impact of AICD in an in vivo tumor model, wt BALB/c mice were injected with DO11/wt, DO11/lpr, or DO11/gld Th1 cells and OVA + tumors. Both DO11/lpr and DO11/gld cells were able to
prevent tumor growth and reject established s.c. tumors more effectively than DO11/wt cells, demonstrating that FasL-mediated AICD limits the effector function of conventional tumor-specific T cells both in vitro and in vivo. Therefore, resistance to AICD can significantly enhance T cell effector activity.

The in vivo efficacy of the DO11/gld cells relative to DO11/wt cells was surprising in light of the in vitro cytotoxic data. The A20 target cells express high levels of Fas and are exceptionally sensitive to FasL. The in vitro data clearly demonstrated that, although DO11/gld cells proliferated strongly in response to A20-tGO cells, they had minimal cytotoxic activity against the tGO target population. These data indicate that extended survival is a much more important parameter for tumor eradication in vivo than the ability to express FasL, and that FasL-independent effector mechanisms play a critical role in tumor immunity, even for FasL-sensitive tumor populations.

This was further demonstrated by injecting mice s.c. with a mixture of A20 and A20-tGO cells. DO11 Th1 cells were able to prevent or delay the growth of tumors in five of six mice (data not shown). As DO11 cells could not directly recognize the A20 tumor cells and could not prevent the outgrowth of tumor in mice challenged with only A20 cells, the elimination of the tumors in the mice challenged with A20 and A20-tGO cells must have occurred through indirect mechanisms, involving inflammatory cytokines and/or the activation of additional effector cells.

A number of studies have pointed to the importance of interactions between CD4 T cells and other effector cells in initiating and maintaining antitumor responses (13, 29–32). CD4 T cells can promote CD8 effector function indirectly, by CD40L-mediated activation of dendritic cells and other APCs (15–17, 33, 34). In addition to their important role in priming CD8 T cells, CD4 T cells can maintain CD8 T cell effector functions by secreting cytokines, such as IL-2, required for CD8 T cell growth and proliferation (35, 36). Moreover, memory CD8 T cells that are generated without CD4 help, by either deletion during the priming phase or in a CD4-deficient host, are defective in their ability to respond to secondary encounters with Ag (37, 38). The role of CD4 T cells in priming and maintaining CD8 cells may be of particular importance in the immunosuppressive environment of tumor-bearing individuals.

Apart from their ability to provide help for CD8 T cells, tumor-specific CD4 T cells can be directly involved in additional effector mechanisms of antitumor immunity. This is demonstrated by both
the ability of adoptively transferred CD4 T cells to mediate rejection of class II-deficient tumors and the decreased antitumor response in mice depleted of CD4 T cells after vaccination, but before tumor challenge (13, 29, 39). The cytokines produced by CD4 Th1 cells, including IL-2, IFN-γ, and TNF-α, can have a profound influence on the outcome of the immune response to a tumor. IFN-γ, the classic Th1 cytokine, has been shown to directly mediate tumor eradication through its antiangiogenic activity, its ability to up-regulate MHC class I expression, and the recruitment and activation of other effector cells such as macrophages (30, 40, 41). Macrophage activation by Th1 effectors leads to the production of reactive oxygen species, including NO and superoxide, which can mediate tumor rejection (29). TNF-α has direct antitumor activity and promotes the release of other proinflammatory cytokines, particularly IL-1, IL-6, and IL-8, as well as promoting protease production (42–45). CD4 cells may also play an important role in the activation of NK cells. IFN-γ production and the engagement of CD40 stimulate IL-12 production by APCs, which promotes Th1 responses and activates NK cells (46, 47).

Activated and rested Th1 cells showed a dramatic difference in their ability to eliminate tumors in vivo, which may be attributed to trafficking preferences of these cells following injection. Activated and rested T cells differentially express cell surface receptors and cytokines that affect their interactions with other cell types, including the vasculature and APCs. Vascular adhesion receptors involved in leukocyte trafficking into tissues, particularly P- and E-selectin ligands, are up-regulated following TCR engagement, and their increased expression may augment the antitumor response following adoptive transfer in vivo. Expression of vascular adhesion and integrin receptors plays a critical role in T cell migration into inflammatory sites (48–51). Resting T cells express an inactive form of the β1 integrins and adhere poorly to β1 integrin extracellular matrix ligands. Activation of T cells results in increased β1 integrin-mediated adhesion (52).

Antigenic modulation remains a significant problem in tumor immunotherapy. Although DO11/lpr and DO11/gld cells were able to cause regression of established tumors, secondary tumors that had lost tGO expression routinely developed in the draining lymph nodes. In vitro, expression of the tGO construct was maintained by the presence of G418 in the culture medium. However, when tumors were established in vivo before T cell transfer, it is likely that tGO-negative variants accumulated that the subsequently injected...
DO11 T cells were unable to recognize. Loss of Ag expression following induction of an immune response is a well-known complication of tumor cell biology (53). The use of target Ags from molecules required for tumor cell growth and/or survival, such as telomerase or the ari hydrocarbon receptor, might provide more stable targets for future studies (54–57). Also, the use of T cell pools specific for multiple Ag targets might overcome the ability of the tumor to evade the immune system. A combination of immunotherapy techniques, including transfer of T cells reactive to multiple Ags, adjuvants such as CpG or heat shock proteins, and cytokines may be necessary to generate an immune response that is strong enough to lead to tumor elimination (58–61). Prevention of AICD significantly enhances T cell effector activity. Targeting Fas expression could add an important weapon to the immunotherapy arsenal for the treatment of cancer.

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References