

Regulatory T Cells in Ovarian Cancer: Biology and Therapeutic Potential

Brian Barnett¹, Ilona Kryczek¹, Pui Cheng², Weiping Zou¹, Tyler J. Curiel¹

Departments of ¹Medicine (Hematology and Medical Oncology) and

²Obstetrics and Gynecology (Gynecologic Oncology), Tulane Medical School, New Orleans, LA, USA

Keywords

Denileukin diftitox (Ontak), immune evasion, immunity, immunotherapy, ovarian cancer, regulatory T cells

Correspondence

Tyler J Curiel, Department of Medicine (Hematology and Medical Oncology), Tulane Medical School, 1430 Tulane Avenue, New Orleans, LA 70112, USA.
E-mail: tcuriel@tulane.edu

Submitted September 6, 2005;
accepted September 26, 2005.

Citation

Barnett B, Kryczek I, Cheng P, Zou W, Curiel TJ. Regulatory T cells in ovarian cancer: biology and therapeutic potential. *Am J Reprod Immunol* 2005; 54:369–377

doi:10.1111/j.1600-0897.2005.00330.x

Tumors express tumor-associated antigens (TAA) and thus should be the object of immune attack. Nonetheless, spontaneous clearance of established tumors is rare.¹ Much work has demonstrated that tumors have numerous strategies either to prevent presentation of TAA, or to prevent TAA presentation in the context of T-cell co-signaling molecules.^{1,2} Thus, it was thought that lack of TAA-specific immunity was largely a passive process: tumors simply did not present enough TAA, or antigen-presenting cells did not have sufficient stimulatory capacity. On this basis, attempts were made to bolster TAA-specific immunity by using optimal antigen-presenting cells or by growing TAA-specific effector T cells *ex vivo* followed by adoptive transfer.³ These approaches met with some success in mouse models of human tumors, and showed some early clinical efficacy in human trials, although long-term efficacy remains to be established, and logistical problems are considerable.⁴ These studies established the concept that experimentally induced TAA-specific immunity is a rational and potentially efficacious means to treat cancer, including ovarian cancer. Nonetheless, recent work demonstrates that lack of naturally induced TAA-specific immunity is not simply a passive process.^{5–12} We discuss recent data clearly demonstrating that ‘tumors actively prevent induction of TAA-specific immunity through induction of TAA-specific tolerance’.¹³ This tolerance is mediated in part by regulatory T cells (Tregs). Means to revert these tolerizing conditions represent a novel anticancer therapeutic stratagem. We discuss Tregs in this regard in human ovarian cancer and present evidence that depleting Treg in human cancer, including ovarian cancer, using denileukin diftitox (Ontak), improves immunity and may be therapeutic.

Introduction

Ovarian cancer is the fifth leading cause of cancer deaths in American women. It usually presents as advanced disease, making cure unlikely. In 2005, it was estimated that there would be 22,200 new cases of ovarian cancer and 16,210 related deaths in the United States.¹⁴ The 2002 SEER database (the most recent date with complete data) estimates that there were 169,875 women alive in the United States

with ovarian cancer on January 1, 2002 (http://seer.cancer.gov/statfacts/html/ovary.html?statfacts_page=ovary.html&x=15&y=18).

Current treatment for advanced stage epithelial ovarian cancer consists of optimal surgical debulking followed by chemotherapy with carboplatin plus paclitaxel. Treatment for stage III or IV disease is rarely curative, with 5-year survival rates under 20%. There is no effective therapy for relapsed or metastatic disease that has failed first-line

therapy.¹⁵ Thus, effective new therapies for ovarian cancer are urgently needed. Our group focuses on novel immune-based strategies to treat ovarian cancer.

The tumor-associated antigen (TAA)-specific immunity has been demonstrated in ovarian cancer.^{16–25} Nonetheless, immune-based therapies for ovarian cancer have generally been clinically ineffective, as for most epithelial tumors.^{21–27} Our prior published works provide a reasonable and plausible explanation for past failures and points to novel immune-based strategies to treat ovarian cancer.^{12,24,25,28} We will review our work, as well as related, work in this regard.

Most studies of cancer immunotherapy to date have focused on augmenting immunity through active or passive strategies. Recent reports demonstrated that CTLA-4 blockade improved immunity and clinical outcomes in mouse²⁹ and human³⁰ melanoma. This latter important work demonstrates that the concept of reversing immunosuppression in cancer has merit as a therapeutic approach and provides further evidence that immune-based therapy will eventually find a meaningful place in the anticancer treatment armamentarium. However, it is unknown how best to accomplish this goal. Newer studies, including our work with Treg depletion discussed shortly, will help point the way to successful application of tumor immunotherapy.

The Immune Environment is Dysfunctional in Ovarian Cancer

We previously demonstrated that the chemokine stromal-derived factor-1 (CXCL12) induced the migration of plasmacytoid dendritic cells (PDC) into the tumor microenvironment in ovarian cancer, and delivered survival signals to PDC. Tumor microenvironmental PDC induced a certain type of Treg with a defined phenotype of Tr1.¹² Tumor microenvironmental PDC induced interleukin (IL)-10 expressing CD8⁺ Treg.³¹ Our subsequent studies demonstrated that tumor microenvironmental vascular endothelial growth factor (VEGF) induced expression of the T-cell co-signaling molecule B7-H1 on myeloid dendritic cells (MDC). This MDC B7-H1 provided a molecular signal for induction of Tregs with a Tr1-like phenotype. We further demonstrated that blocking B7-H1 improved T cell-mediated immune tumor clearance in a mouse model of human ovarian cancer.²⁴ Thus, many

mechanisms of immune suppression appear to converge at the level of inducing Tregs. These studies have revealed potential molecular targets for reversing active tumor-mediated immune evasion and set the stage for novel treatments to enhance antitumor immune therapy for ovarian cancer in human clinical trials.

Regulatory T Cells

CD4⁺ regulatory T cells (Tregs) were recently rediscovered, and shown to inhibit antigen-specific immunity.^{32–35} The best-characterized Treg subset expresses the IL-2 receptor- α chain (CD25). Under homeostatic conditions, these cells arise in the thymus, but may need to re-encounter antigen to become tolerized. Following T-cell receptor ligation, they are inhibitory through soluble cytokines, but may act primarily through contact-dependent mechanisms including CLTA-4.³⁶ CD4⁺ CD25⁺ Tregs also have antigen-independent suppressive effects.³⁷ A distinct phenotypic subset of Tregs is the Tr1 cell (IL-10^{hi}, moderate IL-5, transforming growth factor (TGF)- β and interferon (IFN)- γ , IL-2^{lo}, and IL-4⁻). IL-15 may be critical for their stimulation.³⁶ Tr1 cells suppress in part through IL-10 and TGF- β . CD4⁺ CD25⁺ Tregs help mediate peripheral tolerance in humans and mice, although details, mechanisms of action, and differentiation pathways of distinct Treg types are poorly understood.³⁸ CD4⁺ CD25⁺ Tregs inhibit both naive and memory T cells and can be maintained *in vitro*.³²

Why Tregs may be Elevated in Cancer, and the Problems they Create for the Immune System in Mounting Effective Antitumor Immunity?

Most TAA are self-antigens, and therefore subject to control by peripheral tolerance.³⁹ Thus, it has been proposed that exaggerated self-tolerance may be a critical mediator of suppressed tumor-specific immunity. Emerging evidence suggests that Tregs, particularly CD4⁺ CD25⁺ Tregs, are key mediators of peripheral tolerance.^{40–46} Therefore, engendering a strong antitumor response likely involves breaking Treg-mediated peripheral tolerance to TAA. Consistent with this concept, experimental depletion of Tregs in tumor-bearing mice improves immune-mediated tumor clearance,⁴⁷ improves TAA-specific immunity⁴⁸ and enhances the efficacy of tumor immunotherapy.^{49,50}

Tregs in Cancer

Given the suppressive nature of Tregs *in vitro*, it was reasonable to presume that they suppressed immunity *in vivo*. Evidence for Treg-mediated immune suppression has now been well established in mouse disease models. In tumor-bearing mice, CD4⁺ CD25⁺ Tregs suppress TAA-specific immunity.⁴⁸ B16 melanoma-bearing mice have improved IFN- α -induced tumor immunity when depleted of CD4⁺ CD25⁺ cells.⁵⁰ Blocking CTLA-4 improves tumor immunity better when Tregs are first depleted.⁴⁹ Tumor-bearing humans have elevated numbers of Tregs in blood malignant effusions.^{51–53} These Tregs inhibit non-specific T-cell activation.^{51,53} We just published the first report demonstrating that human CD4⁺ CD25⁺ Tregs inhibit TAA-specific immunity, allow tumor growth in the presence of TAA-specific immunity and predict poor survival in human ovarian cancer.²⁵ We also recently demonstrated that PDC in ovarian cancer induce differentiation of CD8⁺ IL-10⁺ Tregs that inhibit TAA-specific T cells.³¹

Our human studies, viewed in light of prior work in mice led us to predict that depleting CD4⁺ CD25⁺ Tregs would improve TAA-specific immunity in human cancer, and that their depletion would be therapeutic in ovarian cancer. This approach may also be successful in other tumor types, as functional Treg accumulate in many types of human cancer.^{25,43,47,51–58}

CD4⁺ CD25⁺ Cells Accumulate in Malignant Ascites and the Tumor Mass in Ovarian Cancer

In patients with previously untreated malignant ovarian epithelial cancers ($n = 45$), we identified a significant population of CD4⁺ CD25⁺ CD3⁺ T cells (10–17% of CD4⁺ T cells) by fluorescence-activated cell sorter (FACS) in malignant ascites. These were rarely seen in non-malignant ascites. We studied 104 frozen tumor tissues from patients with previously untreated ovarian epithelial cancers and identified substantial numbers of CD4⁺ CD25⁺ T cells by multiple color confocal microscopic analysis in the tumor mass. Stages III–IV tumors had greater CD4⁺ CD25⁺ T-cell numbers than stages I and II. Negligible numbers of CD4⁺ CD25⁺ T cells infiltrated normal ovarian tissues without cancer ($n = 5$). Taken together, our data demonstrate tumor-specific accumulation of CD4⁺ CD25⁺ T cells in malignant human ovarian

tissues. The CD4⁺ CD25⁺ CD3⁺ GITR⁺ CTLA-4⁺ CCR7⁺ CD62L⁺ FOXP3^{high} phenotype of tumor-associated CD4⁺ CD25⁺ T cells that we demonstrated suggests that these cells are Tregs.

Tumor CD4⁺ CD25⁺ T Cells Suppress T-cell Activation *in vitro* and *in vivo*

To test tumor CD4⁺ CD25⁺ T-cell function, we used a well described *in vitro* cellular culture system.^{51,59} Activated tumor ascites CD3⁺ CD25[−] T cells proliferated well and made significant IFN- γ and IL-2. Tumor Treg from ascites or the solid tumor mass significantly inhibited these processes. Based on these functional and phenotypic data, tumor-associated CD4⁺ CD25⁺ T cells are functional Tregs. Tumor CD4⁺ CD25⁺ Tregs spontaneously produced significantly more TGF- β and IL-10 and significantly less IL-2 compared with tumor CD4⁺ CD25[−] T cells (Table I). Thus, tumor CD4⁺ CD25⁺ Tregs may share features in common with thymic-derived natural Tregs and with induced Tr1 Tregs. We determined that ovarian cancer-associated Tregs inhibited TAA-specific T-cell proliferation, cytokine secretion and cytotoxicity in our *in vitro* system.^{12,24} We then used our previously described mouse model²⁴ to demonstrate functional Treg activity *in vivo*, demonstrating that these Tregs inhibited TAA-specific T cell-mediated clearance of autologous tumor.²⁵

Table I Tregs in Ovarian Cancer Exhibit a Tr1 Phenotype

Cytokines	Total CD3 ⁺ CD4 ⁺ T cells	CD25-depleted CD3 ⁺ CD4 ⁺ T cells
TGF- β	250* $\pm$ 109	40 $\pm$ 25
IL-10	80* $\pm$ 32	12 $\pm$ 6.5
IL-2	28* $\pm$ 12	62 $\pm$ 6.5

Total or CD25⁺-depleted tumor CD4⁺ T cells (10⁶/mL) were cultured for 48 hr ($n = 3$). Cytokines (pg/mL) in the supernatants were detected by ELISA (* $P < 0.01$).

CD25⁺ T cells were depleted with Miltenyi beads with approximately 80% efficiency. Thus, actual production of cytokines shown by CD4⁺CD25[−] T cells is likely even lower. These data suggest that CD4⁺CD25⁺ Tregs in ascites may function through IL-10 or TGF- β secretion, suggesting a Tr1 phenotype. ELISA, enzyme-linked immunosorbent assay; TGF, transforming growth factor; IL, interleukin.

Increased Tumor Treg Predict Poor Clinical Outcome

We found a significant ($P < 0.0001$) positive correlation between tumor infiltrating Treg accumulation and overall survival in the 66 patients with ovarian cancer available for such study. Survival for patients in the high Treg group was approximately one-fifth of that in the low Treg group (12.8 months versus 66.4 months; $P < 0.001$), demonstrating significant clinical relevance of tumor Treg infiltration.²⁵

Ontak Depletes Functional Tregs in Patients with Cancer

The above results lead us to hypothesize that depleting Tregs might be beneficial in human cancer, including ovarian cancer. In searching for a suitable agent for this purpose, we identified Ontak (denileukin diftitox), a fusion toxin consisting of IL-2 genetically fused to the enzymatically active and translocating domains of diphtheria toxin.⁶⁰ It is internalized into CD25⁺ cells by endocytosis. The ADP-ribosyltransferase activity of diphtheria toxin is cleaved in the endosome and is translocated into the cytosol where it inhibits protein synthesis, leading to apoptosis.⁶⁰ It is FDA-approved to treat CD4⁺ CD25⁺ cutaneous T-cell leukemia/lymphoma. Based on the phenotypic similarity of CD4⁺ CD25⁺ cutaneous T-cell leukemia/lymphoma cells and CD4⁺ CD25⁺ Tregs, we hypothesized that Ontak would deplete CD4⁺ CD25⁺ Tregs in humans.

We undertook a phase I/II dose-escalation trial of a single i.v. infusion of Ontak to test this concept. This study was approved by the Tulane Institutional Review Board, and all subjects gave written, informed consent. Patients were: (i) a 59-year-old female with stage IV ovarian cancer; (ii) a 41-year-old female with stage IV breast cancer; (iii) a 50-year-old male with stage IIIB squamous cell lung carcinoma; (iv) a 53-year-old female with stage IV ovarian carcinoma. Patients received no cytotoxic drugs, radiation therapy or immune-modulating agents for at least 30 days prior to study. They received 650 mg acetaminophen, 50 mg diphenhydramine, and 25 mg prochlorperazine prior to 60-min i.v. Ontak infusion at 9 µg/kg (patients 1–3) or 12 µg/kg (patient 4). Ontak was well tolerated. Blood was studied before (day 0) and 1 week after treatment, except as noted. Flow cytometry, intracellular cytokine detection, and cell purifications were

performed as we described.²⁵ Experimental differences were determined by *t*-test or chi-squared test as appropriate with $P \leq 0.05$ defined as significant.

Mean blood CD3⁺ CD4⁺ CD25⁺ T-cell prevalence was elevated at 25.3%, but dropped significantly ($P = 0.025$) to 17.7% after Ontak (Fig. 1A). Mean blood CD3⁺ CD4⁺ CD25⁺ cell concentration simultaneously fell from 123/mm³ to 63/mm³ ($P = 0.025$; Fig. 1B). Mean prevalence (0.95–3.0%) and concentration (8–27/mm³) of blood CD3⁺ T cells expressing the Ki-67 proliferation antigen increased following Ontak ($P \leq 0.03$ for each).

Mean blood IFN- γ ⁺ CD3⁺ T-cell prevalence (21.0–36.5%; $P = 0.046$; Fig. 1C) and concentration (173–264/mm³; $P = 0.05$; Fig. 1D) increased after Ontak. We obtained sufficient blood cells from patient 4 to quantify IFN- γ ⁺ CD3⁺ CD8⁺ T cells, whose prevalence (21–37%; Fig. 1E) and concentration (10–23/mm³) increased ($P < 0.05$ for each) following Ontak and remained elevated for a prolonged period. These data are consistent with prolonged immunologic improvement following CD3⁺ CD4⁺ CD25⁺ Treg depletion. We then undertook confirmatory functional studies.

FOXP3 is a forkhead/winged helix protein essential for CD4⁺ CD25⁺ Treg differentiation and function.⁶¹ We demonstrated that only CD3⁺ CD4⁺ CD25⁺ cells express FOXP3 in human cancer.²⁵ We obtained sufficient purified CD3⁺ CD4⁺ CD25⁺ T cells from patient 4 to test *FOXP3* message expression. Strong *FOXP3* message expression in CD3⁺ CD4⁺ CD25⁺ T cells was greatly reduced from 115 to 37 units 5 days after Ontak. Non-selective T-cell depletion cannot explain decreased *FOXP3* message because mean total CD3⁺ T cells (1030/mm³ before, versus 900/mm³ after) and mean CD3⁺ CD8⁺ T cells (450/mm³, comprising 48% of all T cells before, versus 423/mm³, comprising 50% of all T cells after) were not significantly altered. The prevalence and concentration of blood B cells and monocytes were not significantly altered by Ontak (not shown). These data are most consistent with selective depletion of CD3⁺ CD4⁺ CD25⁺ FOXP3⁺ T cells.

We obtained sufficient purified CD3⁺ CD4⁺ CD25⁺ T cells from patient 3 for functional assays. CD3⁺ CD4⁺ CD25⁺ T cells before Ontak were 4.2-fold more potent in suppressing T-cell proliferation compared with cells obtained 30 days after ($P = 0.008$), consistent with depletion of functional CD3⁺ CD4⁺ CD25⁺ Tregs. These data also demonstrate prolonged functional Treg depletion, further supported by increased IFN- γ ⁺ CD3⁺ CD8⁺ T-cell prevalence

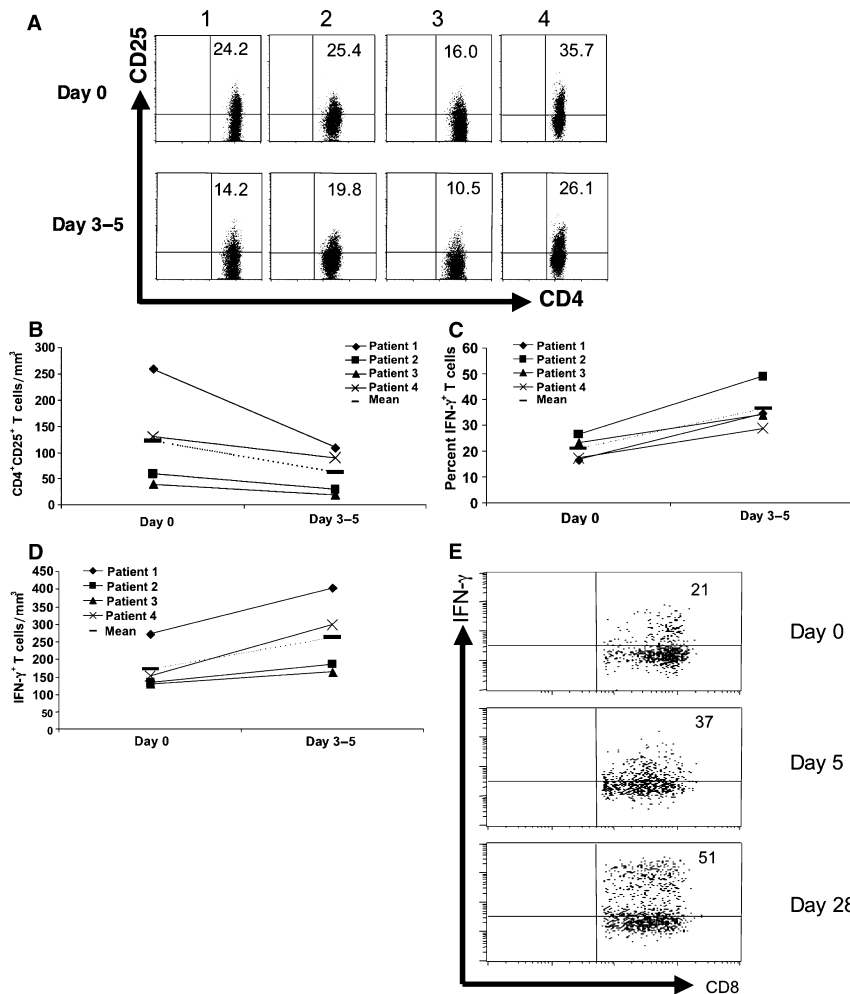


Fig. 1 Ontak reduces blood CD3⁺ CD4⁺ CD25⁺ cell numbers and improves immunity. (A) Flow cytometric CD3-gated analyses of CD4⁺ CD25⁺ cells were performed before (top) and 1 week after (bottom) a single Ontak infusion. Patient number is shown above the corresponding panels. Patient-specific and mean values for blood (B) CD3⁺ CD4⁺ CD25⁺ cell concentration, (C) interferon (IFN)- γ ⁺ CD3⁺ cell prevalence and (D) IFN- γ ⁺ CD3⁺ cell concentration were determined before and 1 week after Ontak. (E) CD3-gated flow cytometric analysis was performed on blood mononuclear cells from patient 4 following intracellular IFN- γ staining at indicated times after the first Ontak infusion at 12 μ g/kg. Quadrant numbers in panels are percentage of gated events.

and reduced CD3⁺ CD4⁺ CD25⁺ cell *FOXP3* message 28 days after Ontak.

This single-dose trial was designed to assess only immunologic end points. Activated effector cells may also express CD25⁴³, and could be depleted as well, which merits further investigation. Our data are consistent with the hypothesis that Treg depletion improves endogenous immunity in cancer patients. Nonetheless, the precise mechanism(s) underlying improved immunity remain to be established. Effects of Treg depletion on clinical outcomes are under study in our ongoing phase II efficacy trial of Ontak

treatment for ovarian cancer. In mouse models for melanoma, Treg depletion augmented CTLA-4 blockade⁴⁹ and augmented effects of IFN- α plus a dendritic cell-based vaccine.⁵⁰ Thus, combination approaches employing Ontak to deplete Tregs, followed by active vaccination may prove more efficacious.

Most cancer immunotherapy focuses on augmenting numbers and function of essential immune cells such as T lymphocytes and dendritic cells.^{13,25} Our data demonstrate that depleting dysfunctional Tregs is a promising strategy that may work alone, but which

also has potential to improve current active immunotherapies, whose successes in cancer treatment have thus far been modest. Ontak represents the first agent effective for human Treg depletion to test such concepts. Depletion of CD4⁺ CD25⁺ Tregs is necessary, but not sufficient to induce autoimmune phenomena,⁶² and we have not observed this problem to date. Nonetheless, the potential for inducing pathologic autoimmunity with this approach requires further study.

Conclusions

Despite a compelling logic, immune therapy for epithelial cancers is rarely effective. Failures of current strategies to induce significant antitumor immunity relate at least in part to the capacity of the tumor to activate tumor-mediated processes. Recent reports from mouse models demonstrate that killing Tregs augments endogenous tumor-specific immunity, and augments the efficacy of active immunization strategies. We recently demonstrated that Treg-mediated immunopathology defeats host antitumor immunity and is associated with poor tumor survival in ovarian cancer.²⁵ Our ongoing clinical trial demonstrates that Treg depletion is feasible in human cancer using Ontak, and is associated with improved immunity. Current data confirm that a single i.v. dose of Ontak at 12 µg/kg reduces phenotypic and functional CD4⁺ CD25⁺ blood Tregs, paving the way for studies of clinical efficacy alone or in combination with additional treatments.

Means to overcome active tumor-mediated immune subversion may finally allow the realization of the benefits of immune-based therapy for cancer.

Acknowledgments

This work was supported by The Ovarian Cancer Research Fund, CA100425, CA105207, CA092562, CA100227, and the Tulane Endowment. Thanks to Ligand Pharmaceuticals for providing Ontak for the clinical trial. Thanks to Tracey Todd, Shuang Wei, Ben Daniel, Michael Brumlik, and Pete Mottram for excellent technical assistance.

References

- 1 Pardoll D: T cells and tumours. *Nature* 2001; 411:1010–1012.
- 2 Albert ML, Sauter B, Bhardwaj N: Dendritic cells acquire antigen from apoptotic cells and induce class I-restricted CTLs. *Nature* 1998; 392:86–89.
- 3 Banchereau J, Briere F, Caux C, Davoust J, Lebecque S, Liu YJ, Pulendran B, Palucka K: Immunobiology of dendritic cells. *Annu Rev Immunol* 2000; 18: 767–811.
- 4 Curiel TJ, Curiel DT: Tumor immunotherapy: inching toward the finish line. *J Clin Invest* 2002; 109:311–312.
- 5 Gabrilovich DI, Nadaf S, Corak J, Berzofsky JA, Carbone DP: Dendritic cells in antitumor immune responses: II. Dendritic cells grown from bone marrow precursors, but not mature DC from tumor-bearing mice, are effective antigen carriers in the therapy of established tumors. *Cell Immunol* 1996; 170:111–119.
- 6 Gabrilovich DI, Corak J, Ciernik IF, Kavanaugh D, Carbone DP: Decreased antigen presentation by dendritic cells in patients with breast cancer. *Clin Cancer Res* 1997; 3:483–490.
- 7 Gabrilovich DI, Ciernik IF, Carbone DP: Dendritic cells in antitumor immune responses: I. Defective antigen presentation in tumor-bearing hosts. *Cell Immunol* 1996; 170:101–110.
- 8 Gabrilovich DI, Chen HL, Girgis KR, Cunningham HT, Meny GM, Nadaf S, Kavanaugh D, Carbone DP: Production of vascular endothelial growth factor by human tumors inhibits the functional maturation of dendritic cells *Nat Med* 1996; 2:1096–1103 (erratum appears in *Nat Med* 1996; November 2 (11):1267).
- 9 Gabrilovich D, Ishida T, Oyama T, Ran S, Kravtsov V, Nadaf S, Carbone DP: Vascular endothelial growth factor inhibits the development of dendritic cells and dramatically affects the differentiation of multiple hematopoietic lineages in vivo. *Blood* 1998; 92:4150–4166.
- 10 Chomarat P, Banchereau J, Davoust J, Palucka AK: IL-6 switches the differentiation of monocytes from dendritic cells to macrophages. *Nat Immunol* 2000; 1:510–514.
- 11 Menetrier-Caux C, Montmain G, Dieu MC, Bain C, Favrot MC, Caux C, Blay JY: Inhibition of the differentiation of dendritic cells from CD34(+) progenitors by tumor cells: role of interleukin-6 and macrophage colony-stimulating factor. *Blood* 1998; 92:4778–4791.
- 12 Zou W, Machelon V, Coulomb-L'Hermin A, Borvak J, Nome F, Isaeva T, Wei S, Krzysiek R, Durand-Gasselin I, Gordon A, Pustilnik T, Curiel DT, Galanaud P, Capron F, Emilie D, Curiel TJ: Stromal-derived factor-1 in human tumors recruits and alters the function of plasmacytoid precursor dendritic cells. *Nat Med* 2001; 7:1339–1346.

- 13 Zou W: Immunosuppressive networks in the tumour environment and their therapeutic relevance. *Nat Rev Cancer* 2005; 5:263–274.
- 14 Jamal A, Murray T, Ward E, Samuels A: Cancer statistics, 2005. *CA Clin J Cancer* 2005; 55:10–30.
- 15 Disis ML, Rivkin S: Future directions in the management of ovarian cancer. *Hematol Oncol Clin North Am* 2003; 17:1075–1085.
- 16 Albert ML, Darnell JC, Bender A, Francisco LM, Bhardwaj N, Darnell RB: Tumor-specific killer cells in paraneoplastic cerebellar degeneration. *Nat Med* 1998; 4:1321–1324.
- 17 Gong J, Nikrui N, Chen D, Koido S, Wu Z, Tanaka Y, Cannistra S, Avigan D, Kufe D: Fusions of human ovarian carcinoma cells with autologous or allogeneic dendritic cells induce antitumor immunity. *J Immunol* 2000; 165:1705–1711.
- 18 Ioannides CG, Fisk B, Pollack MS, Frazier ML, Taylor Wharton J, Freedman RS: Cytotoxic T-cell clones isolated from ovarian tumour infiltrating lymphocytes recognize common determinants on non-ovarian tumour clones. *Scand J Immunol* 1993; 37:413–424.
- 19 Peoples GE, Goedegebuure PS, Smith R, Linehan DC, Yoshino I, Eberlein TJ: Breast and ovarian cancer-specific cytotoxic T lymphocytes recognize the same HER2/neu-derived peptide. *Proc Natl Acad Sci U S A* 1995; 92:432–436.
- 20 Wagner U, Schlebusch H, Kohler S, Schmolling J, Grunn U, Krebs D: Immunological responses to the tumor-associated antigen CA125 in patients with advanced ovarian cancer induced by the murine monoclonal anti-idiotypic vaccine ACA125. *Hybridoma* 1997; 16:33–40.
- 21 Disis ML, Gooley TA, Rinn K, Davis D, Piepkorn M, Cheever MA, Knutson KL, Schiffman K: Generation of T-cell immunity to the HER-2/neu protein after active immunization with HER-2/neu peptide-based vaccines. *J Clin Oncol* 2002; 20:2624–2632.
- 22 Knutson KL, Schiffman K, Cheever MA, Disis ML: Immunization of cancer patients with a HER-2/neu, HLA-A2 peptide, p369–377, results in short-lived peptide-specific immunity. *Clin Cancer Res* 2002; 8:1014–1018.
- 23 Qian HN, Liu GZ, Cao SJ, Feng J, Ye X: The experimental study of ovarian carcinoma vaccine modified by human B7-1 and IFN-gamma genes. *Int J Gynecol Cancer* 2002; 12:80–85.
- 24 Curiel TJ, Wei S, Dong H, Alvarez X, Cheng P, Mottram P, Krzysiek R, Knutson KL, Daniel B, Zimmermann MC, David O, Burow M, Gordon A, Dhurandhar N, Myers L, Berggren R, Hemminki A, Alvarez RD, Emilie D, Curiel DT, Chen L, Zou W: Blockade of B7-H1 improves myeloid dendritic cell-mediated antitumor immunity. *Nat Med* 2003; 9:562–567.
- 25 Curiel TJ, Coukos G, Zou L, Alvarez X, Cheng P, Mottram P, Evdemon-Hogan M, Conejo-Garcia JR, Zhang L, Burow M, Zhu Y, Wei S, Kryczek I, Daniel B, Gordon A, Myers L, Lackner A, Disis ML, Knutson KL, Chen L, Zou W: Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival. *Nat Med* 2004; 10:942–949.
- 26 Disis ML, Knutson KL, Schiffman K, Rinn K, McNeel DG: Pre-existent immunity to the HER-2/neu oncogenic protein in patients with HER-2/neu overexpressing breast and ovarian cancer. *Breast Cancer Res Treat* 2000; 62:245–252.
- 27 Murray JL, Gillogly ME, Przepiorka D, Brewer H, Ibrahim NK, Booser DJ, Hortobagyi GN, Kudelka AP, Grabstein KH, Cheever MA, Ioannides CG: Toxicity, immunogenicity, and induction of E75-specific tumor-lytic CTLs by HER-2 peptide E75 (369–377) combined with granulocyte macrophage colony-stimulating factor in HLA-A2+ patients with metastatic breast and ovarian cancer. *Clin Cancer Res* 2002; 8:3407–3418.
- 28 Knutson K, Curiel T, Salazar L, Disis M: Immunologic principles and immunotherapeutic approaches in ovarian cancer. *Hematol Oncol Clin North Am* 2003; 17:1051–1073.
- 29 Phan GQ, Yang JC, Sherry RM, Hwu P, Topalian SL, Schwartzentruber DJ, Restifo NP, Haworth LR, Seipp CA, Freezer LJ, Morton KE, Mavroukakis SA, Duray PH, Steinberg SM, Allison JP, Davis TA, Rosenberg SA: Cancer regression and autoimmunity induced by cytotoxic T lymphocyte-associated antigen 4 blockade in patients with metastatic melanoma. *Proc Natl Acad Sci U S A* 2003; 100:8372–8377.
- 30 Hodi FS, Mihm MC, Soiffer RJ, Haluska FG, Butler M, Seiden MV, Davis T, Henry-Spires R, MacRae S, Willman A, Padera R, Jaklitsch MT, Shankar S, Chen TC, Korman A, Allison JP, Dranoff G: Biologic activity of cytotoxic T lymphocyte-associated antigen 4 antibody blockade in previously vaccinated metastatic melanoma and ovarian carcinoma patients. *Proc Natl Acad Sci U S A* 2003; 100:4712–4717.
- 31 Wei S, Kryczek I, Zou L, Daniel B, Cheng P, Mottram P, Curiel T, Lange A, Zou W: Plasmacytoid dendritic cells induce CD8+ regulatory T cells in human ovarian carcinoma. *Cancer Res* 2005; 65:5020–5026.
- 32 Levings MK, Sangregorio R, Roncarolo MG: Human cd25(+)cd4(+) T regulatory cells suppress naive and memory T cell proliferation and can be expanded in vitro without loss of function. *J Exp Med* 2001; 193:1295–1302.

- 33 Piccirillo CA, Shevach EM: Cutting edge: control of cd8(+) T cell activation by cd4(+)cd25(+) immunoregulatory cells. *J Immunol* 2001; 167:1137–1140.
- 34 Dieckmann D, Plottner H, Berchtold S, Berger T, Schuler G: Ex vivo isolation and characterization of CD4(+)CD25(+) T cells with regulatory properties from human blood. *J Exp Med* 2001; 193:1303–1310.
- 35 Jonuleit H, Giesecke-Tuettgenberg A, Tuting T, Thurner-Schuler B, Stuge TB, Paragnik L, Kandemir A, Lee PP, Schuler G, Knop J, Enk AH: A comparison of two types of dendritic cell as adjuvants for the induction of melanoma-specific T-cell responses in humans following intranodal injection. *Int J Cancer* 2001; 93:243–251.
- 36 Roncarolo MG, Levings MK, Traversari C: Differentiation of T regulatory cells by immature dendritic cells. *J Exp Med* 2001; 193:F5–F9.
- 37 Thornton AM, Shevach EM: Suppressor effector function of CD4+CD25+ immunoregulatory T cells is antigen nonspecific. *J Immunol* 2000; 164:183–190.
- 38 Gavin MA, Clarke SR, Negrou E, Gallegos A, Rudensky A: Homeostasis and anergy of CD4(+)CD25(+) suppressor T cells in vivo. *Nat Immunol* 2002; 3:33–41.
- 39 Overwijk WW, Theoret MR, Finkelstein SE, Surman DR, de Jong LA, Vyth-Dreese FA, DelleMijn TA, Antony PA, Spiess PJ, Palmer DC, Heimann DM, Klebanoff CA, Yu Z, Hwang LN, Feigenbaum L, Kruisbeek AM, Rosenberg SA, Restifo NP: Tumor regression and autoimmunity after reversal of a functionally tolerant state of self-reactive CD8+ T cells. *J Exp Med* 2003; 198:569–580.
- 40 Sakaguchi S, Sakaguchi N, Asano M, Itoh M, Toda M: Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. *J Immunol* 1995; 155:1151–1164.
- 41 Wood KJ, Sakaguchi S: Regulatory T cells in transplantation tolerance. *Nat Rev Immunol* 2003; 3:199–210.
- 42 von Herrath MG, Harrison LC: Antigen-induced regulatory T cells in autoimmunity. *Nat Rev Immunol* 2003; 3:223–232.
- 43 Shevach EM: CD4+ CD25+ suppressor T cells: more questions than answers. *Nat Rev Immunol* 2002; 2:389–400.
- 44 Taylor PA, Noelle RJ, Blazar BR: CD4(+)CD25(+) immune regulatory cells are required for induction of tolerance to alloantigen via costimulatory blockade. *J Exp Med* 2001; 193:1311–1318.
- 45 Taylor PA, Lees CJ, Blazar BR: The infusion of ex vivo activated and expanded CD4(+)CD25(+) immune regulatory cells inhibits graft-versus-host disease lethality. *Blood* 2002; 99:3493–3499.
- 46 Taylor PA, Friedman TM, Korngold R, Noelle RJ, Blazar BR: Tolerance induction of alloreactive T cells via ex vivo blockade of the CD40: CD40L costimulatory pathway results in the generation of a potent immune regulatory cell. *Blood* 2002; 99:4601–4609.
- 47 Shimizu J, Yamazaki S, Sakaguchi S: Induction of tumor immunity by removing CD25+CD4+ T cells: a common basis between tumor immunity and autoimmunity. *J Immunol* 1999; 163:5211–5218.
- 48 Tanaka H, Tanaka J, Kjaergaard J, Shu S: Depletion of CD4+ CD25+ regulatory cells augments the generation of specific immune T cells in tumor-draining lymph nodes. *J Immunother* 2002; 25:207–217.
- 49 Suttmuller RP, van Duivenvoorde LM, van Elsas A, Schumacher TN, Wildenberg ME, Allison JP, Toes RE, Offringa R, Melief CJ: Synergism of cytotoxic T lymphocyte-associated antigen 4 blockade and depletion of CD25(+) regulatory T cells in antitumor therapy reveals alternative pathways for suppression of autoreactive cytotoxic T lymphocyte responses. *J Exp Med* 2001; 194:823–832.
- 50 Steitz J, Bruck J, Lenz J, Knop J, Tuting T: Depletion of CD25(+) CD4(+) T cells and treatment with tyrosinase-related protein 2-transduced dendritic cells enhance the interferon alpha-induced, CD8(+) T-cell-dependent immune defense of B16 melanoma. *Cancer Res* 2001; 61:8643–8646.
- 51 Woo EY, Yeh H, Chu CS, Schlienger K, Carroll RG, Riley JL, Kaiser LR, June CH: Regulatory T cells from lung cancer patients directly inhibit autologous T cell proliferation. *J Immunol* 2002; 168:4272–4276.
- 52 Woo EY, Chu CS, Goletz TJ, Schlienger K, Yeh H, Coukos G, Rubin SC, Kaiser LR, June CH: Regulatory CD4(+)CD25(+) T cells in tumors from patients with early-stage non-small cell lung cancer and late-stage ovarian cancer. *Cancer Res* 2001; 61:4766–4772.
- 53 Liyanage UK, Moore TT, Joo HG, Tanaka Y, Herrmann V, Doherty G, Drebin JA, Strasberg SM, Eberlein TJ, Goedegebuure PS, Linehan DC: Prevalence of regulatory T cells is increased in peripheral blood and tumor microenvironment of patients with pancreas or breast adenocarcinoma. *J Immunol* 2002; 169:2756–2761.
- 54 Javia LR, Rosenberg SA: CD4+CD25+ suppressor lymphocytes in the circulation of patients immunized against melanoma antigens. *J Immunother* 2003; 26:85–93.
- 55 Somasundaram R, Jacob L, Swoboda R, Caputo L, Song H, Basak S, Monos D, Peritt D, Marincola F, Cai D, Birebent B, Bloome E, Kim J, Berencsi K,

- Mastrangelo M, Herlyn D: Inhibition of cytolytic T lymphocyte proliferation by autologous CD4+/CD25+ regulatory T cells in a colorectal carcinoma patient is mediated by transforming growth factor-beta. *Cancer Res* 2002; 62:5267–5272.
- 56 Wolf AM, Wolf D, Steurer M, Gastl G, Gunsilius E, Grubeck-Loebenstien B: Increase of regulatory T cells in the peripheral blood of cancer patients. *Clin Cancer Res* 2003; 9:606–612.
- 57 Sasada T, Kimura M, Yoshida Y, Kanai M, Takabayashi A: CD4+CD25+ regulatory T cells in patients with gastrointestinal malignancies: possible involvement of regulatory T cells in disease progression. *Cancer* 2003; 98:1089–1099.
- 58 Francois Bach J: Regulatory T cells under scrutiny. *Nat Rev Immunol* 2003; 3:189–198.
- 59 Baecher-Allan C, Viglietta V, Hafler DA: Inhibition of human CD4(+)CD25(+high) regulatory T cell function. *J Immunol* 2002; 169:6210–6217.
- 60 Foss FM: DAB(389)IL-2 (Ontak): a novel fusion toxin therapy for lymphoma. *Clin Lymphoma* 2000; 1:110–116; discussion 117.
- 61 Fontenot JD, Gavin MA, Rudensky AY: Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. *Nat Immunol* 2003; 4:330–336.
- 62 McHugh RS, Shevach EM: Cutting edge: depletion of CD4+CD25+ regulatory T cells is necessary, but not sufficient, for induction of organ-specific autoimmune disease. *J Immunol* 2002; 168:5979–5983.