Plasmacytoid Dendritic Cells Induce CD8\(^+\) Regulatory T Cells In Human Ovarian Carcinoma

Shuang Wei,\(^1\) Ilona Kryczek,\(^1,2\) Linhua Zou,\(^1\) Ben Daniel,\(^1\) Pui Cheng,\(^1\) Peter Mottram,\(^1\) Tyler Curiel,\(^1\) Andrzej Lange,\(^2\) and Weiping Zou\(^1\)

\(^1\)Tulane University Health Science Center, New Orleans, Louisiana and \(^2\)Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wroclaw, Poland

Abstract

To directly dissect the role of each immune component in human tumor immunopathogenesis, we have studied the interaction between dendritic cells and T cells in the tumor environment of patients with ovarian carcinoma. We previously reported that functional plasmacytoid dendritic cells, but not functionally mature myeloid dendritic cells, accumulated in tumor microenvironments. We now show that tumor ascites plasmacytoid dendritic cells induce tumor-associated antigen–specific CD8\(^+\) T cells with effector functions. Strikingly, tumor ascites plasmacytoid dendritic cells induced interleukin-10 (IL-10) and CD45RO\(^+\)CD8\(^+\) regulatory T cells. Four characteristics have been identified in tumor plasmacytoid dendritic cell–induced CD8\(^+\) regulatory T cells: (a) induction of CD8\(^+\) regulatory T cells is independent of CD4\(^+\)CD25\(^+\) CD45RO\(^+\)CD8\(^+\) regulatory T cells; (b) CD8\(^+\) regulatory T cells significantly suppress myeloid dendritic cell–mediated tumor-associated antigen–specific T cell effector functions through interleukin-10; (c) repetitive myeloid dendritic cell stimulation can recover CD8\(^+\) regulatory T cell–mediated tumor cell proliferation, but not T cell effector function; (d) CD8\(^+\) regulatory T cells express functional CCR7, and efficiently migrate with lymphoid homing chemokine MIP-3\(\beta\). Primary suppressive CD45RO\(^+\)CD8\(^+\) regulatory T cells are found in the tumor environment of patients with ovarian cancers. Thus, tumor-associated plasmacytoid dendritic cells contribute to the tumor environmental immunosuppressive network. Collectively, tumors manipulate tumor microenvironmental dendritic cell subset distribution and function to subvert tumor immunity. The data are relevant to understanding tumor immunopathology as well as reevaluating tumor immunotherapeutic strategies. (Cancer Res 2005; 65(12): 5020-6)

Introduction

Human cancers are able to prime tumor-specific immunity. There exist tumor-specific T lymphocytes in blood, tumor tissues, and lymph nodes in cancer patients. Isolated tumor-associated antigen–specific T cells can kill autologous tumor cells. Functional tumor-associated antigen–specific T cells can be established from tumor T cells (1–5). Furthermore, studies have shown increases in tumor-associated antigen–specific cytotoxic CD8\(^+\) T cells without evidence of clinical regression in some patients (2, 6, 7). This suggests that tumor-associated antigen–specific T cells might be functionally inhibited in vivo. The underlying mechanisms were extensively reviewed (8).

Dendritic cells play a critical role in establishing tumor immunity. However, because of the rarity of dendritic cells in vivo, primary dendritic cell studies are confined to in vitro differentiated myeloid dendritic cells. Published, detailed studies of functional human dendritic cells in tumor environment are rare (9). Furthermore, another dendritic cell subset, plasmacytoid dendritic cells are technically more difficult to study. T cell activation pattern induced by plasmacytoid dendritic cells are largely determined in the allogeneic system. Little is known of the role of tumor environmental plasmacytoid dendritic cells in tumor immunity (9, 10). These limitations may significantly underestimate the impact of the dendritic cell system in tumor pathology. We have studied malignant ascites of patients with ovarian carcinoma. Malignant ascites contains viable tumor and immune cells, and serves as a model for the tumor microenvironment (9–13). We identified a significant population of plasmacytoid dendritic cells in malignant ascites. In an allogeneic system, normal blood plasmacytoid dendritic cells (14, 15) and tumor plasmacytoid dendritic cells (9) induce interleukin (IL)-10 expressing suppressive T cells. In this report, we further studied the pathologic interaction between tumor ascites T cells and tumor ascites plasmacytoid dendritic cells, and its impact on tumor-associated antigen–specific T cell immunity.

Materials and Methods

Human subjects. We studied previously untreated patients with epithelial ovarian carcinomas, International Federation of Gynecology and Obstetrics stages I, II, III, and IV. Patients gave written, informed consent. Clinical specimens were collected at the Tulane University, New Orleans. The study was approved by local Institutional Review Boards.

Ascites, ascites cells, and tumor-draining lymph nodes. We collected and prepared single cells from ascites, blood, and lymph nodes as previously described (9, 16). CD3, CD3\(\text{4+}\), CD16, CD19, and CD56-expressing cells were depleted using paramagnetic beads (Miltenyi, Auburn, CA), and plasmacytoid dendritic cells were sorted with FACSAria gating on CD4\(\text{4+}\)CD123\(\text{hi+}\)HLA-DR\(\text{bright}\)CD11c\(^-\) cells. Cell populations were ≥99% pure by flow cytometry analysis (fluorescence-activated cell sorting, FACS). Tumor ascites T cells were obtained by depletion CD14, CD16, CD19, and CD56, and CD123-expressing cells using microbeads (Miltenyi), and sorting CD45\(^-\) cells by flow cytometry. Cellular purity was ≥98% as determined by FACS staining with anti-human CD3 antibody. Antibodies for flow cytometry and depletion were purchased from BD Pharmingen, San Jose, CA. Autologous ascites cells were used throughout this study.

Peripheral blood cells. Peripheral blood was collected aseptically from normal volunteers or patients by venipuncture or by cytophoresis, and mononuclear cells were then obtained by ficoll-hypaque density centrifugation. Blood plasmacytoid dendritic cells were obtained as described for tumor ascites plasmacytoid dendritic cells. Cells were ≥98% pure as determined by FACS.

Note: S. Wei and I. Kryczek contributed equally to this work.

Requests for reprints: Weiping Zou, Tulane University Health Science Center, Section of Hematology and Medical Oncology, 1430 Tulane Avenue, New Orleans, LA 70112-2699. Phone: 504-988-3562; Fax: 504-988-5483; E-mail: wzou@tulane.edu.

© 2005 American Association for Cancer Research.

Cancer Res 2005; 65(12): 5020-6

www.aacrjournals.org
Tumor Plasmacytoid Dendritic Cells Induce CD8\(^+\) Regulatory T Cells

In vitro tumor-associated antigen–specific T cell stimulation. Tumor myeloid dendritic cells were differentiated from tumor macrophages as previously described (17). Tumor plasmacytoid dendritic cells were sorted from ascites as we previously described (9). Dendritic cells were activated with CD40L (200 ng/mL) for 24 hours (17), and loaded with three distinct HLA-A2-binding Her-2/neu peptides (13, 18) at 5 μg/mL each: p369-384 (KIFGSLAFLEPSDFGDPA), p688-703 (RRLLQETULVEPTPS), and p971-984 (ELYSEF5MRADPO). Peptide-loaded myeloid dendritic cells (tumor-associated antigen–myeloid dendritic cell) or peptide-loaded plasmacytoid dendritic cells (tumor-associated antigen–plasmacytoid dendritic cells; 10\(^{4}-10^{5}/\)mL) were used to stimulate autologous tumor ascites T cells (5 × 10\(^{5}/\)mL) in the presence of IL-2 (50 units/mL; R & D Systems, Minneapolis, MN) for 6 days (one round of stimulation). In some experiments, CD25\(^+\) or CCR7\(^+\) ascites T cells were initially depleted with magnetic beads, and CD25\(^+\) or CCR7\(^+\) T cells were then stimulated with tumor-associated antigen–plasmacytoid dendritic cells. T cells might receive several rounds of the identical stimulation as noted. T cell phenotypes, tumor-associated antigen–specific T cell proliferation, cytokines, and cytotoxic activity were detected as we described (9, 16). In some cases, T cells were stained with mouse anti-human CCR7-PE (clone 150503, mouse IgG2a, D) mouse anti-human CD8-APC (289-13,604, IgG2a, Pharmingen) and anti-human IL-10 (JES-19F1, rat anti-human IgG2a, Pharmingen). At least 5,000 gated events per condition were analyzed using CellQuest software (Becton Dickinson, Mountain View, CA).

In vitro allogeneic T cell immunosuppression assay. Normal peripheral blood CD3\(^+\) T cells (4 × 10\(^{5}/\)mL) were stimulated with 2.5 μg/mL anti-human CD3 (BD Pharmingen) and blood monocytes (1 × 10\(^{5}/\)mL) in the presence of different concentrations of freshly sorted allogeneic CCR7\(^+\)CD45RO\(^+\)CD8\(^+\) T cells from peripheral blood in normal donors or ascites in patients with ovarian cancer. Seventy-two hours after coculture, T cell proliferation was evaluated with thymidine incorporation.

In vitro tumor-associated antigen–specific immunosuppression assay. After two rounds of stimulation with tumor-associated antigen–plasmacytoid dendritic cells, ascites T cells (0.5-2 × 10\(^{5}/\)mL; on day 13) were added into tumor-associated antigen–myeloid dendritic cells (10\(^{4}-10^{5}/\)mL) and primary ascites T cell (5 × 10\(^{5}/\)mL) coculture for 6 days. Tumor-associated antigen–specific T cell proliferation and cytokines were detected as we described (9, 13, 16). In some cases, anti-human IL-10 receptor (250 ng/mL; R&D Systems) and anti-human IL-10 (clone 14-2–3-8, rat anti-human IL-10, Pharmingen) were added into the coculture.

Migration assay. After two rounds of stimulation with tumor-associated antigen–plasmacytoid dendritic cells, tumor-associated antigen–myeloid dendritic cells, day 13 T cells (2 × 10\(^{5}/\)mL) were initially depleted with 500 ng/mL mouse anti-human CCR7 monoclonal antibody (clone 150503, mouse IgG2a, R & D), mouse anti-human CCR7 monoclonal antibody (clone C55-155, mouse IgG2a, BD Pharmingen) and human anti-human CCR7 (clone 3D5, BD Pharmingen) were added into the coculture.

Results

Tumor plasmacytoid dendritic cells and myeloid dendritic cells induce distinct T cell activation. To determine the pathologic significance of tumor environmental plasmacytoid and myeloid dendritic cells on tumor-associated antigen–specific immunity, we used our previously described in vitro tumor-associated antigen–specific culture system (9, 13, 16). Myeloid dendritic cells were differentiated in vitro from ascites macrophages of HLA-A2\(^+\) patients as described in ref. (17), and loaded with HLA-A2-specific Her-2/neu peptides (tumor-associated antigen–myeloid dendritic cells; refs. 13, 18). Autologous CD40L-activated tumor-associated antigen–myeloid dendritic cells induced specific T cell activation as expected (Fig. 1).

To study the function of tumor plasmacytoid dendritic cells in the context of tumor-associated antigen–specific T cell immunity, we sorted tumor plasmacytoid dendritic cells from ascites (9, 20). The functional identity of plasmacytoid dendritic cells were confirmed by their capacity to produce copious amounts of type I IFN after adenovirus activation (9). Tumor plasmacytoid dendritic cells were loaded with HLA-A2-specific Her-2/neu peptides (tumor-associated antigen–plasmacytoid dendritic cell). Autologous CD40L-activated tumor-associated antigen–plasmacytoid dendritic cells induced a modest T cell proliferation. Tumor plasmacytoid dendritic cells were significantly less efficient in inducing T cell proliferation than myeloid dendritic cells (n = 4; *, P < 0.001 for all; Fig. 1A). Strikingly, tumor plasmacytoid dendritic cells induced strong T cell IL-10 (221 ± 53 pg/mL; Fig. 1B), little T cell IFN-\(\gamma\) (Fig. 1C), and poor cytotoxic activity (Fig. 1D; n = 4; *, P < 0.001 for all, compared with myeloid dendritic cell). No significant T cell IL-4, IL-5, IL-13 transcription factors and poor cytotoxic activity (Fig. 1D; n = 4; *, P < 0.001 for all, compared with myeloid dendritic cell). No significant T cell IL-4, IL-5, IL-13 and transforming growth factor-\(\beta\) (TGF-\(\beta\)) were detected. Primary tumor ascites tumor-associated antigen–plasmacytoid dendritic cells (without CD40L activation) did not induce important T cell proliferation (<1,000 c.p.m.) and cytokine production (IL-10, <30 pg/mL; IFN-\(\gamma\), <0.1 ng/mL). The results indicate that tumor plasmacytoid and myeloid dendritic cells induced distinct T cell responses.

Tumor ascites contain CD4\(^+\)CD25\(^+\)FOXP3\(^+\) T cells (CD4\(^+\) regulatory T cells; ref. 13). To determine whether CD4\(^+\) regulatory T cells affect IL-10 production induced by tumor plasmacytoid dendritic cells, we did similar experiments by initially deleting CD25\(^+\) ascites T cells with magnetic beads, and stimulated CD25\(^+\) ascites T cells with tumor-associated antigen–plasmacytoid dendritic cells. We detected similar level of IL-10 (195 ± 73 pg/mL versus 221 ± 53 pg/mL; n = 4) in the supernatant from CD25\(^+\) ascites T cell and tumor-associated antigen–plasmacytoid dendritic cell coculture (P = 0.078, compared with no CD25 deletion). The data suggest that CD4\(^+\) regulatory T cells are not essential for tumor plasmacytoid dendritic cells to induce IL-10-expressing T cells.

Tumor plasmacytoid dendritic cells induced IL-10-CCR7\(^+\)CD45RO\(^+\)CD8\(^+\) T cells. We next examined the phenotype of IL-10-expressing T cells induced by tumor plasmacytoid dendritic
cells. Intracellular staining showed that tumor plasmacytoid dendritic cells induced IL-10⁺CD8⁺ T cells (n = 4; Fig. 2A). Multiple-color FACS analysis further showed that 100% of IL-10⁺ T cells (gate 1) were CCR7⁺CD45RO⁺CD8⁺ T cells and IL-10⁻CD8⁺ T cells (gate 2) were CCR7⁻CD45RO⁺ T cells, and IL-10⁻CD8⁻ cells (gate 3) were largely CCR7⁻CD45RO⁺ cells (Fig. 2A).

FACS analysis revealed that primary tumor ascites contained CCR7⁺CD45RO⁺CD8⁺ T cells (Table 1). The level of CD45RO⁻CCR7⁺CD8⁺CD3⁺ cells was significantly higher in ascites, blood, and draining lymph nodes in patients with ovarian cancer than that in blood from normal donors (P < 0.01 for all; Table 1). Furthermore, the level of CCR7⁺CD45RO⁺CD8⁺CD3⁺ cells was also significantly higher in malignant ascites and tumor-draining lymph nodes than in peripheral blood (P < 0.01) in the same individual patients (Table 1).

To determine whether original ascites CCR7⁺CD8⁺ T cells affected the induction of IL-10⁺CCR7⁺CD45RO⁺CD8⁺ T cells by tumor plasmacytoid dendritic cells, we did the similar experiments by initially deleting CCR7⁺ ascites T cells with magnetic beads, and stimulated CCR7⁻ ascites T cells with tumor-associated antigen–plasmacytoid dendritic cells. We detected similar levels of IL-10 (243 ± 83 pg/mL versus 221 ± 53 pg/mL) in the supernatant from CCR7⁻ ascites T cell and tumor-associated antigen–plasmacytoid dendritic cell coculture (P = 0.12, compared with no CCR7 depletion). The data suggest that original CCR7⁺CD8⁺ T cells are not essential for tumor plasmacytoid dendritic cells to induce IL-10-expressing T cells.

**Tumor plasmacytoid dendritic cell–induced IL-10⁺CD8⁺ T cells migrate with MIP-3β.** To further determine whether CCR7 is functional in tumor plasmacytoid dendritic cell–induced IL-10⁺CD8⁺ T cells, we conducted a chemotaxis assay with MIP-3β (CCL19), the ligand for CCR7. We observed that a fraction of tumor plasmacytoid dendritic cell–activated T cells efficiently migrated with MIP-3β, but not with MIP-3α and medium (data not shown), and neutralizing mouse anti-human CCR7 monoclonal antibodies efficiently blocked this migration (n = 4; *P < 0.001; Fig. 2B). FACS analysis revealed that the migrated cells were uniquely CCR7⁺CD8⁺ T cells (Fig. 2B). Tumor myeloid dendritic cell–activated T cells poorly migrated in these conditions. CCR7 was solely expressed on IL-10⁺CD8⁺ T cells (Fig. 2A). Thus, tumor plasmacytoid dendritic cell–induced IL-10⁺CD8⁺ T cells expressed functional CCR7, which would allow them to return to the tumor-draining lymph nodes.

**Tumor plasmacytoid dendritic cell–induced IL-10⁺CCR7⁺CD8⁺ T cells are suppressive.** We next studied whether tumor plasmacytoid dendritic cell–induced IL-10⁺CCR7⁺CD8⁺ T cells are suppressive. To this end, autologous tumor ascites T cells were initially activated with tumor-associated antigen–plasmacytoid dendritic cells as described (Fig. 1A). Then, different concentrations of tumor plasmacytoid dendritic cell–activated T cells were added into autologous tumor-associated antigen–myeloid dendritic cell and T cell coculture system. As expected, myeloid dendritic cells induced strong tumor-associated antigen–specific T cell proliferation in the absence of plasmacytoid dendritic cell–activated T cells (Fig. 3A and B). Interestingly, tumor plasmacytoid dendritic cell–activated T cells significantly inhibited myeloid dendritic cell–mediated, Her2/neu-specific T cell proliferation in a dose-dependent manner (n = 4; *P < 0.05; **P < 0.01, compared with controls; Fig. 3A). Tumor-associated antigen–specific T cell IFN-γ production was also suppressed (n = 4; *P < 0.01; Fig. 3C). Interestingly, mouse neutralizing anti-human IL-10 receptor antibody significantly recovered T cell functions (Fig. 3B and C). The data indicate that tumor plasmacytoid dendritic cell–induced CD8⁺ T cells are suppressive and that IL-10 is critical for this suppression.

**Myeloid dendritic cell stimulation fails to recover tumor plasmacytoid dendritic cell–elicited poor T cell function.** Myeloid dendritic cells have been used as immune-boosting vaccines to treat patients with cancers. It is unknown whether repetitive myeloid dendritic cell vaccination could override the T cell immunosuppressive function induced by tumor plasmacytoid dendritic cells. To test this possibility, autologous tumor ascites T cells were initially stimulated with tumor plasmacytoid dendritic cells followed by repetitive myeloid dendritic cell stimulation. As expected, repetitive myeloid dendritic cell stimulation (without plasmacytoid dendritic cells) induced strong tumor-associated antigen–specific T cell proliferation (n = 4; Fig. 3D). Repetitive plasmacytoid dendritic cell stimulation (without myeloid dendritic cells) induced a modest tumor-associated antigen–specific T cell proliferation (n = 4; Fig. 3D). Interestingly, after two rounds of myeloid dendritic cell stimulation, T cells prestimulated with tumor plasmacytoid dendritic cells exhibited strong T cell proliferation and cellular divisions, comparable to T cells that received three rounds of myeloid dendritic cell stimulation (Fig. 3D and E). However, after myeloid dendritic cell stimulation, the T cells previously stimulated with tumor plasmacytoid dendritic cells produced IL-10 (170 ± 68 pg/mL) and expressed little IFN-γ (0.12 ± 0.05 ng/mL), which was largely observed in the first two cell divisions (5 ± 2% IFN-γ⁺ T cells; Fig. 3E). The IL-10-expressing cells were CD8⁺ T cells, similar to tumor plasmacytoid dendritic cell stimulation alone.

**Figure 1.** Distinct T cell activation induced by tumor plasmacytoid and myeloid dendritic cells. Primary tumor ascites T cells (5 × 10⁶/ml) received two rounds of stimulation with different concentrations of autologous tumor-associated antigen–plasmacytoid dendritic cells or tumor-associated antigen–myeloid dendritic cells as we described in Materials and Methods. A, T cell proliferation was detected by thymidine incorporation. B, IL-10 was detected in the culture supernatant by ELISA. C, IFN-γ was detected in the culture supernatant by ELISA. D, tumor T cell–mediated specific lysis of T2 cells was detected by FACS as we described in Materials and Methods. T cell cytokines and CD8⁺ T cell activity were shown when tumor ascites T cells (5 × 10⁶/ml) were stimulated with 10⁴/mL tumor-associated antigen–dendritic cells (n = 4; *, P < 0.001, compared with plasmacytoid dendritic cells).
lymph nodes in the same individual patients ($n = 8$) and from peripheral blood in normal donors ($n = 5$). Direct intracellular staining revealed a negligible level of IL-10 protein in the sorted T cells. However, real-time reverse transcriptase-PCR showed high levels of IL-10 mRNA in CCR7$^+$CD45RO$^+$CD8$^+$ T cells from peripheral blood, primary tumor ascsites, and primary tumor-draining lymph node in patients with ovarian cancer, whereas normal blood CCR7$^+$CD45RO$^+$CD8$^+$ T cells expressed a negligible level of IL-10 mRNA ($*, P < 0.001$, compared with normal blood; Fig. 4A). Furthermore, the level of IL-10 mRNA was at least 2-fold higher in CCR7$^+$CD45RO$^+$CD8$^+$ T cells from tumor ascsites than that from blood and lymph nodes (Fig. 4A). All the CCR7$^+$CD45RO$^+$CD8$^+$ T cells expressed comparable, but low levels of IFN-γ, whereas activated normal T cells expressed high IFN-γ (Fig. 4B). Altogether, IL-10 is highly expressed in primary CCR7$^+$CD45RO$^+$CD8$^+$ T cells in vivo in patients with ovarian carcinomas.

We further tested whether tumor primary CCR7$^+$CD45RO$^+$CD8$^+$ T cells were functionally distinct from normal blood CCR7$^+$CD45RO$^+$CD8$^+$ T cells. To this end, CCR7$^+$CD45RO$^+$CD8$^+$ T cells were sorted from normal blood and ovarian tumor ascsites, and cultured with normal allogeneic blood T cells in the presence of anti-CD3 stimulation. Strikingly, CCR7$^+$CD45RO$^+$CD8$^+$ T cells from tumor ascsites, but not from normal blood, suppressed T cell proliferation in a dose-dependent manner ($P < 0.01$ for all; Fig. 4C). Thus, tumor environmental CCR7$^+$CD45RO$^+$CD8$^+$ T cells are functionally suppressive T cells (CD8$^+$ regulatory T cells). These CD8$^+$ regulatory T cells might be induced by tumor environmental plasmacytoid dendritic cells (Fig. 2).

**Discussion**

Published, detailed functional studies of dendritic cells and T cells in human tumor environment are scarce. Here, we studied the interaction between plasmacytoid dendritic cells and T cells in the tumor microenvironment in ovarian carcinomas. We show that tumor plasmacytoid dendritic cells induce regulatory CD8$^+$ T cells.

There are several proposals to explain why the immune system fails to control tumor progression, including deletions or mutations

Table 1. CD45RO$^+$CCR7$^+$CD8$^+$ T cells in ovarian cancer patients

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>Number of patients</th>
<th>Tumor type</th>
<th>Number of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor ascsites</td>
<td>33</td>
<td>Tumor blood</td>
<td>15</td>
</tr>
<tr>
<td>Tumor lymph node</td>
<td>8</td>
<td>Normal blood</td>
<td>8</td>
</tr>
</tbody>
</table>

| CD45RO$^+$ | 59 ± 11$^*$ | 38 ± 16$^*$ | 45 ± 7$^*$ | 19 ± 6$^*$ |
| CCR7$^+$ | 41 ± 15     | 48 ± 6     | 92 ± 6     | 62 ± 12    |
| CD45RO$^+$CCR7$^+$ | 22 ± 8$^*$ | 12 ± 5$^*$ | 20 ± 5$^*$ | 5 ± 3      |

Note: Fresh T cells were isolated from normal blood, or from ascsites, blood, and draining lymph nodes in patients with ovarian cancer, and stained with mouse anti-human CD45RO, CCR7, CD3, and CD8, and then analyzed by flow cytometry analysis. Results were expressed as the percentage of marked cells in CD8$^+$CD3$^+$ population (mean ± SE). $^*$ $P < 0.01$, as compared with normal blood.
endothelial growth factor inhibit dendritic cell–differentiation, thought to contribute to tumor immunopathogenesis. Tumor-associated antigen–specific immunosuppression (13). Apart from these possibilities, the dendritic cell system is involved in tumor environmental CD4+CD25+FOXP3+ T cells contribute to immunosuppression (23), and producing inhibitory cytokines such as TGF-β and IL-10. We recently showed that tumor environmental CD4+CD25+FOXP3+ T cells contribute to tumor-associated antigen–specific immunosuppression (13). Normal blood plasmacytoid dendritic cells are shown to induce immunosuppressive T cells in an allogeneic T cell reaction (14, 15). We previously showed that tumor environmental plasmacytoid dendritic cells promoted tumor vascularization (10), and induced IL-10-expressing T cells (9). We now show that tumor plasmacytoid dendritic cells induce CD8 regulatory T cells in the context of tumor-specific immunity. Although the mechanisms whereby tumor plasmacytoid dendritic cells induce CD8 regulatory T cells remain to be defined, we have identified four characteristics for these plasmacytoid dendritic cell–induced IL-10+CD8+ T cells: (a) induction of CD8+ regulatory T cells is independent on CD4+ regulatory T cells; (b) CD8+ regulatory T cells significantly suppress myeloid dendritic cell–mediated tumor-associated antigen–specific T cell effector functions through IL-10; (c) tumor myeloid dendritic cells cannot recover the suppressive functions; (d) CD8+ regulatory T cells express functional CCR7 and efficiently migrate with lymphoid homing chemokine MIP-3β. Furthermore, primary functional IL-10+CCR7+CD8+ T cells are found in blood, malignant ascites, and tumor-draining lymph nodes in patients with ovarian cancers. The data suggests that tumor plasmacytoid dendritic cells may induce IL-10+CCR7+CD8+ regulatory T cells in vivo.

CCR7 was used to categorize central memory T cells (CD45RO+CCR7+) versus effector memory T cells (CD45RO-CCR7-; ref. 28). Simultaneous staining of peripheral blood cells with CD45RO and CCR7 reveals a high heterogeneity in distribution of these markers (29). Functional variables are suggested to describe different T cell subsets (30). We show that tumor plasmacytoid dendritic cell–induced IL-10+ T cells exhibit a central memory T cell phenotype (CCR7+CD45RO+; ref. 28). These cells are functionally suppressive and migrate in response to MIP-3β. Furthermore, the in vivo primary counterpart (IL-10+CD8+CD45RO+CCR7+ T cells) is found in patients with ovarian cancers. Analogously,
CD8+CD45RO+CCR7+ T cells in ovarian tumor-bearing patients are able to inhibit T cell activation. Based on the functional definition of regulatory T cells (31), ovarian tumor–associated CD8+CD45RO+CCR7+ T cells are regulatory CD8+ T cells. It remains to be defined whether this population is suppressive in patients with other tumors. Furthermore, normal blood CD8+-CD45RO+CCR7+ T cells are not suppressive. Therefore, CD8+CD45RO+CCR7+ T cells are not a common phenotype and marker for defining CD8+ regulatory T cells. In fact, CD8+ regulatory T cells with distinct phenotypes have been reported. For instance, CD8+CD28+ alloantigen-specific T suppressor cells were observed in human heart transplant recipients (32). Human CD8+CD25+ thymocytes are reported to mediate suppressive effects through CTLA4 and TGF-β in normal donors (33). Murine CD8+ regulatory T cells with central memory phenotype were also reported to be a novel CD8+ regulatory T cell population (34). Our data suggest that tumor plasmacytoid dendritic cells would contribute to CD8+ regulatory T cell induction. CD8+ regulatory T cells may inhibit tumor-associated antigen–specific effector T cell immunity at the functional sites (tumor sites), and further home to lymph node and suppress tumor-associated antigen–specific naive T cell central priming at the priming sites.

No immunogenic (functionally mature) myeloid dendritic cells are detected in malignant ascites, suggesting that their absence is immunopathologically relevant, and means to introduce them into the tumor might be therapeutic. However, if myeloid dendritic cells cannot recover T cell effector functions after plasmacytoid dendritic cell induction, as shown in this study, blocking plasmacytoid dendritic cell tumor trafficking or functions may be essential prior to boosting tumor immunity by myeloid dendritic cells. Our data, in conjunction with the work from other groups (35–42), may at least partially explain why tumor-bearing hosts often have established weak tumor immunity, not sufficient to control disease progress, and why only moderate clinical benefits are observed in myeloid dendritic cell–based immunotherapy.

In summary, our study indicates that tumors manipulate tumor environmental dendritic cell subset distribution and functions to subvert tumor immunity (8). Multiple cellular and molecular layers of the suppressive network have been imposed in the tumor microenvironment in patients with cancers (8, 35–42). We suggest that a combination therapy with subverting immunosuppressive mechanisms, including blocking plasmacytoid dendritic cell tumor trafficking or function, is a promising, yet little-studied strategy for treating human cancers.

Acknowledgments
Received 11/11/2004; revised 3/14/2005; accepted 3/30/2005.
Grant support: The Department of Defense (OC020173), the National Cancer Institute (CA092562, CA100227), and the Louisiana Cancer Research Consortium.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
We thank Roy Weiner and Jules Puschett for their constant support.

References


Plasmacytoid Dendritic Cells Induce CD8⁺ Regulatory T Cells In Human Ovarian Carcinoma

Shuang Wei, Ilona Kryczek, Linhua Zou, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/65/12/5020

Cited articles
This article cites 42 articles, 16 of which you can access for free at:
http://cancerres.aacrjournals.org/content/65/12/5020.full.html#ref-list-1

Citing articles
This article has been cited by 59 HighWire-hosted articles. Access the articles at:
/content/65/12/5020.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.