

Research Paper

Regulatory T Cells and Cytokines in Malignant Pleural Effusions Secondary to Mesothelioma and Carcinoma

Peter DeLong²

Richard G. Carroll¹

Adam C. Henry²

Tomoyuki Tanaka²

Sajjad Ahmad²

Michael S. Leibowitz¹

Daniel H. Serman²

Carl H. June¹

Steven M. Albelda²

Robert H. Vonderheide^{1,*}

¹Abramson Family Cancer Research Institute; and the ²Thoracic Oncology Research Laboratory; University of Pennsylvania School of Medicine; Philadelphia, Pennsylvania USA

*Correspondence to: Robert H. Vonderheide; University of Pennsylvania School of Medicine; 551 BRB II/III; 421 Curie Blvd.; Philadelphia, Pennsylvania 19104 USA; Tel.: 215.573.5540; Fax: 215.573.2652; Email: rhv@mail.med.upenn.edu

Received 02/23/05; Accepted 03/01/05

Previously published online as a *Cancer Biology & Therapy* E-publication: <http://www.landesbioscience.com/journals/cbt/abstract.php?id=1644>

KEY WORDS

malignant pleural effusion, mesothelioma, lung cancer, breast cancer, T regulatory cells, cytokines, transforming growth factor β

ABBREVIATIONS

PBL peripheral blood lymphocyte
TGF transforming growth factor beta
Treg T regulatory cells

ACKNOWLEDGEMENTS

This work was supported by NCI grant PO1 CA 66726.

ABSTRACT

Immunotherapy against a variety of malignancies, including pleural-based malignancies, has shown promise in animal models and early human clinical trials, but successful efforts will need to address immunosuppressive factors of the tumor and host, particularly certain cytokines and CD4⁺ CD25⁺ regulatory T cells (Treg). Here, we evaluated the cellular and cytokine components of malignant pleural effusions from 44 patients with previously diagnosed mesothelioma, non-small cell lung cancer (NSCLC), or breast cancer and found significant differences in the immune profile of pleural effusions secondary to mesothelioma vs. carcinoma. Although a high prevalence of functionally suppressive CD4⁺ CD25⁺ T cells was found in carcinomatous pleural effusions, mesothelioma pleural effusions contained significantly fewer CD4⁺ CD25⁺ T cells. Activated CD8⁺ T cells in pleural fluid were significantly more prevalent in mesothelioma than carcinoma. However, there is clear patient-to-patient variability and occasional mesothelioma patients with high percentages of CD4⁺ CD25⁺ pleural effusion T cells and low percentages of CD8⁺ CD25⁺ pleural effusion T cells can be identified. Mesothelioma pleural effusions contained the highest concentrations of the immunosuppressive cytokine transforming growth factor (TGF)- β . Thus, the contribution of cellular and cytokine components of immunosuppression associated with malignant pleural effusions varies by tumor histology and by the individual patient. These results have implications for the development of immunotherapy directed to the malignant pleural space, and suggest the need to tailor immunotherapy to overcome immunosuppressive mechanisms in tumor environments.

INTRODUCTION

Malignancy in the pleural space portends a dismal prognosis, with life expectancy of six to nine months. A high percentage of malignancies in the pleural space are associated with effusions, and current therapy consists largely of palliative measures designed to drain or obliterate the pleural space.^{1,2} Immunotherapy is a promising strategy which has been shown to be effective against mouse models of various thoracic tumors³ including non-small cell lung cancer (NSCLC) and mesothelioma, and has demonstrated some effect in human trials for both of these diseases.⁴ Intrapleural immunotherapy has been tested in human clinical trials for pleural-based malignancies⁵ and is ongoing at our cancer center.

Early results from these efforts, and other approaches in cancer immunotherapy, make clear the need to address immuno-suppressive factors of the tumor and host, particularly immuno-suppressive cytokines (such as TGF- β)⁶ and the recently described CD4⁺ CD25⁺ regulatory T cell (Treg) that infiltrate tumors and suppress immunity.⁷ In the 1980's, North demonstrated the existence of a T cell-mediated mechanism of immuno-suppression that prevented adoptive tumor-sensitized T cells from mediating regression of tumors in recipient mice.^{8,9} This suppressor notion was largely abandoned in the 1990's, but has now resurfaced in mouse models and in human studies. In humans, a naturally occurring subpopulation of Treg cells constitutively expressing CD25 (the IL-2 receptor- α chain) comprises approximately 5–15% of peripheral blood CD4⁺ T cells.¹⁰ Treg cells inhibit autoimmune diabetes, prevent inflammatory bowel disease, mediate transplantation tolerance, impede antitumor immunity and prevent the expansion of other T cells in vivo.^{7,11,12} In particular, human Treg cells markedly inhibit CD8⁺ T cell activation.¹³

Since our initial description of functionally suppressive Treg cells in human NSCLC and ovarian tumors,^{14,15} numerous other studies have identified CD4⁺ CD25⁺ Treg cells in the microenvironment of other cancers, including breast, pancreatic, and colon cancer.¹⁶⁻¹⁸ Treg cells comprise up to 30% of tumor infiltrating lymphocytes. In fact, no tumor type has been identified that lacks Treg cells. It has been hypothesized that depletion of Treg cells in vivo would enhance the induction of tumor immunity.^{19,20} In mouse models,

injection of anti-CD25 antibody compared to control immunoglobulin markedly enhances response to vaccination and induction of anti-tumor immunity²¹⁻²⁴ by depleting CD4⁺ CD25⁺ cells in vivo.

In malignant pleural effusions, the prevalence of Treg cells measured in concert with levels of immunosuppressive cytokines is not known. In this study, we evaluated malignant pleural effusions from patients with mesothelioma, NSCLC, or breast cancer to characterize and compare lymphoid subsets and cytokine milieu. We demonstrate that there are significant differences in the cellular and cytokine components of pleural effusions secondary to mesothelioma as compared to those secondary to carcinoma. In particular, mesothelioma pleural effusions include significantly fewer CD4⁺ CD25⁺ T cells but have the highest levels of TGF- β of the tumors we studied.

MATERIALS AND METHODS

Collection of patient samples. Pleural fluid was obtained from 44 cancer patients undergoing thoracenteses at the Thoracic Surgery, Pulmonary, or Interventional Bronchoscopy Services at the Hospital of the University of Pennsylvania. Peripheral blood (10 ml) was obtained by phlebotomy. The protocols were approved by the University of Pennsylvania's Institutional Review Board, and signed, written informed consent was obtained from each patient. Patients with previously diagnosed malignant mesothelioma (n = 7), NSCLC (n = 11), or breast cancer (n = 26) were enrolled.

Sample preparation. Pleural fluid specimens were mixed with 3.8% sodium citrate. Cytopathological analysis was performed at the Department of Pathology of the Hospital of the University of Pennsylvania and confirmed malignancy in each case. After centrifugation at 1,200 g for seven minutes at room temperature, mononuclear cells were isolated from the cell pellet using Ficoll centrifugation. Supernatant material from the first centrifugation were recentrifuged at high speed and the supernatant frozen at -70°C for cytokine analysis. Mononuclear cells from phlebotomy samples were isolated by Ficoll centrifugation.

Cell surface phenotype analysis. Four-color flow cytometric analysis of pleural fluid mononuclear cells was performed using monoclonal antibody (mAb) and isotype controls as described previously.²⁵ Antibodies conjugated with FITC, PE, PerCP or APC were purchased from BD Biosciences (San Jose, CA) (CD3, CD4, CD8, CD11c, CD14, CD27, CD28, CD45, CD45RA, CD45RO, CD62L, CD123), Immunotech (Marseille, France) (CD83), or Dako (Carpinteria, CA) (CD19, CD25). Flow cytometry was performed using a Becton Dickinson FACS Calibur flow cytometer (BD Biosciences, San Jose, CA).

Proliferation assays. Ninety-six well plates were coated with 1 μ g/ml anti-CD3 (OKT3) and 1 μ g/ml anti-CD28 Ab (clone 9.3) overnight at 37°C and then thoroughly washed. Lymphocytes from pleural effusions were stained with CD4-APC mAb and CD25-FITC mAb and sorted for CD4⁺ CD25⁻ and CD4⁺ CD25⁺ fractions using a MoFlo Cell Sorter (DakoCytomation, Fort Collins, CO). Autologous peripheral blood lymphocytes (PBL) in complete media (RPMI 1640 containing 10% fetal calf serum, 15 μ g/ml gentamicin, and 2 mmol/L glutamine) were plated in triplicate at 5×10^4 cells per well, and sorted populations of CD4⁺ CD25⁺ or CD4⁺ CD25⁻ cells in complete media (or media alone) were added (2×10^4

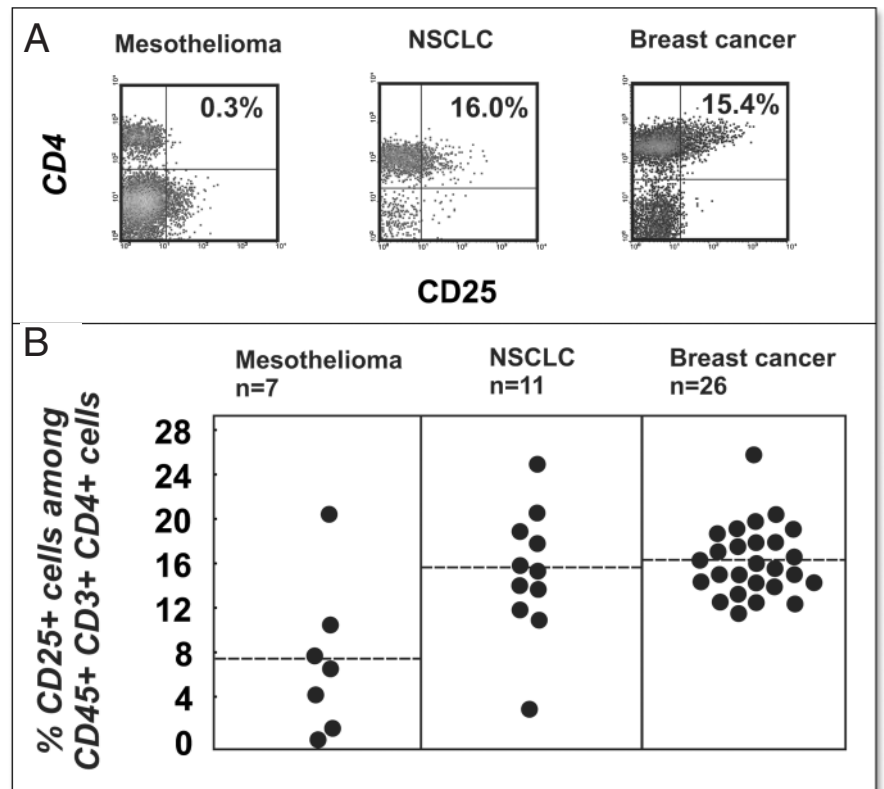


Figure 1. Lymphocytes from pleural effusions due to mesothelioma have significantly fewer CD4⁺ CD25⁺ T cells than effusions due to NSCLC or breast cancer. (A) Representative dot plots of flow cytometric analysis of lymphocytes from malignant pleural effusions of patients with mesothelioma, NSCLC, and breast cancer. (B) Data from flow cytometric analysis of lymphocytes from all malignant pleural effusion samples evaluated for CD45⁺ CD3⁺ CD4⁺ CD25⁺ T cells grouped by tumor histology.

cells per well). On day 3, [³H]thymidine was added (1 μ Ci/well) and plates were harvested 18 h later. Experiments were performed in triplicates, and cpm is shown \pm 1 SD.

Cytokine analysis. Concentrations of cytokines in pleural fluid were measured by commercially available enzyme linked immunosorbent assay (ELISA) kits for interleukin (IL)-1- β , IL-2, IL-4, IL-6, IL-10, IL-12, IL-15, VEGF, GM-CSF, PGE2, TNF- α , IFN- β , IFN- γ (Pharmingen, San Diego, CA; or R&D Systems Inc, Minneapolis, MN) according to the manufacturer's instructions. Bioactive TGF- β was determined by the mink lung epithelial cell assay (MLEC) as previously described.²⁶

Statistical analysis. Statistical analyses of lymphoid subsets were analyzed with unpaired Student's t. Comparisons of mean cytokine levels between groups were made by calculating the analysis of variance using StatView (Cary, NC). Statistical significance was set at $p < 0.05$. Results are expressed as mean \pm 1 SD.

RESULTS

Flow cytometric analysis of pleural effusion lymphocytes. Malignant pleural effusions from 44 patients with mesothelioma, NSCLC, or breast cancer were analyzed by flow cytometry for phenotypic evidence of CD4⁺ CD25⁺ Treg, as previously described.^{14,15} After isolation of pleural fluid mononuclear cells, we gated on CD45⁺ CD3⁺ T cells and analyzed them for coexpression of CD4 and CD25 (Fig. 1A). Pleural effusions secondary to NSCLC or breast cancer had a statistically higher prevalence of CD4⁺ CD25⁺ T cells than mesothelioma (15.0% \pm 5.9% and 15.9% \pm 3.2%, respectively, versus 7.8% \pm 6.8%, $p < 0.05$ for both comparisons) (Fig. 1B).

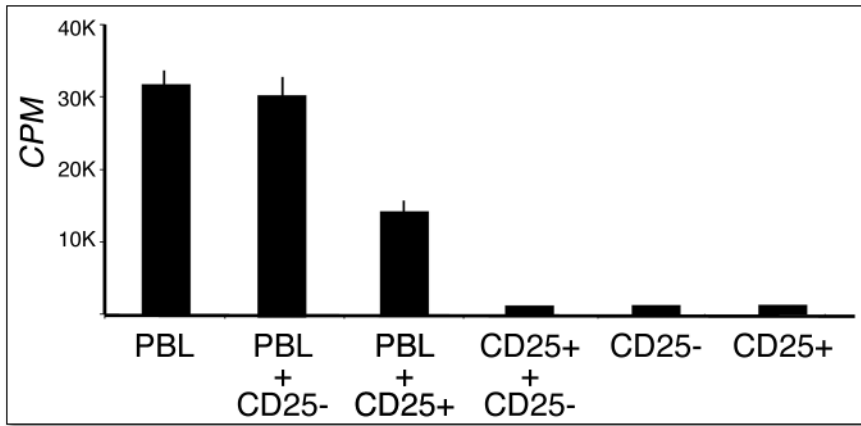


Figure 2. CD4⁺ CD25⁺ T cells from carcinomatous pleural effusions inhibit the proliferation of autologous PBLs. PBL from patients proliferated markedly to anti-CD3 and anti-CD28 stimulation alone (PBL), or in combination with pleural effusion CD4⁺ CD25⁻ cells (PBL + CD25⁻). Coincubation with pleural effusion CD4⁺ CD25⁺ T cells markedly inhibited proliferation (PBL + CD25⁺). Data is representative of three experiments for carcinoma patients.

Table 1 Cytokine concentrations in malignant pleural effusions

Cytokine	Mesothelioma		NSCLC		Breast Cancer	
	Mean (pg/ml)	SD	Mean (pg/ml)	SD	Mean (pg/ml)	SD
IL-1-β	<0.1		<0.1		<0.1	
IL-2	<0.1		<0.1		<0.1	
IL-4	<0.1		<0.1		<0.1	
IL-6	783	313	366	221	372	176
IL-10	76	18	28	8	33	7
IL-12	<0.1		<0.1		<0.1	
IL-15	<0.1		<0.1		<0.1	
GM-CSF	<0.1		<0.1		<0.1	
VEGF	1755	495	788	231	1029	316
PGE2	66	12	99	31	71	34
IFN-γ	<0.1		<0.1		<0.1	
IFN-β	<0.1		<0.1		<0.1	
TGF-β	1964	468	293	112	309	106
TNF-α	<0.1		<0.1		<0.1	

For two patients with mesothelioma, <3.0% CD4⁺ CD25⁺ pleural fluid T cells were observed whereas none of the patients with NSCLC or breast cancer had <3.0% CD4⁺ CD25⁺ pleural fluid T cells. However, some heterogeneity was noted, including one patient with sarcomatoid mesothelioma who had 21.0% CD4⁺ CD25⁺ T cells in the pleural fluid. In the peripheral blood of mesothelioma patients (n = 6), we found that the prevalence CD4⁺ CD25⁺ T cells was not significantly different than the prevalence of CD4⁺ CD25⁺ T cells in the blood of breast cancer patients (n = 14) (13.6% ± 6.4% vs. 13.3% ± 8.3%, P > 0.05).

To assess whether CD4⁺ CD25⁺ cells in pleural effusions of nonmesothelioma cancer patients exhibit suppressive function associated with Treg cells, we evaluated the effect of CD4⁺ CD25⁺ cells on polyclonal in vitro stimulation of autologous PBL. Although PBL from patients proliferated markedly to anti-CD3/anti-CD28 stimulation alone, coincubation with pleural effusion

CD4⁺ CD25⁺ cells markedly inhibited proliferation (Fig. 2). In contrast, equal numbers of pleural effusion CD4⁺ CD25⁻ cells coincubated with autologous PBL failed to suppress anti-CD3/anti-CD28 proliferation. This data demonstrates that cells with this phenotype are also functionally growth suppressive.

Other differences in lymphoid subsets between NSCLC or breast cancer vs. mesothelioma were also observed in pleural effusions. Most notably, the prevalence of CD8⁺ CD25⁺ cells among pleural effusion T cells was higher in mesothelioma (10.2% ± 6.2%) than either NSCLC (2.0% ± 1.2%) or breast cancer (3.1% ± 2.3%) (P < 0.05 for both comparisons), suggesting a more active state of the CD8⁺ cytotoxic T cell compartment in mesothelioma pleural effusions compared to NSCLC or breast cancer (Fig. 3A and B). CD45⁺ CD11c⁺ CD14⁻ myeloid dendritic cells (DC) were identified at the same prevalence among lymphocytes from pleural effusions due to mesothelioma (10.6% ± 16.2%), NSCLC (8.0% ± 2.6%), and breast cancer (11.4% ± 4.2%) (P > 0.05 for each comparison), but the percentage of mature DC among pleural effusion myeloid DC (based on CD83 expression) was greater in mesothelioma (17.5% ± 13.6%) than in breast cancer (8.9% ± 3.1%) (P < 0.05). In NSCLC, the percentage of mature myeloid DC was not statistically different than the percentage in mesothelioma. CD45⁺ CD123⁺ CD14⁻ plasmacytoid DC were also identified at the same prevalence among lymphocytes from pleural effusions due to mesothelioma (5.6% ± 3.4%), NSCLC (3.6% ± 2.2%), and breast cancer (5.0% ± 6.0%) (P > 0.05 for each comparison), and the percentage of mature CD83⁺ DC among pleural effusion plasmacytoid DC was 16.2% ± 13.9%, 18.0% ± 11.3%, and 23.0% ± 14.5%, respectively; P > 0.05 for all comparisons). There was large patient-to-patient heterogeneity of the maturation state of plasmacytoid DC in pleural effusions; for each histology, there was at least one patient with 0.0% CD83⁺ plasmacytoid DC and at least one patient with >30% CD83⁺ plasmacytoid DC.

There was no statistical difference in the prevalence of pleural effusion B cells (CD19⁺ CD3⁻ CD14⁻) or natural killer cells (CD56⁺ CD3⁻ CD8⁻) between the three tumor types. There were also no differences between the three tumor types with regard to percent of CD4⁺ T cells or CD8⁺ T cells expressing T cell memory markers including CD45RA, CD45RO, CD28, CD27 or CD62L.

Few patients with mesothelioma were treated with chemotherapy before the development of pemetrexed, in contrast to the routine use of chemotherapy for patients with either breast cancer or NSCLC. This raised the possibility that the results obtained in the flow cytometric analysis of Treg cells and activated CD8⁺ cells might be related to treatment with chemotherapy. To test this hypothesis, the treatment status of each patient was compared to our flow cytometric results. Because none of the patients with mesothelioma received chemotherapy, and all but one of the patients with breast cancer received chemotherapy, these groups were uninformative. However, only five of 11 patients with NSCLC had received chemotherapy. There were no significant differences in the prevalence of Treg cells in NSCLC patients who had received chemotherapy vs. patients who had not (data not shown).

Pleural effusion cytokine analysis. Pleural fluid was also assayed for the concentration of 14 cytokines. Levels of 9 of these cytokines were below the limit of detection in each patient regardless of tumor type, including IL-1-β, IL-2, IL-4, IL-12, IL15, GM-CSF, IFN-γ, IFN-β and TNF-α. Measurable levels of IL-6, IL-10, VEGF, PGE2 and TGF-β were detected and several significant differences in cytokine levels between mesothelioma vs. NSCLC or breast cancer were detected (Table 1). Most notably, we found TGF-β levels to be more than six times higher on average in pleural effusions secondary to mesothelioma (1964 pg/ml, SD 468) compared to either NSCLC (293 pg/ml, SD 112) or breast cancer (309 pg/ml, SD 106) (P < 0.05 for each comparison). Mesothelioma pleural effusions also had higher levels of VEGF,

IL-6, and IL-10 than those of NSCLC or breast cancer, but these differences—although statistically significant—were modest.

DISCUSSION

Refined knowledge of the lymphoid and cytokine milieu of malignant pleural effusions may assist in the development of immunogene therapies targeting this space. Malignant pleural fluid has been studied in the past as if it were a single entity, and the cellular and cytokine components have generally not been studied by grouping them according to tumor type.²⁷⁻²⁹ For example, a recent study by Atanackovic et al. examined adhesion molecules and chemokine receptors on lymphocytes from 16 malignant effusions due to eight different tumor types.²⁷ Here, we demonstrate that there are significant differences in the regulatory and activated lymphoid components of pleural effusions secondary to mesothelioma as compared to those secondary to NSCLC or breast cancer. We found that in contrast to NSCLC and breast cancer, which harbor significant numbers of functionally suppressive Treg cells, mesothelioma pleural effusions contain significantly fewer numbers of CD4⁺ CD25⁺ Treg cells. However, there is clear patient-to-patient variability and occasional mesothelioma patients with high percentages of CD4⁺ CD25⁺ T cells can be identified. In contrast, the cytokines TGF- β , VEGF, and IL-10—each with well-documented immunosuppressive effects^{6,30,31}—were found in mesothelioma pleural effusions at levels significantly higher than NSCLC or breast cancer. Although Treg have been implicated in the production of TGF- β , numerous other cells can also produce this cytokine. Most notably, malignant mesothelioma cells themselves have been shown to produce significant levels of TGF- β .^{32,33} Thus, the contribution of cellular vs. cytokine components of immunosuppression associated with malignant pleural effusions varies by tumor histology such that optimal therapeutic agents for immune intervention in the pleural space may be cancer type-specific. These findings suggest that the desired clinical elimination of immune suppression in cancer is not an effort that depends strictly on elimination of Tregs but that depletion of cytokines may be equally valid depending on the histology and the patient.

The main finding in this study is that mesothelioma pleural effusions have a relatively low prevalence of CD4⁺ CD25⁺ Treg cells and a relatively high prevalence of activated CD8⁺ T cells. In contrast, both NSCLC and breast cancer pleural effusions include relatively high percentages of Treg cells and low percentages of activated CD8⁺ cells. This inverse relationship appeared independent of prior therapy with chemotherapy. Treg comprise approximately 5–15% of peripheral blood CD4⁺ T cells in normal individuals,¹⁰ but there is an increased pool of such cells in the peripheral blood of cancer patients.¹⁶ Physiologically, CD4⁺ CD25⁺ Treg cells inhibit autoimmune reactions but in the setting of cancer, impede immunity and inhibit activation of host lymphocytes.^{7,11-13} CD4⁺ CD25⁺ but not CD4⁺ CD25⁻ T cells isolated from human tumor lesions actively suppress polyclonal proliferation of autologous peripheral T cells, similar to

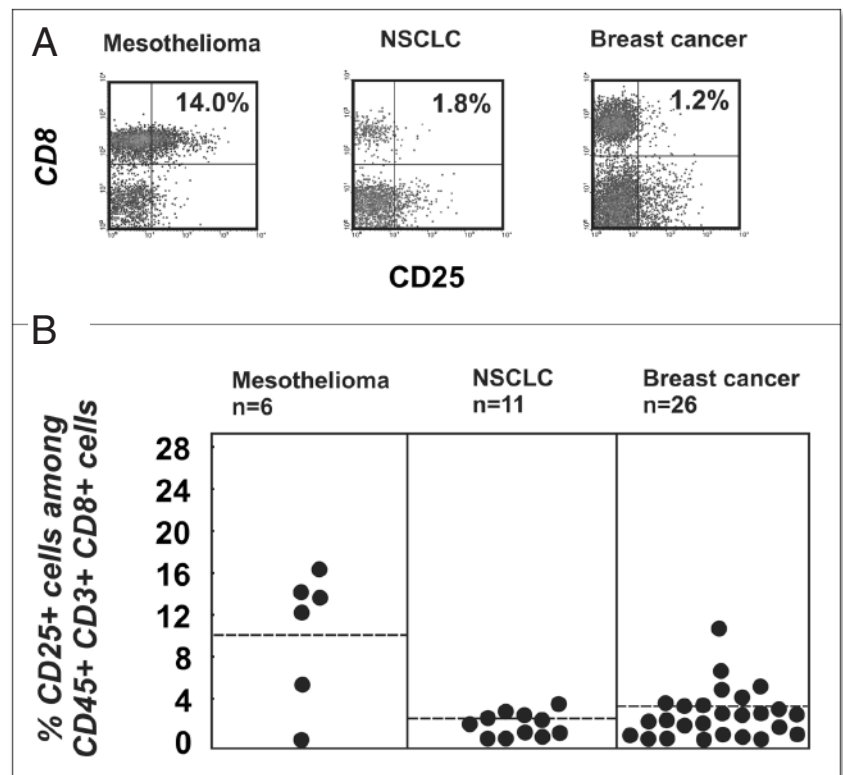


Figure 3. Lymphocytes from pleural effusions secondary to mesothelioma have significantly more CD8⁺ CD25⁺ cells than effusions due to NSCLC or breast cancer. (A) Representative dot plots of flow cytometric analysis of lymphocytes from malignant pleural effusions of patients with mesothelioma, NSCLC, and breast cancer. (B) Data from flow cytometric analysis of lymphocytes from all malignant pleural effusion samples evaluated for CD45⁺ CD3⁺ CD8⁺ CD25⁺ T cells are grouped by tumor histology.

the suppression we observed in this study using CD4⁺ CD25⁺ cells from malignant pleural effusions. Among tumor infiltrating lymphocytes and in draining lymph nodes, Treg cells comprise up to 30% of lymphocytes, as shown in many epithelial tumors including NSCLC, breast cancer, ovarian cancer, pancreatic cancer and nearly every other tumor examined. Thus, it was not surprising to identify functionally suppressive CD4⁺ CD25⁺ in NSCLC and breast cancer pleural effusions in study. However, the significantly lower prevalence of Treg cells identified in mesothelioma was unexpected. An issue that is not completely resolved is whether the difference between mesothelioma Treg and carcinoma Treg is qualitative in addition to quantitative. For example, it remains possible that mesothelioma Treg cells are more potent at suppression of an MLR on a cell-for-cell basis.

It has been hypothesized that removal of Treg cells will enhance the induction of tumor immunity^{19,20} and the efficacy of immunotherapy. Injection of mice with anti-CD25 antibody compared to control immunoglobulin depleted animals of CD25⁺ T cells and markedly enhances response to vaccination and anti tumor immunity²¹⁻²⁴ by depleting CD4⁺CD25⁺ cells in vivo. Cyclophosphamide has been used in both humans and mice to deplete Treg cells in vivo prior to vaccination, and remains an agent under intensely active study. Such maneuvers represent a growing effort to augment immuno-stimulatory experimental therapies (such as vaccines or adoptive immunotherapy) with agents that inhibit immune inhibitors, including but not limited to Treg cells. Our data suggest that in mesothelioma, there is a rationale to explore therapeutic

modulation of immuno-suppressive cytokines such as TGF- β instead of or in addition to modulation of Treg cells.

Our data also imply that different tumors trigger different responses from the host immune system. Immune therapies that work for one tumor type, or one patient, may not be effective against another tumor type, or in other patients. The immunological environment created by each tumor will need to be characterized with attention paid to the point, or points, at which the immune system fails to mount an effective anti-tumor response. As our ability to manipulate specific stages of the immune response grows, rational combinations of immune therapy will require detailed knowledge of the immunobiology of each tumor type.

References

- Sterman DH, Kaiser LR, Albelda SM. Advances in the treatment of malignant pleural mesothelioma. *Chest* 1999; 116:504-20.
- Antony VB, Lodenkemper R, Astoul P, Boutin C, Goldstraw P, Hott J, Rodriguez Panadero F, Sahn SA. Management of malignant pleural effusions. *Eur Respir J* 2001; 18:402-19.
- Odaka M, Sterman DH, Wiewrodt R, Zhang Y, Kiefer M, Amin KM, Gao GP, Wilson JM, Barsoum J, Kaiser LR, Albelda SM. Eradication of intraperitoneal and distant tumor by adenovirus-mediated *interferon-beta* gene therapy is attributable to induction of systemic immunity. *Cancer Res* 2001; 61:6201-12.
- Nemunaitis J, Sterman D, Jablons D, Smith IIJD JW, Fox B, Maples P, Hamilton S, Borellini F, Lin A, Morali S, Hege K. Granulocyte-macrophage colony-stimulating factor gene-modified autologous tumor vaccines in nonsmall-cell lung cancer. *J Natl Cancer Inst* 2004; 96:326-31.
- Astoul P, Viallat JR, Laurent JC, Brandely M, Boutin C. Intrapleural recombinant IL-2 in passive immunotherapy for malignant pleural effusion. *Chest* 1993; 103:209-13.
- Wahl SM, Chen W. TGF-beta: How tolerant can it be? *Immunol Res* 2003; 28:167-79.
- Sakaguchi S, Sakaguchi N, Shimizu J, Yamazaki S, Sakihama T, Itoh M, Kuniyasu Y, Nomura T, Toda M, Takahashi T. Immunologic tolerance maintained by CD25+ CD4+ regulatory T cells: Their common role in controlling autoimmunity, tumor immunity, and transplantation tolerance. *Immunol Rev* 2001; 182:18-32.
- Berendts MJ, North RJ. T-cell-mediated suppression of anti-tumor immunity. An explanation for progressive growth of an immunogenic tumor. *J Exp Med* 1980; 151:69-80.
- Dye ES, North RJ. T cell-mediated immunosuppression as an obstacle to adoptive immunotherapy of the P815 mastocytoma and its metastases. *J Exp Med* 1981; 154:1033-42.
- Jonuleit H, Schmitt E, Stassen M, Tuettenberg A, Knop J, Enk AH. Identification and functional characterization of human CD4(+)CD25(+) T cells with regulatory properties isolated from peripheral blood. *J Exp Med* 2001; 193:1285-94.
- Salomon B, Lenschow DJ, Rhee L, Ashourian N, Singh B, Sharpe A, Bluestone JA. B7/CD28 costimulation is essential for the homeostasis of the CD4+CD25+ immunoregulatory T cells that control autoimmune diabetes. *Immunity* 2000; 12:431-40.
- Shevach EM. Regulatory T cells in autoimmunity. *Annu Rev Immunol* 2000; 18:423-49.
- Camara NO, Sebille F, Lechler RI. Human CD4+CD25+ regulatory cells have marked and sustained effects on CD8+ T cell activation. *Eur J Immunol* 2003; 33:3473-83.
- 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 nonsmall cell lung cancer and late-stage ovarian cancer. *Cancer Res* 2001; 61:4766-72.
- Woo EY, Yeh H, Chu CS, Schlienger K, Carroll RG, Riley JL, Kaiser LR, June CH. Cutting edge: Regulatory T cells from lung cancer patients directly inhibit autologous T cell proliferation. *J Immunol* 2002; 168:4272-6.
- 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; 9:606-12.
- 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; 169:2756-61.
- Marshall NA, Christie LE, Munro LR, Culligan DJ, Johnston PW, Barker RN, Vickers MA. Immunosuppressive regulatory T cells are abundant in the reactive lymphocytes of Hodgkin lymphoma. *Blood* 2004; 103:1755-62.
- Gallimore A, Sakaguchi S. Regulation of tumour immunity by CD25+ T cells. *Immunology* 2002; 107:5-9.
- Shevach EM. CD4+ CD25+ suppressor T cells: More questions than answers. *Nat Rev Immunol* 2002; 2:389-400.
- Sutmoller 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; 194:823-32.
- Pasare C, Medzhitov R. Toll pathway-dependent blockade of CD4+CD25+ T cell-mediated suppression by dendritic cells. *Science* 2003; 299:1033-6.
- 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; 163:5211-8.
- Onizuka S, Tawara I, Shimizu J, Sakaguchi S, Fujita T, Nakayama E. Tumor rejection by in vivo administration of anti-CD25 (interleukin-2 receptor alpha) monoclonal antibody. *Cancer Res* 1999; 59:3128-33.
- Vonderheide RH, Domchek SM, Schultze JL, George DJ, Hoar KM, Chen DY, Stephens KF, Masutomi K, Loda M, Xia Z, Anderson KS, Hahn WC, Nadler LM. Vaccination of cancer patients against telomerase induces functional antitumor CD8+ T lymphocytes. *Clin Cancer Res* 2004; 10:828-39.
- Abe M, Harpel JG, Metz CN, Nunes I, Loskutoff DJ, Rifkin DB. An assay for transforming growth factor-beta using cells transfected with a plasminogen activator inhibitor-1 promoter-luciferase construct. *Anal Biochem* 1994; 216:276-84.
- Atanackovic D, Block A, de Weerth A, Faltz C, Hossfeld DK, Hegewisch-Becker S. Characterization of effusion-infiltrating T cells: Benign versus malignant effusions. *Clin Cancer Res* 2004; 10:2600-8.
- Robinson E, Segal R, Vesely Z, Mekori T. Lymphocyte subpopulations in peripheral blood and malignant effusions of cancer patients. *Eur J Cancer Clin Oncol* 1986; 22:191-3.
- Oka M, Yoshino S, Hazama S, Shimoda K, Suzuki T. The characterization of peritoneal and pleural exudate cells from malignant effusions. *Surg Today* 1993; 23:500-3.
- Ohm JE, Carbone DP. VEGF as a mediator of tumor-associated immunodeficiency. *Immunol Res* 2001; 23:263-72.
- Fickenscher H, Hor S, Kupers H, Knappe A, Wittmann S, Sticht H. The interleukin-10 family of cytokines. *Trends Immunol* 2002; 23:89-96.
- Fitzpatrick DR, Bielefeldt-Ohmann H, Himbeck RP, Jarnicki AG, Marzo AL, Robinson BW. Transforming growth factor-beta: Antisense RNA-mediated inhibition affects anchorage-independent growth, tumorigenicity and tumor-infiltrating T-cells in malignant mesothelioma. *Growth Factors* 1994; 11:29-44.
- Marzo AL, Fitzpatrick DR, Robinson BW, Scott B. Antisense oligonucleotides specific for transforming growth factor beta2 inhibit the growth of malignant mesothelioma both in vitro and in vivo. *Cancer Res* 1997; 57:3200-7.