Different flavors of regulatory T-cell subsets in patients with cancer and their role in tumor escape

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Treg subsets promote tumor escape from the host immune system

- Types of regulatory T cells:
  - **nTreg**: CD4⁺CD25<sup>high</sup>Foxp3<sup>high</sup>
    1. thymus derived
    2. suppress immune responses against “self” by mechanisms involving contact inhibition
  - **Tr1 cells**: CD4⁺CD25<sup>neg</sup>IL10⁺TGF-β<sub>1</sub>⁺
    1. induced in the periphery upon Ag presentation
    2. suppress immune responses through IL-10 and TGF-β<sub>1</sub> secretion

- an increased frequency of Treg in the tumor and in the peripheral circulation of patients with HNSCC was previously reported by us:
  - Albers AE *et al.*, Cancer Immunology Immunotherapy, 2005;54: 1072-81
  - Schaefer C *et al.*, British Journal of Cancer, 2005;92: 913-20

- In patients with ovarian cancer, accumulations of Treg at the tumor site were associated with shorter survival (Curiel, Nat Med 2004)
Methods

- **Cell source:** PBMC and TIL from HNSCC patients or PBMC from NC
- **Single-cell sorting:** $\text{CD}4^+\text{CD}25^{\text{high}}$
  $\text{CD}4^+\text{CD}25^{\text{neg}}$
- **Phenotype:** gate on $\text{CD}3^+\text{CD}4^+ (\text{Tr1})$ or $\text{CD}4^+\text{CD}25^{\text{high}} (\text{nTreg})$
  rare-event multicolor flow cytometry
  multicolor immunofluorescence microscopy
- **Suppressor function:**
  CFSE-labeled autologous $\text{CD}4^+\text{CD}25^{\text{neg}}$ responder cells (R) + Treg (S)
  added at 1S:1R, 1S:5R, 1S:10R ratios
- **Mechanisms of suppression:**
  - Transwell system
  - neutralizing antibody in suppressor assays
  - IL-10, TGF-$\beta_1$ in cells, in supernatants (Flow, Luminex)
- **Associations with the disease stage and/or progression**
**CD25^{high} nTreg are expanded in HNSCC patients vs. NC**

- **NC**
  - 1.7% CD25^{high}
  - 0.3% CD4

- **HNSCC**
  - 5% CD25^{high}
  - 2% CD4

*Graph showing gated CD4^{+}CD25^{+} T cells with p<0.001.*

- NC (n=15)
- PBMC (n=35)
- TIL (n=15)
Phenotypic characteristics of CD25^{high} nTreg in different compartments (HNSCC patients)

- **PBMC**
  - Gated on CD3+CD4+
  - IL-10
    - 31%
  - Foxp3
    - 70%
  - TGF-β1
    - 60%
  - CD4+CD25^{high} Foxp3+CD62L+CCR7+

- **TIL**
  - IL-10
    - 72%
  - Foxp3
    - 93%
  - TGF-β1
    - 96%
  - CD4+CD25^{high} Foxp3+GITR+IL-10+TGF-β1+
CD4<sup>+</sup>CD25<sup>+</sup> nTreg among TIL at the tumor site
Suppressor function of CD4$^{+}$CD25$^{\text{high}}$ nTreg is cell contact- and cytokine- dependent
CD4^+CD25^{high} Treg in PBMC of HNSCC patients expand after oncologic therapy

- **% positive cells**
  - AD: n=10
  - NED: n=25
  - p<0.0059

- **% suppression of proliferation**
  - 1S:1R
  - 1S:5R
  - 1S:10R
  - p<0.0038
  - p<0.0011
Phenotypic characteristics of CD4$^+$ Tr1 cells in the circulation or TIL in HNSCC patients

PBMC: CD4$^+$CD25$^{\text{neg}}$Foxp3$^+$CD122$^+$IL-10$^+$TGF-$\beta_1$$^+$
TIL: CD4$^+$CD25$^{\text{neg}}$CD132$^+$IL-10$^+$TGF-$\beta_1$$^+$
Tr1 precursors *in situ* at the tumor site expressing suppressive molecules

**CD4**

**CD132**

**TGF-β**

**overlay**

**x 600**

**CD4**

**CD25**

**Foxp3**

**overlay**

**x 600**
Suppressor activity of Tr1 precursors or Tr1 cells is cytokine-dependent but cell contact-independent.
Marker expression and function of Tr1 cells in HNSCC patients is associated with the T stage

IL-10

TGF-β1

Foxp3

suppression

% positive cells

% suppression

T1/T2  T3/T4

T1/T2  T3/T4

T1/T2  T3/T4

T1/T2  T3/T4

p<0.0001

p=0.0004

p<0.0001

p=0.001

p=0.0004
Conclusions

- Treg in the blood and in the tumor of patients with HNSCC have a distinct phenotype and elevated suppressor activity relative to Treg in NC.
- Both nTreg and Tr1 assemble at the tumor site.
- nTreg-mediated suppression is contact dependent while that mediated by Tr1 is cytokine (IL-10 and TGF-β) dependent.

- In HNSCC patients:
  - nTreg expansion and regulatory activity is higher in NED than in AD.
  - Tr1 expansion and regulatory activity increase with tumor stage.
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