Dendritic Cells Loaded with Stressed Tumor Cells Elicit Long-Lasting Protective Tumor Immunity in Mice Depleted of CD4+CD25+ Regulatory T Cells

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Citation:

Prasad, S.; Farrand, K.; Matthews, S.; Chang, J.; McHugh, R.; Ronchese, F., *Journal of Immunology*, **174** (1), 90-98, 2005.

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BE.450 Paper Presentation

March 2, 2005

Background – Immune Cell Lineage

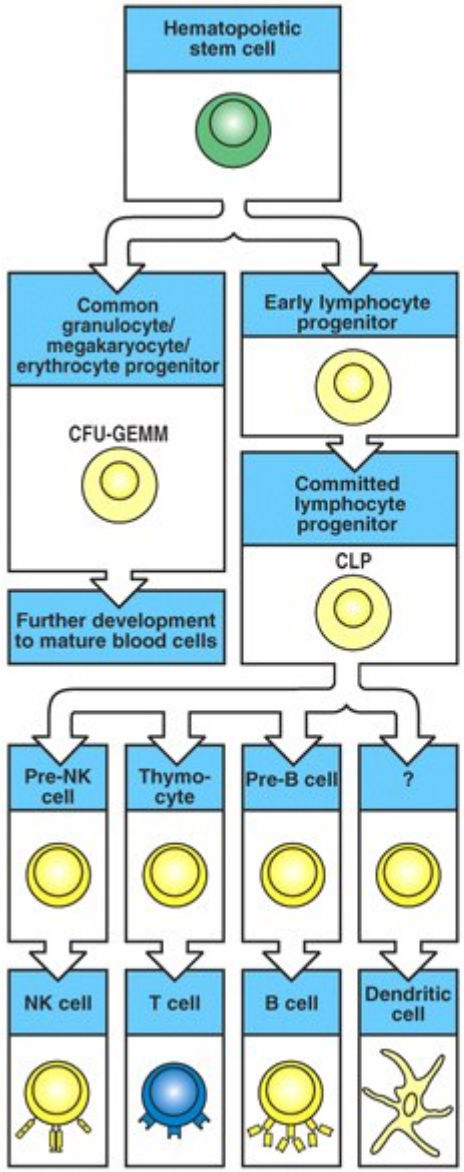
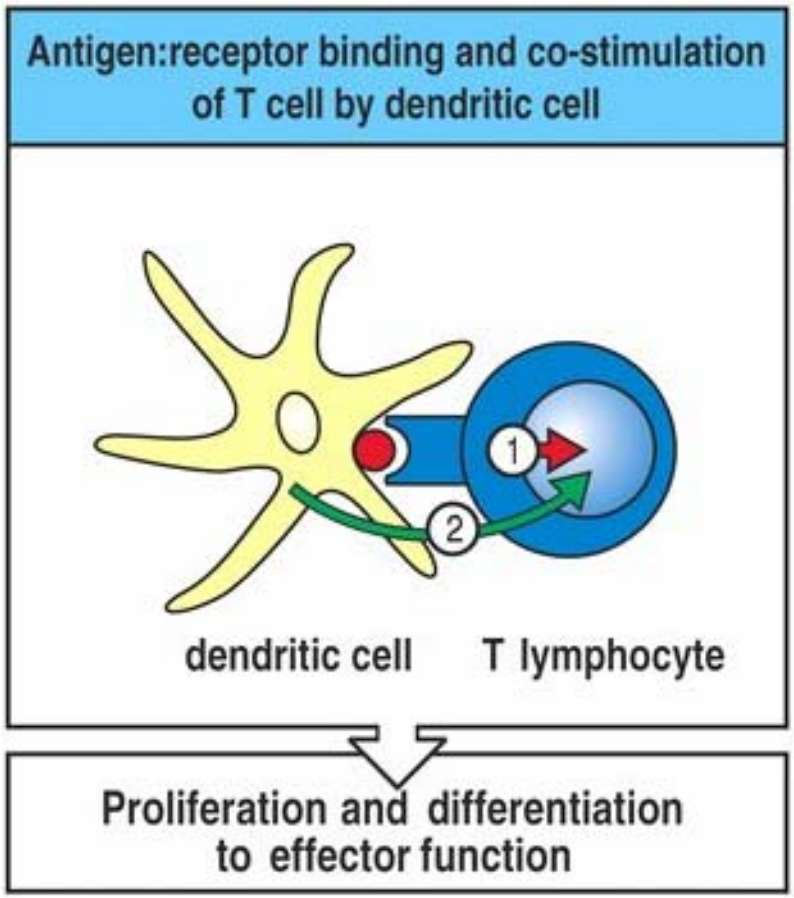


Figure 7-3 Immunobiology, 6/e. (© Garland Science 2005)

Background – Dendritic Cell



- MHC Class I
- MHC Class II
- Co-stimulatory molecules:
  - CD40
  - CD80
  - CD86

Background – MHC Class I Pathway

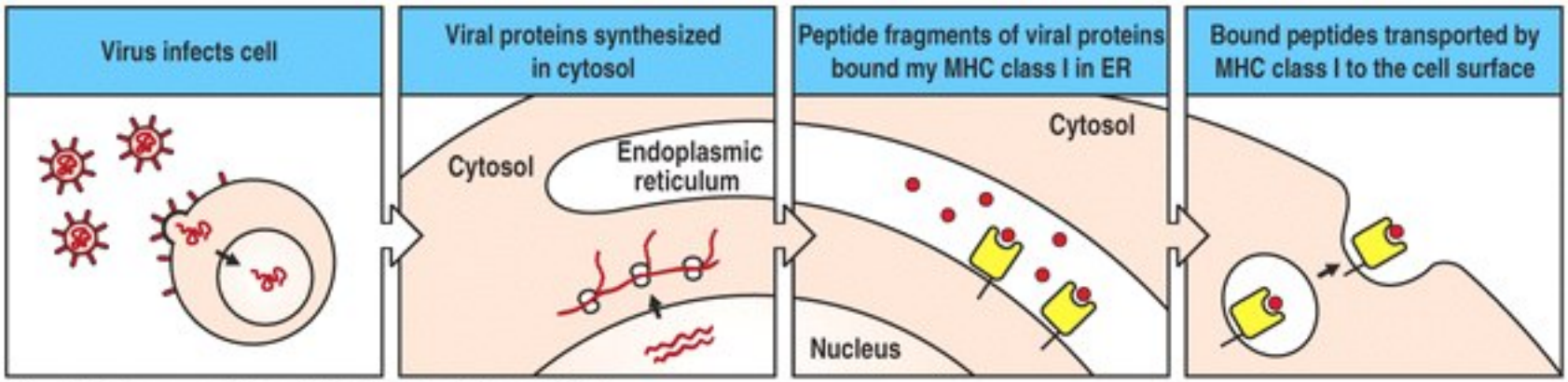
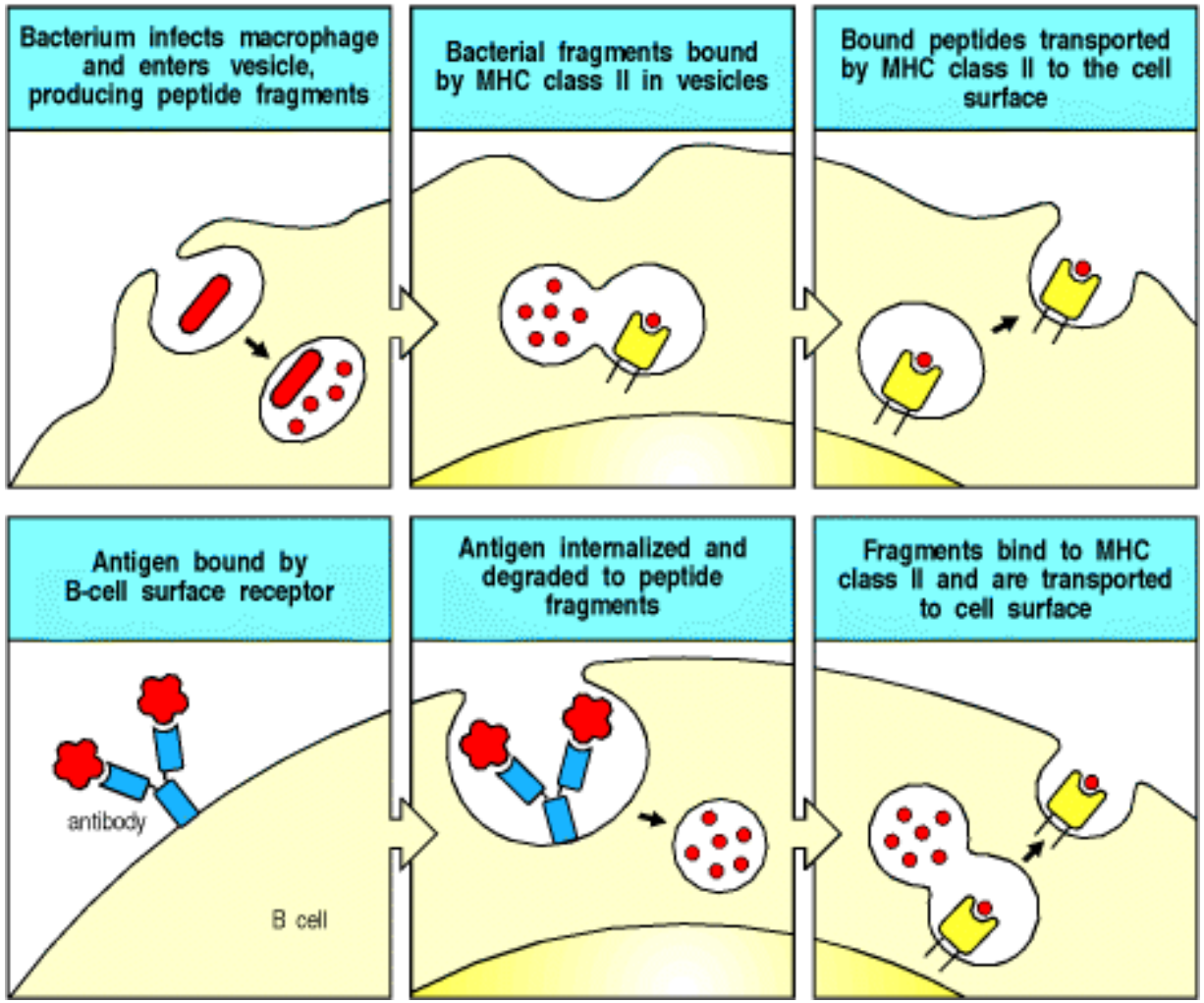


Figure 1-28 Immunobiology, 6/e. (© Garland Science 2005)

Background – MHC Class II Pathway



Background – CD8+ & CD4+ T cells

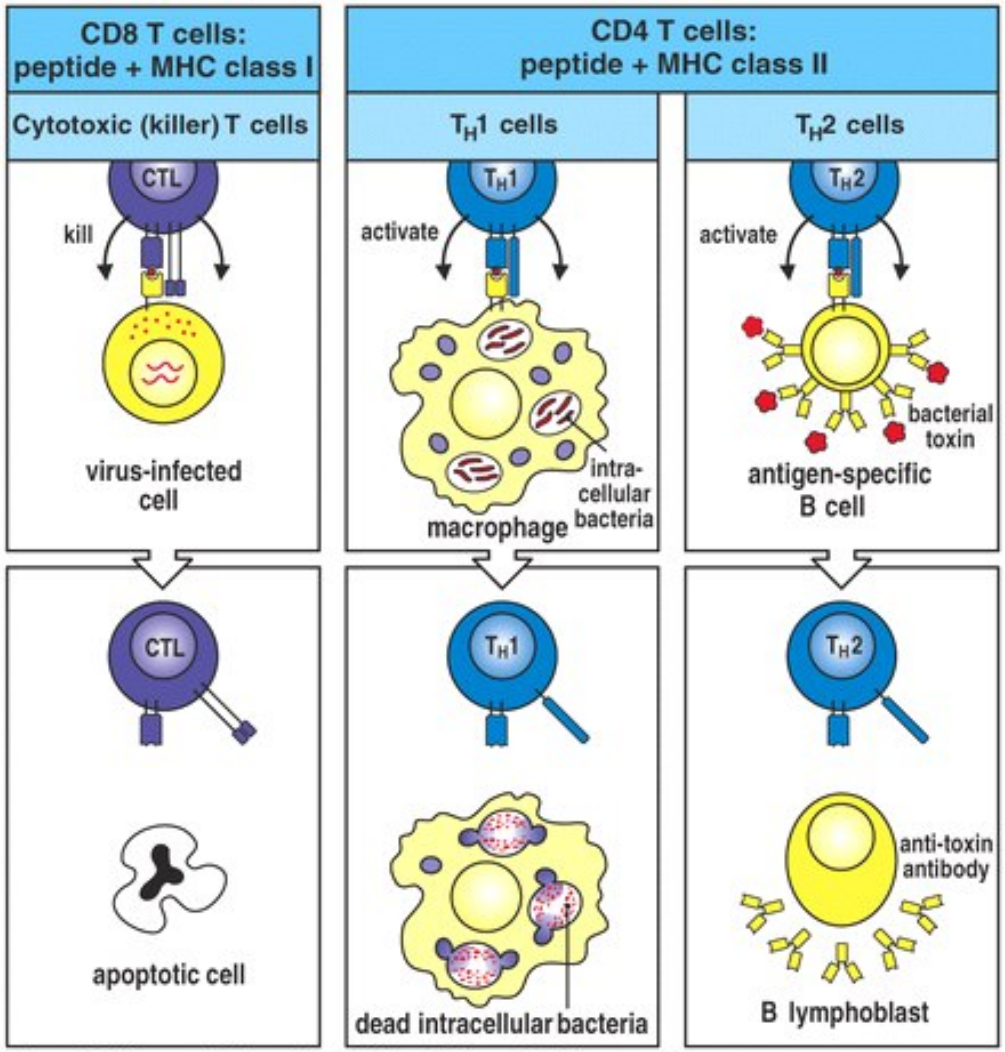
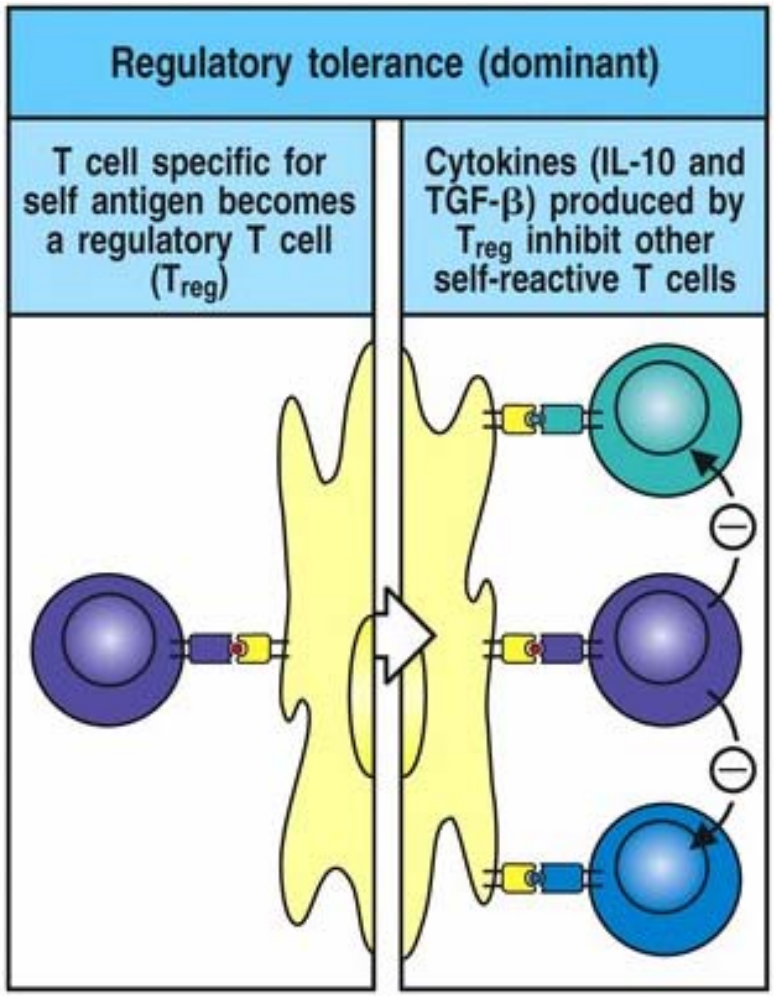


Figure 8-27 Immunobiology, 6/e. (© Garland Science 2005)

Background – CD4+ CD25+ Regulatory T Cells



- "low frequency and unknown specificity of naturally occurring CD4+CD25+ T_{reg} cells limit their immediate application"

  - *Nature Reviews Immunology* **5**, 100 (2005)

Jaeckel, E. *et al*. Antigen specific FoxP3-transduced T-cells can control established type 1 diabetes. *Diabetes* **54**, 306–310 (2005)

Mekala, D. J. & Geiger, T. L. Immunotherapy of autoimmune encephalomyelitis with re-directed CD4+CD25+ T-lymphocytes. *Blood* **105**, 2090–2092 (2004)

Figure 1 – Objective and Methods

Objective:

Determine if heat shock and gamma irradiation induce apoptosis and necrosis in B16-F10 melanoma cells.

Methods:

- **Heat shock:** Incubation @ 43°C for 1 hr
- **Gamma irradiation:** 200 Gy from cesium source (usually ^{137}Cs)
- **Flow cytometry:**
  - Incubation @ 37°C & 5% CO_2 for further 20 – 24 hrs
  - Annexin V staining for apoptosis and necrosis
  - Propidium iodide staining for apoptosis

Figure 1 – Methods, cont.

Annexin V/PI staining for apoptosis/necrosis:

- R1 = Live cells
- R2 = Apoptotic cells
- R3 = Necrotic cells

Annexin V binds phosphatidylserine

Information obtained from:
http://www.roche-applied-science.com
/pack-insert/1858777a.pdf

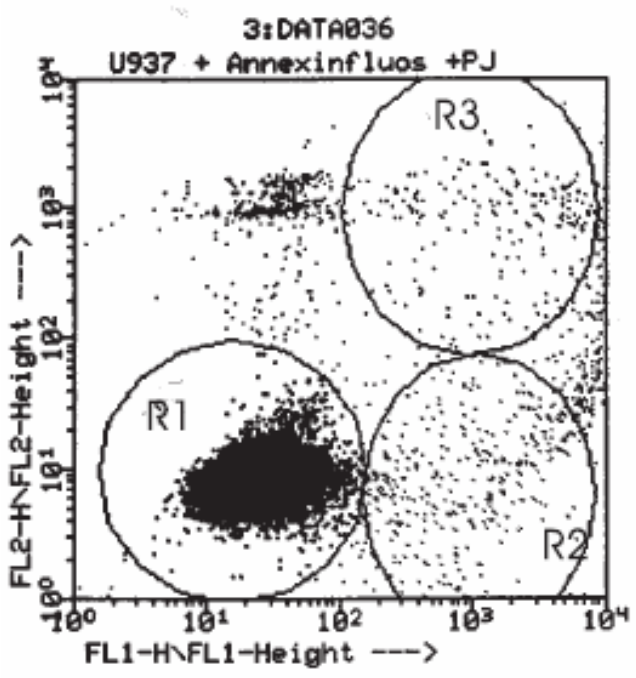


Figure 1 - Results

- Stressed cells:
  - ↓ cell size (FSC)
  - ↑ granularity (SSC)
  - Consistent with apoptosis
- Significant apoptosis and necrosis induced by heat shock/irradiation, maximal after 20 hrs.

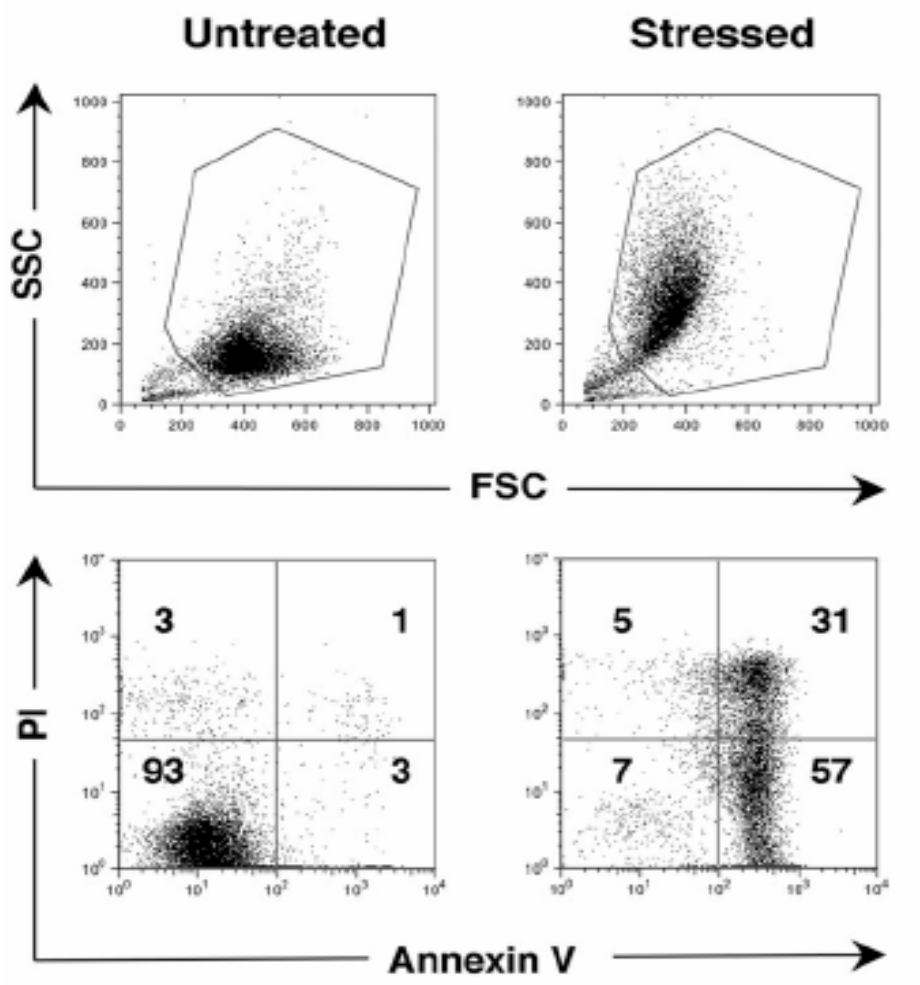


Figure 1 – Discussion

- Advantages of using whole stressed tumor cells:
  - Avoid issues concerning Ag preparation and DC loading
  - Potential broad array of tumor Ags
- Cells were stressed:
  - Induction of apoptosis for receptor-mediated uptake:
    - CD36, $\alpha_V\beta_5$ integrin, phosphatidylserine receptor, HSP receptor
  - Induction of necrosis:
    - More potential sources of tumor Ag

Figure 2 – Objective and Methods

Objective:

Determine if stressed melanoma cells are taken up by DCs.

Methods:

- **DC CFSE labeling:**
  - Harvest, wash and resuspend in PBS (1 x 10^6 cells/mL)
  - Add equal volume of 2.5 μM CFSE for 8 min
  - Quench with equal volume of FCS and wash with cIMDM
- **B16-F10 CTO labeling:**
  - 10 mM CTO in cIMDM for 8 min
  - Wash and stress by heat shock and irradiation
  - Incubate for 2 hrs
- **1:1 co-incubation of DC & B16-F10 for 48 hrs**
- **Flow cytometry analysis**

Figure 2 - Results

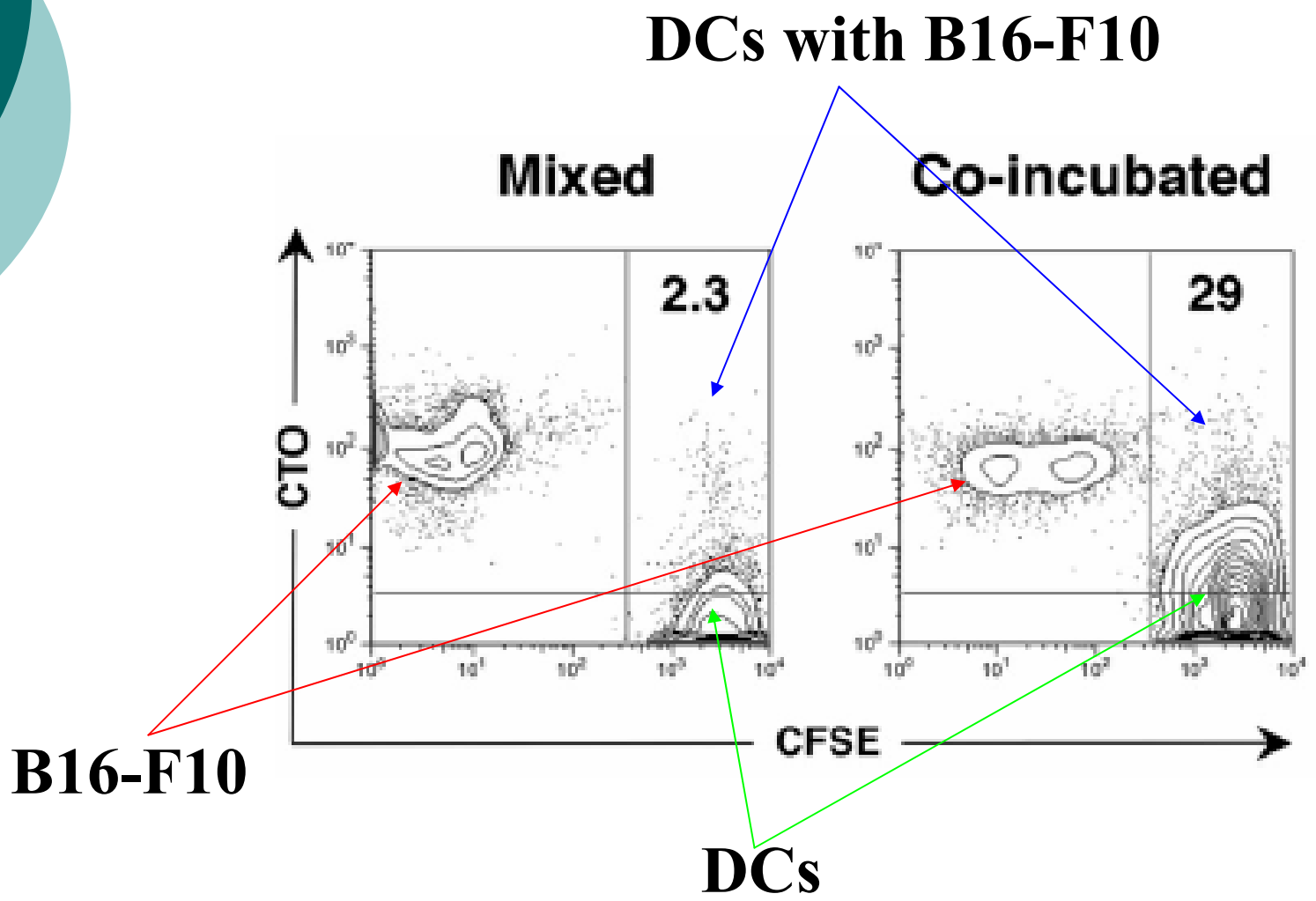


Figure 3 – Objective and Methods

Objective:

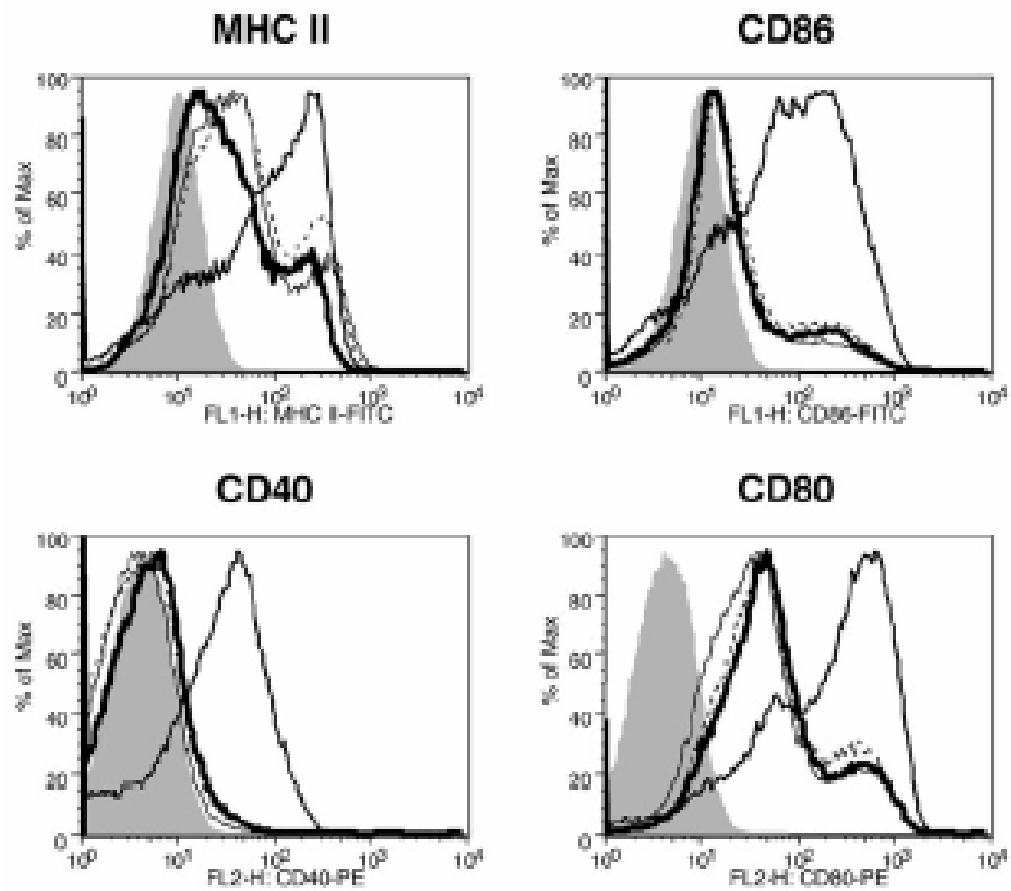
Determine if exposure to stressed melanoma cells induces DC maturation.

Methods:

- DC incubated with stressed B16-F10 for 48 hrs @ 1:1 ratio
- Positive control: 100 ng/mL LPS during last 24 hrs
- Flow cytometry analysis:
  - Blocked FcγRII with 2.4G2
  - MHC Class II
  - CD40, CD80, CD86

Figure 3 - Results

- Shaded histograms: unstained cells
- Thin line: untreated DC
- Dashed line: mock treated DC
- Medium line: LPS
- Thick line: Co-cultures



- LPS induces robust maturation.
- Stressed tumor cells have no detectable effect on DC maturation.

Figure 3 – Discussion

- DC cultures were kept “clean” of contaminating endotoxin
  - PCR analyses for presence of mycoplasma
- Heat shock of B16-F10 to induce DC maturation through up-regulated HSP not seen
- DC maturation was not induced *in vitro* or *in vivo*
  - LPS-treated DCs + stressed B16-F10 induced stronger anti-tumor response.

Figure 4 – Objective and Methods

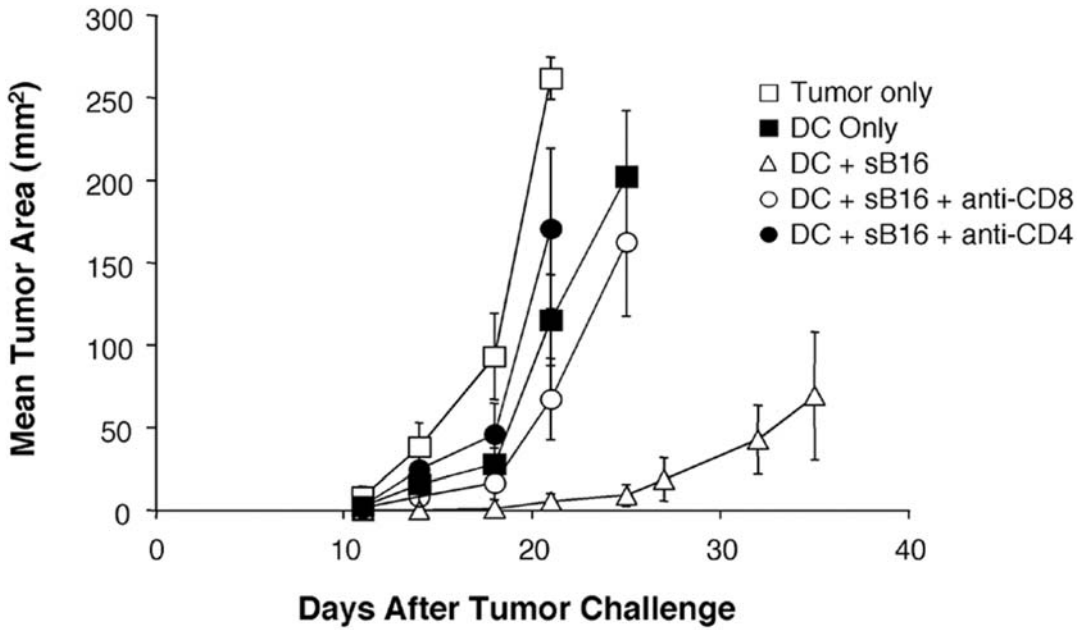
Objective:

Determine if tumor growth is retarded by DC vaccination with various vaccination regimens.

Methods:

- **Day 0:** 5 mice/group were left untreated, or vaccinated with
  - Stressed B16.F10 melanoma cells only
  - DC only
  - DC + stressed melanoma cells
  - DC + stressed melanoma cells + anti-CD8 (days -3, -2, -1)
  - DC + stressed melanoma cells + anti-CD4 (days -3, -2, -1)
- **Day 7:** All mice challenged with B16-F10 tumor cells, and monitored thereafter

Figure 4 - Results



- Only DC + stressed B16-F10 was able to induce tumor retardation.
- Depletion of CD8+ and CD4+ T cells resulted in the inability to retard tumor growth.

Figure 4 – Discussion

- Tumor retardation requires:
  - CD8+ activity
    - CTL activity – perforin-dependent lysis / IFN-γ secretion
  - CD4+ activity
    - Thought to provide help to CTL via cytokines
    - Direct role in tumor rejection – TNF α etc.

Figure 5 – Objective and Methods

Objective:

Determine if pretreatment of DCs with LPS improves the effect of vaccination with DC and stressed B16.F10 melanoma cells.

Methods:

- **Day 0:** 5 mice/group were left untreated, or vaccinated with
  - Stressed B16.F10 melanoma cells only
  - DC only
  - DC only + LPS
  - DC + stressed melanoma cells
  - DC + stressed melanoma cells + LPS
- **Day 7:** All mice challenged with B16-F10 tumor cells, and monitored thereafter

Figure 5 - Results

- Both untreated and LPS treated DC were able to induce tumor retardation, but LPS-treated DCs showed stronger results.
- DCs still showed tumor retardation in the absence of stressed melanoma cells.

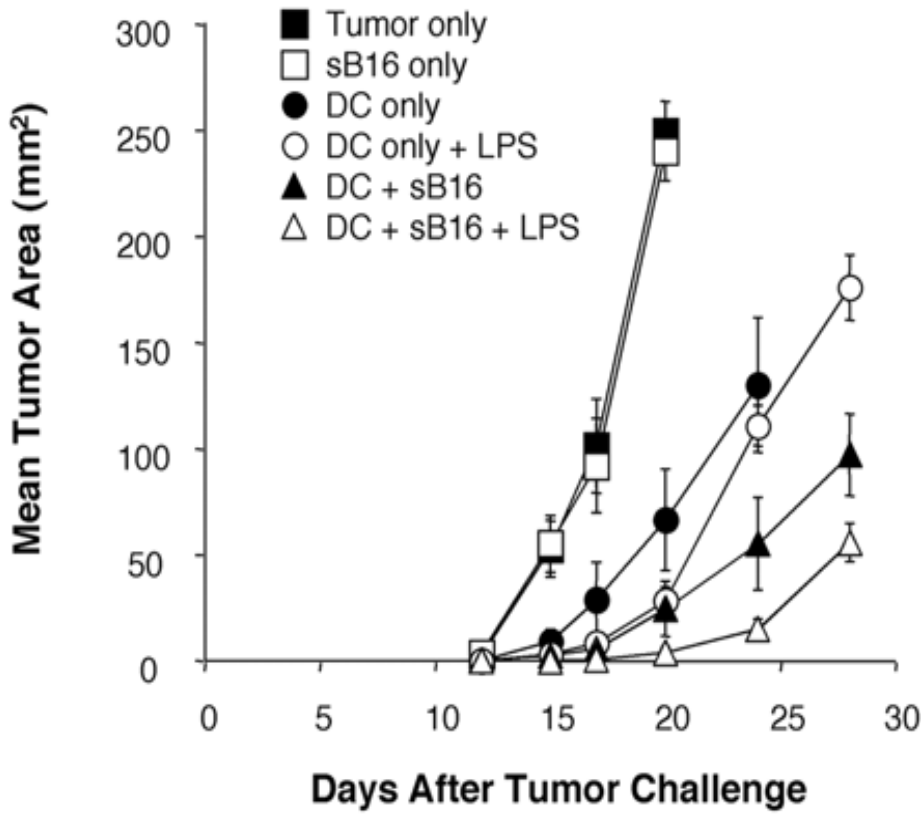


Figure 5 – Discussion

- Stronger immune response with LPS treatment is likely due to:
  - DC's higher expression of costimulatory molecules
  - Better ability to migrate to the draining lymph node after injection
    - Results in enhanced interaction with Ag-specific T cell populations
- Even in the absence of LPS, DCs were mature enough to initiate a protective immune response.

Figure 6 – Objective and Methods

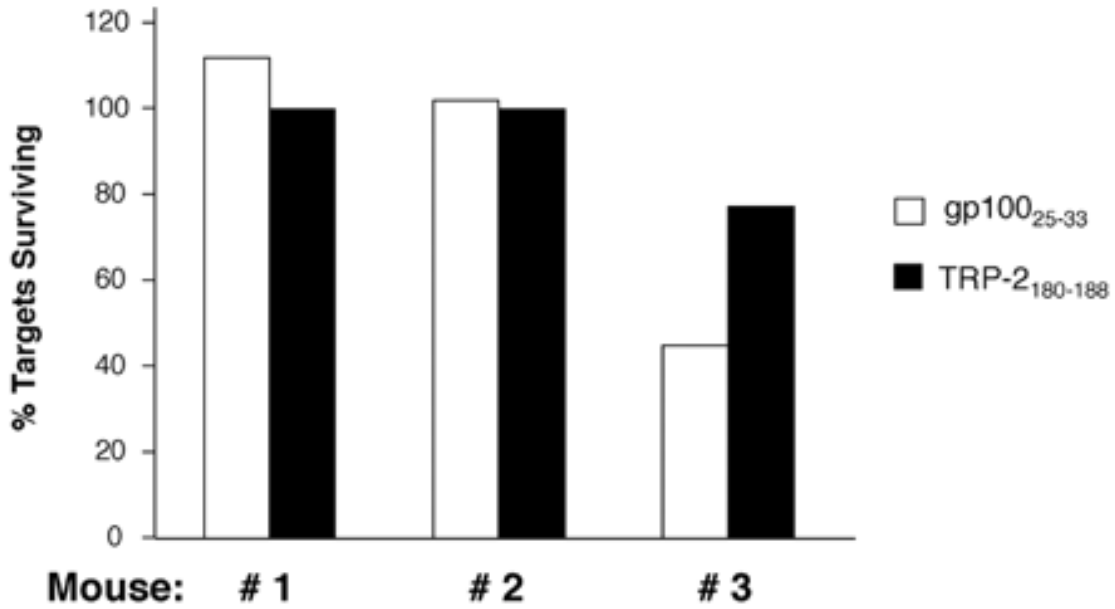
Objective:

Determine if vaccination with DC and stressed melanoma cells elicits a CTL response specific for known melanoma Ags (TRP-$2_{181\text{-}188}$ or $gp100_{25\text{-}33}$).

Methods:

- **In vivo cytotoxicity assay:**
  - Used mice which had rejected a challenge with B16-F10 melanoma cells following vaccination; mice were boosted, as initially vaccinated.
  - After 48hrs, mice were injected with equal numbers of unpulsed or peptide-pulsed, fluorochrome-labelled splenocytes (target cells).
  - After 48hrs, presence of target cells in lymph notes was detected by flow cytometry.
  - Survival based on ratio of peptide-pulsed to unpulsed target cells compared to control cells.

Figure 6 – Results



- Some cytotoxicity displayed in only 1 of 3 mice.
- CTL response specific for these known melanoma antigens probably not involved in protection; response likely directed at other antigens.

Figure 6 - Discussion

- TRP-$2_{181\text{-}188}$ and $gp100_{25\text{-}33}$ are found in both normal melanocytes and melanomas.
  - Ag-specific T cells against these epitopes have been documented in melanoma immunity and autoimmune situations.
- Tumor-protective CD8+ T cells activated by DC/stressed tumor cell vaccine may be directed at melanoma epitopes **not** shared with normal melanocytes.
- Lack of autoimmune effects may be due to suboptimal activation of DCs, which leads to inadequate activation of specific CD8+ T cells.

Figure 7 – Objective and Methods

Objective:

Determine if the protective effect of DC/stressed tumor cell vaccine is enhanced by prior depletion of CD4+CD25+ T_{reg}.

Methods:

- **Day 0:** Mice were left untreated, or vaccinated with
  - Stressed B16.F10 melanoma cells only
  - DC only
  - DC only + anti-CD25 (day -1)
  - DC + stressed melanoma cells
  - DC + stressed melanoma cells +anti-CD-25

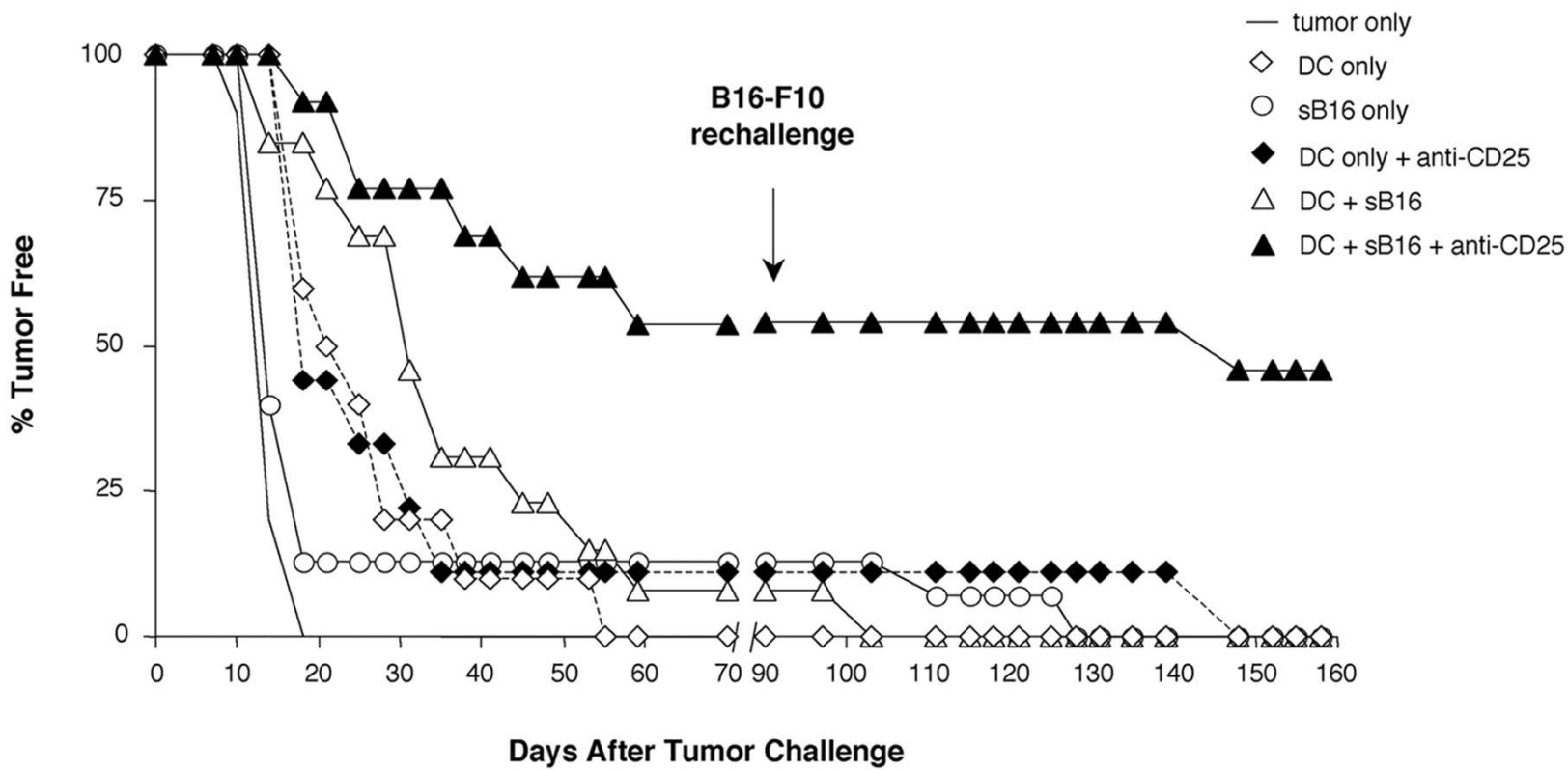
Figure 7 – Objective and Methods, cont.

Methods, cont.:

- **Day 7:** All mice challenged with B16-F10 tumor cells, and monitored thereafter.
  - Scored as tumor positive when tumors were >3mm^2
- **Day 90:** Tumor-free mice received second B16-F10 tumor cell challenge, and monitored thereafter.

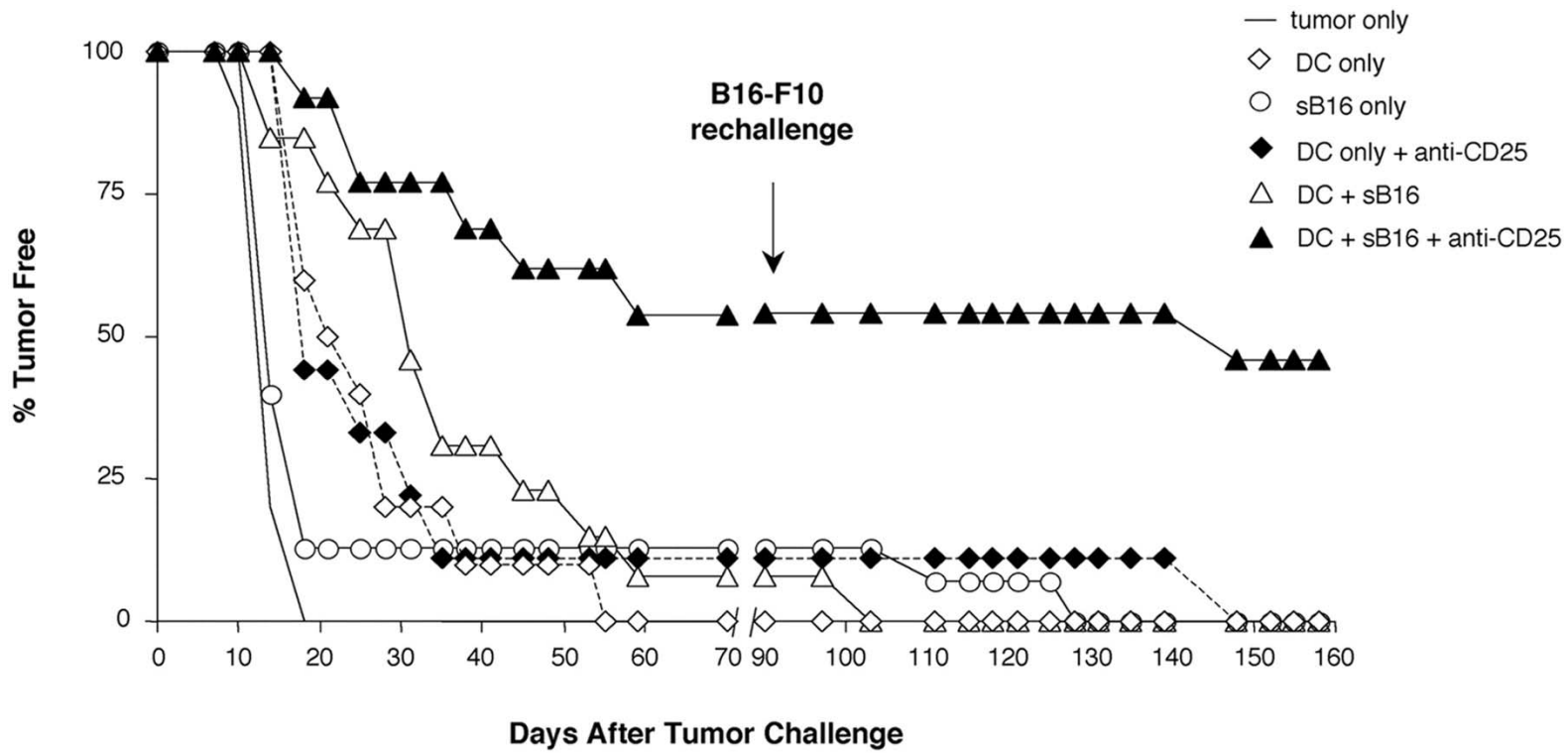
- Each line is a group of 9-15 mice.

Figure 7 - Results



- **Over 50%** of mice depleted of T_{reg} and immunized with DC and stressed tumor cells were tumor-free for **>60 days**; in DC/stressed tumor group and in DC only and anti-CD25 control group, only **10%** tumor-free.

Figure 7 – Results, cont.



- After day 90 challenge, most mice in T_{reg} depleted group remained tumor free. All mice in other groups developed tumors.
- Thus, depletion of T_{reg} enhances impact of vaccination and long-term memory with DC and stressed tumor cells.

Figure 7 - Discussion

- Use of self-Ags can lead to preferential activation of T_{reg}, and therefore suppression of anti-tumor immune response.
- DC / stressed tumor cell vaccine may contain significant self-Ags, so T_{reg} depletion may be required to allow an effective response against these self-Ags.
- Other authors have reported that T_{reg} depletion is required for a strong response with DC and non-self-Ag.
  - May be a general phenomenon important for both self and non-self-Ags.

Figure 8 – Objective and Methods

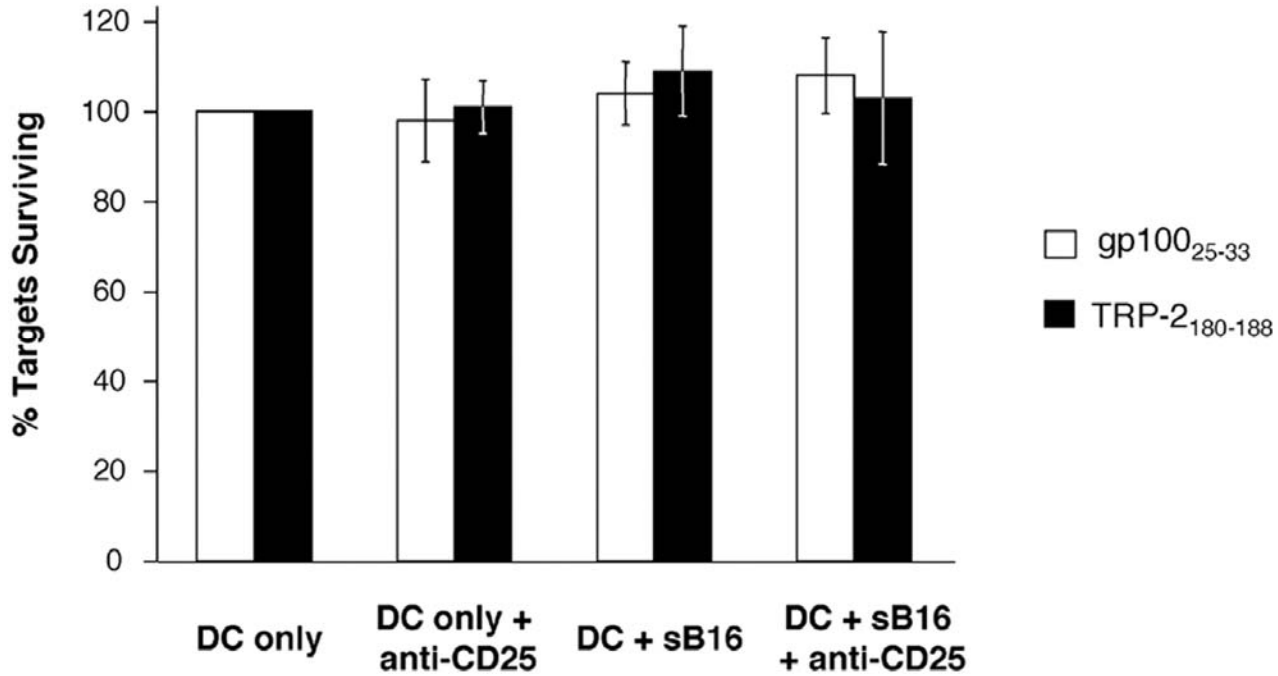
Objective:

Determine if vaccination with DC and stressed melanoma cells elicits a CTL response specific for melanoma Ags TRP-$2_{181\text{-}188}$ or $gp100_{25\text{-}33}$, implicated in autoimmune vitiligo, when T_{regs} have been depleted.

Methods:

- **In vivo cytotoxicity assay:**
  - Mice injected with saline or anti-CD25 mAb PC61 on day -1, and vaccinated with DC and stressed tumor cells, or DC only, on day 0.
  - Assay procedure involving fluorochrome-labeled unpulsed and peptide-pulsed target cells as previously described in Figure 6. Evaluated survival after 72 hrs.

Figure 8 - Results



- No detectable response to known melanoma antigens.
- Depletion of T_{regs} resulted in induction of strong tumor-protective memory T cell responses by DC/stressed tumor cell vaccine, but no detectable increase in risk of autoimmune effects.

Main Conclusions

- Dendritic cells loaded with stressed melanoma tumor cells activated tumor-protective CD8+ and CD4+ T cells which retarded tumor growth.
  - Prophylactic vaccine
- Potency of vaccine was increased by treatment of dendritic cells with LPS to induce DC maturation.
- Concomitant depletion of T_{reg} resulted in full tumor rejection and long-lasting tumor immunity in most tumor-inoculated mice.

Comments

- Susceptibility of DC to inhibitory function of T_{reg} will depend on DC maturation status.
  - Could have evaluated the impact of

    DC + sB16 + anti-CD25 (+/-) **LPS**
- Did not fully address retardation seen with DC only (ex. Figure 5).
- Is DC maturation advantageous?
- Practicality of T_{reg} depletion in human system?
  - Autoimmune response?