Chronic Mild Thermal Stress Stimulates Tumor Growth and Depresses the Anti-Tumor Immune Response

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How is the immune system regulated by body temperature?
Mild (Fever-Range) Hyperthermia Affects Many Aspects of the Tumor Microenvironment
THERMOREGULATION- A unique automomic system

• Evolved to regulate a stable core over wide range of ambient temperatures, heat loads from work, and fever

• One of the only systems that also relies on behavior and conscious awareness of the environment to achieve regulation
Effectiveness of Human Thermoregulation
The stress response

• Integrated definition
  – Stressor (internal or external stimulus)
  – Stress perception in the brain
  – Physiologic adaptions ("fight-or-flight")

• Internal stimuli
  – Emotions (depression, fear)
  – Anxiety (crowding, isolation)

• External stimuli
  – Chemical (toxins)
  – Biological (infection, inflammation, metabolic)
  – Physical (temperature, pain)

Joel et al, Nature Reviews Neuroscience 2009
Glaser et al, Nature Reviews Immunology 2005

Sympathetic Nervous System (SNS)-Norepinephrine (adrenalin)
Hypothalamic-Pituitary-Adrenal axis (HPA-axis)
Hormonal and “hard-wired” mediators of stress
Sensitivity to ambient temperature depends on surface/volume ratio.

Mice are very sensitive to ambient temperature.

Mice easily gain, or lose, heat from their environment.

Why Humans Have No Fur
And How Evolving Bare Skin Led to Big Brains

“Impossible” Colors See Hues That Can’t Exist.

Plus:
Seafloor Oases
Whale Carcasses Sustain Ecosystems

Mysteries of How a STAR IS BORN

HIGH-MPG CARS
Gasoline-Engine Innovations

STOPPING INFECTIONS
New Ways to Elude Bacterial Invaders
Housing temperature for laboratory mice—
An unexpected tool for studying cold stress and cancer

- The thermoneutral temperature for laboratory mice is between 29-31°C (~84-88°F)
  - At thermoneutrality, little or no extra metabolism is required to maintain body temperature.

- But IACUC certified facilities must select and maintain a temperature between 20-26°C (~68-78°; until 2011, 18-24 C permitted). (Guide for the Care and Use of Laboratory Animals, 8th Ed, 2011)
  - The standard temperature ranges in Europe and Asia are even lower (approx. 18-24°C).
  - The temperature in the animal facility at RPCI is maintained between 22-23°C (approx. 72 °C)
Mice in a huddle- A typical scene in the mouse room at ST

Photo by K. Kokolus and S. Sexton, RPCI
Mice at ST and TT maintain a normal body temperature

Kokolus et al, PNAS 2013
STATIC DISCHARGE RATE OF CUTANEOUS WARM AND COLD FIBERS

Hensel, 1969

Pain threshold

Hensel, 1969
Maintaining a Normal Body Temperature (37°C) in Mice Requires a Significant Increase in Metabolic Rate when Housed ST

Adapted from Specter et al, Handbook of Biological Data 1956
Silva et al, Physiological Reviews 2006
Canon et al, Journal of Experimental Biology 2011
Cold Stress Induces Norepinephrine Production to Facilitate Heat Production

Cold Stress

$\Delta$HEAT

UCP1

The uncoupling protein in mitochondria drives adaptive thermogenesis


Cannon et al, Physiology Reviews 2006

Adapted from Karp et al, Journal of Experimental Medicine 2012
Laboratory mice feel even more cold after tumor implantation!

Using a thermal preference apparatus to assess thermal comfort

Katie Kokolus, PhD

n=5, p=0.01
Older mice (> 18 months) prefer a warmer temperature (~34 °C)

Bonnie Hylander, PhD
Plasma and Tumor Norepinephrine Levels are Increased in Mice Housed at ST compared with TT

Naïve and tumor-bearing mice maintained at ST and TT for 3-4 weeks → Collect plasma and tumor lysates → Analyze by ELISA for norepinephrine concentrations

**Graphs:**
- **MIA PaCa-2:**
  - ST: 80 pg of NE/mg of total protein
  - TT: 50 pg of NE/mg of total protein
  - n=13
  - *p<0.05

- **BxPC-3:**
  - ST: 100 pg of NE/mg of total protein
  - TT: 50 pg of NE/mg of total protein
  - n=6
  - **p<0.01

- **Pan02:**
  - ST: 25 pg of NE/mg of total protein
  - TT: 20 pg of NE/mg of total protein
  - n=6
  - p=0.19

- **12424:**
  - ST: 10 pg of NE/mg of total protein
  - TT: 5 pg of NE/mg of total protein
  - n=5
  - p=0.17
How is tumor growth influenced by ambient temperature?

Standard Temperature (ST) → 22°C (~72°F)
Thermoneutral Temperature (TT) → 30°C (~86°F)

- Ambient temperature consistently maintained
- Humidity controlled with an air pump
- 12 hour light/dark cycle (6 AM – 6 PM)
Alleviating Cold Stress by Housing Mice at TT Significantly Improves Tumor Control

Kokolus et al, PNAS 2013
Tumor growth is significantly slower in mice housed at thermoneutrality and this depends on the immune system

Kokolus et al, PNAS 2013

* p < 0.05
**** p < 0.0001
Mice Housed at TT Develop Fewer Metastastic Tumors (4T1 tumor model)

Kokolus et al., PNAS 2013
Carcinogen (methylcholanthrene) induced tumor formation is prevented or delayed at TT

Kokolus et al, PNAS 2013
CD8+ T Cells in the Tumor Microenvironment are Increased at TT

N = 5 - 6; * p < 0.05, *** p < 0.001

Kokolus et al., - PNAS 2013
Are immunosuppressive cells impacted by cold stress?
Myeloid derived suppressor cells suppress antitumor immunity through a variety of diverse mechanisms.
Decreased Spleen Mass in Tumor Bearing Mice at TT

The spleen is a critical repository for MDSCs prior to their trafficking to the tumor microenvironment. (Cortez-Retamozo et al., 2012)

Significant Reduction in MDSC in Tumor Bearing Mice at TT

Flow Cytometry (Spleen)

N=10/group

** p<0.01

Kokolus et al., PNAS 2013
Does blocking β-adrenergic signaling in mice at ST or TT impact tumor growth?
Propranolol treatment converts the ST phenotype to that seen at TT.

Effect depends upon presence of immune system!

(A) BALB/c - 4T1 Tumor

(B) SCID - 4T1 Tumor

Mark Bucsek, Katie Kokolus
The sympathetic system can impact immune cells via mechanisms now being defined.
Conclusions: Ambient temperature impairs the anti-tumor immune response

- Chronic thermal stress results in reduced CD8+ T cell infiltration and increased Treg/MDSC
- Increased levels of NE in tumors
- Can be reversed by TT or β-blockers
- Causes environmentally induced resistance to cytotoxic therapies

• *Thermal damage may include the impact of chronic mild thermal stress*
Growing recognition of an ominous role for norepinephrine in tumor progression

- Adrenergic signaling protects ovarian tumor cells from anoikis-apoptosis due to detachment (Sood et al 2010; Cole & Sood, Clin Canc Res, 2012)

- NE increases VEGF, IL-6, and MMPs in ovarian and pancreatic cancers leading to increase metastasis. (Thakar et al, Nature Medicine 2006; Lutgendorf et al, Clinical Cancer Research 2003; Guo et al, Oncology Reports 2009)

- NE downregulates MHC class 1 and B7-1 on tumor cells leading to immune escape. (Wang et al, Plos One 2009)

- NE enhances prostate tumor survival by upregulating the expression of MAPK, and inactivating the apoptotic molecule, BAD. (Sastry, JBC 2007; Hassan et al, JCI 2012)

- Activation of β-adrenergic receptors promotes the formation of primary tumors and metastases. (Campbell et al PloS One 2012; Magnon et al Science 2013)
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