Stress Responses: Diverse Impact on Immunity, Cancer and Therapy

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Advanced Immunology
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STRESS RESPONSE in Advanced Immunology?

• Since the 40s and 50s, GC hormones have been prescribed clinically because of their inherent and powerful anti-inflammatory properties..
• 1950 Nobel Prize in Medicine: Edward Kendall, Tadeus Reichstein and Philip Hench for their studies on the hormones of the adrenal cortex.
• Based on their early observations, it has become very clear that GC hormones can regulate a wide variety of immune cell functions.
• Modulate expression of: cytokines, chemoattractants, adhesion-molecules, and impact immune cell trafficking, lymphocyte proliferation and differentiation, and effector function.
• How do stress hormones mediate this wide spectrum of immunological influence??
• HSPs- UPR, Stress Response Proteins: Relationship to immune response?
How does stress influence the immune system?

• Psychoneuroimmunology- PNI
• Focuses on the interactions among the central nervous system (CNS), the endocrine system and the immune system, and the impact that these interactions have on health.
• --a complex network of signals that function in bi-directional communication among these 3 system.
• The hypothalamic-pituitary-adrenal (HPA) and the sympathetic-adrenal medullary (SAM) axes are the two major pathways through which the immune system can be altered.
Immune system can respond to stress

- Lymphocytes, monocytes, or macrophages and granulocytes, exhibit receptors for many neuroendocrine products of the HPA and SAM axes, such as cortisol and catecholamines, which can cause changes in cellular trafficking, proliferation, cytokine secretion, antibody production and cytolysis.
Ecoimmunology- a new discipline related to PNI

• The study of environmental influences on immune function

• Involves the fields of ecology and evolutionary biology.

• Goal-to understand immune function within a broadly integrative, organismal context, but from a more evolutionary perspective,

• Involves the study on interactions and trade-offs between immunity and other life history traits, both within and across individuals, populations and species. (see review by Demas and Carlton, 2015)
Goal of Ecoimmunology

• To understand extrinsic and intrinsic factors leading to changes in immune system function and how these changes contribute to disease susceptibility across a wide range of animal species (Usually natural populations, not model systems)
The immune and nervous systems communicate with and regulate each other.
Stressor

Hypothalamus

Corticotropin-releasing hormone
Pituitary gland

Brain

Adrenocorticotrophic hormone

'Hard-wiring' sympathetic innervation

Prolactin and growth hormone

Adrenal gland
Cortex
Medulla

Lymph node

Other physiological pathways for stress

• Endogenous opioids diminish NK cell cytotoxicity,

• Substance P, a neuropeptide is able to reduce inflammatory responses by suppressing IL-16 production by eosinophils.

• Whereas the anti-inflammatory function of GC center on NF-kB inhibition, the GR most likely interferes with other transcriptional regulators. (AP-1 and NF-AT.)
<table>
<thead>
<tr>
<th>Hormone</th>
<th>Expression of receptors by immune cells</th>
<th>Examples of effects on cell function</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucocorticoids</td>
<td>T and B cells, neutrophils, monocytes and macrophages</td>
<td>Inhibit inflammation; inhibit the production of IL-12 by antigen-presenting cells; induce a shift from production of T_{h}1 to T_{h}2 cytokines</td>
<td>87,88</td>
</tr>
<tr>
<td>Substance P</td>
<td>T and B cells, eosinophils, mast cells, monocytes and macrophages</td>
<td>Stimulates mitogen-induced blastogenesis; increases trafficking of cells from lymph nodes to peripheral blood; stimulates monocytes to produce several cytokines, such as IL-1, IL-6 and TNF</td>
<td>89</td>
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<tr>
<td>Neuropeptide Y</td>
<td>T and B cells, dendritic cells, monocytes and macrophages</td>
<td>Can downregulate antibody production to T-cell-dependent antigens by its effect on dendritic cells, and T and B cells</td>
<td>90</td>
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<tr>
<td>Corticotropin-releasing hormone</td>
<td>T cells, monocytes and macrophages</td>
<td>Increases production of IL-1 by monocytes; evidence for autocrine and/or paracrine modulation of inflammation</td>
<td>91</td>
</tr>
<tr>
<td>Prolactin</td>
<td>T and B cells, granulocytes, NK cells, monocytes and macrophages</td>
<td>Can stimulate lymphoid-cell clonal expansion; might function as an in vitro co-mitogen for NK cells and macrophages</td>
<td>92,93</td>
</tr>
<tr>
<td>Growth hormone</td>
<td>T and B cells, NK cells, monocytes and macrophages</td>
<td>Helps to maintain competence of T and B cells, and macrophages; stimulates antibody production and NK-cell activity</td>
<td>94</td>
</tr>
<tr>
<td>Catecholamines (adrenaline and noradrenaline)</td>
<td>T and B cells, NK cells, monocytes and macrophages</td>
<td>Induce a shift to a T_{h}2 response, involving antigen-presenting cells and T_{h}1 cells</td>
<td>95</td>
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<tr>
<td>Serotonin</td>
<td>T and B cells, NK cells, monocytes and macrophages</td>
<td>Modulates the synthesis of IFN-γ by NK cells; stimulates the production of IL-16 (a chemotactic factor) by T cells</td>
<td>96</td>
</tr>
</tbody>
</table>

FN-γ, interferon-γ; IL, interleukin; NK, natural killer; T_{h}, T helper; TNF, tumour-necrosis factor.
Is there an analogous role for autonomic nerves?
The sympathetic nervous system controls tumor growth in mice: Norepinephrine (NE)

Magnon/Frenette Science 341, 1236361 (2013)
Stress promotes tumor growth and metastasis

• SNS stress response releases catecholamines (NE, Epi)

• NE/Epi signal through β-AR to promote tumor growth by several mechanisms (Cole & Sood, Clin Canc Res, 2012)

• Adrenergic signaling protects ovarian tumor cells from anoikis- apoptosis due to detachment (Sood et al 2010)

• Enhances prostate tumor survival by inactivating (phosphorylating) the pro-apoptotic molecule, BAD. (Sastry, JBC 2007; Hassan et al, JCI 2012)
• Standard temperature (ST) – 19-22°C (66-72°F, RT for humans)
• Thermoneutral zone – the range of ambient temperatures in which minimal metabolic energy is expended for warmth (or cooling)
• Thermoneutral temperature (TT) – 30–32°C (86-90°F) (Gordon, Temp Regulation in Laboratory Rodents, 1993).
• Mice will select TT if given the opportunity

Laboratory Mice are Cold Stressed Due to Housing Temperature

Martin et al, PNAS 2010
Guide for the Care and Use of Laboratory Animals 8th Ed, 2011
Karp, JEM 2012
Generation of extra body heat through adaptive thermogenesis uses a lot of energy.

At standard ambient temperature mice:

- Eat more
- Have higher BP, heart and breathing rates
- Have increased oxygen consumption

Guyton & Hall, 2002; Cannon, 2004; Gordon, 2012
Cold Stress Induces Norepinephrine Production to Facilitate Heat Production

Cold Stress

$\Delta$HEAT

$\uparrow$UCP1

The uncoupling protein in mitochondria drives adaptive thermogenesis

Nguyen et al, Nature 2011
UCP1 Mediates Adaptive Thermogenesis by Uncoupling Oxidative Respiration
NE Levels are increased in mice housed at ST compared with TT

Eng et al, Nat Comm 2015
Tumor growth is inhibited at TT and this depends on the immune system.

Kokolus et al, PNAS 2013

* p < 0.05
**** p < 0.0001
Increased activation of CD8$^+$ T cells in the tumors of mice housed at TT

N=5/group *p<0.05

Kokolus et al., - PNAS 2013
β-AR blockade improves tumor growth control and depends on the immune system

Propranolol: β1/β2 adrenergic receptor blocker

Mark Bucsek, manuscript in prep
How are other therapies affected by adrenergic signaling at ST?

Pro-survival molecules are increased in tumor cells at ST; Reversed by blockade of the β-ARs with propranolol (Pr)

MIA PaCa-2

<table>
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<th>ST</th>
<th>TT</th>
<th>ST+Pr</th>
<th>TT+Pr</th>
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<tr>
<td>pBAD^{S112}</td>
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<td>BAD</td>
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<td>BCL-X_{L}</td>
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<td>BCL-2</td>
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<td>β-Actin</td>
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</table>

Eng et al, Nat Comm 2015
Blockade of β-adrenergic signaling increases therapeutic sensitivity at ST

Eng et al, Nat Comm 2015
Physiological stress can alter diseases being modeled in laboratory mice

See also:
David Masopust Lab paper on the impact of adding pet store mice to cages of inbred mice, Nature, April, 2016

Demas and Carlton, Brain Behav Immun, 2015
Housing temperature affects mouse biology and experimental outcomes:

**PHYSIOLOGY**
- NE Levels (44, 46)
- Food Intake (7, 84)
- Adaptive Thermogenesis (71)
- Brown Fat Physiology (10, 37, 40)

**METABOLISM**
- Sleep (79, 94)
- Obesity (63, 66, 67)
- Insulin Production (46)
- Cardiovascular (27, 28)
- Organ Size, tail length (29)
- Basal Metabolism (7, 12, 84)

**HEMATOPOIETIC STEM CELLS**
- Radiation Sensitivity (88)
- Graft vs Host Disease (48)
- Bone Marrow Differentiation?

**CANCER**
- Chemotherapy (44)
- Immunosuppression (42)
- Growth and Metastasis (42)
- Anti-tumor Immunity (42, 47)
- Radiation?
- Immunotherapy?
- GEM Tumor Models?

**IMMUNITY/INFLAMMATION**
- Fever (89)
- Cytokines (43, 66)
- HSP induction (93)
- Atherosclerosis (70)
- Vascular Inflammation (70)
- Macrophage Polarization (43)

**OTHER MODELS?**
- Ageing?
- Exercise?
- Microbiome?
- Social or Restraint Stress?
- Other Experimental Stressors?

**HYLANDER & REPASKY, TRENDS IN CANCER, 2016**
1962
- Italian scientist Ferrucio Ritossa discovers new puffing pattern in the giant chromosomes found in *Drosophila* salivary glands: many of the normal (pre-existing) ‘puffs’ were gone, and he saw new ones. He later found that a colleague had increased the temperature of the incubator. Also observed new RNA synthesis

1974
- Alfred Tissières ‘rediscovered’ these results and observed new pattern of (radiolabeled) protein synthesis following a heat-shock by SDS PAGE

1982
- Several heat-shock inducible genes are cloned

1985
- Alfred Goldberg shows that production of abnormal proteins in *E. coli* activates the heat-shock response

1986
- Richard Voellmy discovers the trigger for the heat-shock (stress) response

1988
- Sambrook and Gething discover the Unfolded Protein Response (UPR) of the endoplasmic reticulum
- Molecular chaperone functions and structures are being elucidated
In the beginning

HSPs and Drosophila

Protection from teratogenesis
Stress Proteins

• Stress proteins are highly conserved throughout all living organisms
• Differentially compartmentalized and functionally regulated
• Constitutively expressed and can be induced under certain stress conditions
• Heat shock proteins (HSPs) and glucose regulated proteins (GRPs) are two major groups
• Act as molecular chaperones in protein maturation and function regulation
Stress Proteins and Stress Response

Cytoplasm, Nucleus, Mitochondria
hsp27
hsp40
hsp60
hsp70
hsp90
hsp110

Endoplasmic Reticulum (ER)
grp78(Bip)
grp94(gp96)
grp170

Inhibitors of glycosylation; hypoxia; calcium ionophores reductive stress

ER damage

heat oxidizing agents cytoplasmic & mitochondrial damage
Heavy Metals
Mammalian stress proteins: heat shock proteins & glucose (hypoxia) regulated proteins

Heat

Hypoxia

hsp110
hsp90
hsp70
hsp60
hsp28
grp170
grp94/gp96
grp78

Sciandra et al. PNAS 1984; Shen et al. PNAS 1987
How do HSPs protect the cell?

• Inhibition of protein aggregation that arises as a result of protein unfolding during physical (e.g. heat) or pathological (e.g. neurodegenerative diseases) stress: HOLDING

• Refolding the denatured substrate proteins using multi-component protein machines: FOLDING

• Directing damaged substrate proteins to degradation pathways.
Hsp110 and grp170 are highly effective in chaperoning large protein substrates: inhibition of heat induced Luciferase aggregation.
FOLDING

• Once the denatured protein is bound by the hsp (molecular chaperone), it can then be refolded with the assistance of other chaperones.
• For example: refolding of denatured Luciferase and Other Reporter Proteins
Stress Functions and Non-Stress Functions Are Related

- Holding, Folding, and Degradation activities are essential at all times in cells, during stress or in natural cellular processes.
- Holding, folding, assembly and translocation of newly synthesized proteins and multiprotein ensembles, targeting to cellular organelles, membranes, secretory pathway, etc.
Stress Proteins as Danger Signals

- HSPs and GRPs are released from damaged and dying cells.
- These stress proteins will carry peptide or protein elements related to cell damage, e.g., mutated proteins, viral proteins, etc.
- These stress proteins may therefore act as a ‘natural’ vaccine in-situ.
- Stress Proteins are one group of a broader set of natural ‘Danger Signals’ that are released by damaged cells and alert the immune system by binding to receptors on antigen-presenting cells and stimulating immune responses.
Chaperones, stress and evolution?

- Forces that govern protein folding may exert a profound effect on how genotypes are translated into phenotypes.
- HSPs promote the correct folding and maturation of proteins in the cell.
- Hsp90-abundant and highly specialized chaperone that works on “metastable” signal transducers that are key regulators of a broad spectrum of biological processes.
- The folding of “clients” of Hsp90 are very sensitive to changes in the external and internal environment of the cell.

See details in Lindquist, 2010
Figure 1. Examples of developmental abnormalities associated with Hsp90 deficits in Drosophila. (A) Thickened wing veins, (B) transformed second leg with an ectopic sex comb, (C) deformed eye with an extraantenna, (D) disorganized abdominal tergites, (E) notched wings, (F) extraneous tissue growing out of tracheal pit.
# Agents/treatments that induce the stress response

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Probable effects</th>
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<tbody>
<tr>
<td>temperature shifts (e.g., heat-shock)</td>
<td>protein denaturation</td>
</tr>
<tr>
<td>heavy metals, arsenite, iodoacetamide</td>
<td>reaction with sulfhydryl groups, conformational changes in protein</td>
</tr>
<tr>
<td>anoxia, hydrogen peroxide, superoxide ions, free radicals</td>
<td>oxygen toxicity, free radical fragmentation of proteins</td>
</tr>
<tr>
<td>proteasome inhibitors (lactacystin)</td>
<td>inhibition of proteolysis</td>
</tr>
<tr>
<td>amytal, azide, dinitrophenol, ionophores, rotenone, antimycin</td>
<td>inhibition of oxidative phosphorylation, changes in redox state, covalent modification of proteins</td>
</tr>
<tr>
<td>hydroxylamine</td>
<td>cleavage of asparagine-glycine bonds</td>
</tr>
<tr>
<td>ethanol</td>
<td>translation errors</td>
</tr>
<tr>
<td>amino acid analogs, puromycin</td>
<td>abnormal proteins</td>
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</tbody>
</table>
Stress response: cellular changes

- transcriptional upregulation of the synthesis of some genes
  - major proteins produced have the following sizes:
    - <30, 40, 60, 70, 90, ~100 kDa
- downregulation of the production of most proteins
- most dramatic example:
  - Pyrodictium occultum thermosome (chaperonin) accumulates to ~70% or more of total cellular protein; other proteins not produced

- acquired thermotolerance
What triggers the cell stress response?

- direct sensing of external agents, e.g., heat, by protein(s)?
- indirect sensing of external agents? translational downregulation of the production of most proteins

Experiment: co-injection of purified proteins and hsp gene reporter

<table>
<thead>
<tr>
<th>Injected in Xenopus oocytes</th>
<th>β-galactosidase activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>normal temperature (21ºC)</td>
<td>NO</td>
</tr>
<tr>
<td>heat shock (36.5ºC)</td>
<td>YES</td>
</tr>
<tr>
<td>construct + native protein</td>
<td>NO</td>
</tr>
<tr>
<td>construct + denatured protein</td>
<td>YES</td>
</tr>
</tbody>
</table>

Regulation of the stress response: prokaryotes

- Stress-inducible genes have promoters that differ from genes that are not induced under stress conditions:
  - Sometimes part of an operon, e.g., GroEL-GroES or DnaK-DnaJ-GrpE

- $\sigma^{70}$ (sigma-70) binds RNAP and activates transcription of genes

- $\sigma^{32}$ (sigma-32) can also assemble with RNAP, but it is unstable and rapidly degraded under physiological conditions; under stress conditions (e.g., heat-shock), it is stabilized and it assembles with RNAP

- RNAP-$\sigma^{32}$ binds HS promoters, upregulating their transcription

- DnaK (Hsp70), DnaJ (Hsp40) and GrpE $E.\ coli$ mutants have a constitutively active stress response
physiological stress physiological

chaperones

inactive Transcription Factor

active Transcription Factor

folded proteins denatured proteins

denatured proteins

denatured proteins

folded/proteins

feedback control
Heat-shock transcription factor (HSF) binds heat-shock elements (HSE). The HSE has been conserved throughout evolution, from yeast to humans.

HSEs consist of the sequences nGAAn and its complement nTTCn, and occur in tandem (multiple copies).

- An example of a potent, bi-directional heat-shock promoter is from the C. elegans Hsp16-1 and Hsp16-48 operon:

```
CATGAGCAT          T
GAA   TTCTAAGAT
TG    T
TCGAAATTTCTAGAG
TATA

GTACTCGTA          A
CTT   AAAGATCTT
AGCTTAAAGATCTC
TACA

M  S  L...
```

- At least 2-3 HSE sequences are required for HSF binding; HSF binding to HSEs is cooperative: the more HSEs present, the stronger the binding.

- Many heat-shock protein promoters have been used to control gene expression.

- *e.g.*, nematode biosensor
Regulation of the stress response:

HSF

Inactive heat shock transcription factor (HSF) monomer

HSF has a Winged Helix-Turn-Helix Motif

HSF DNA binding domain (monomer) in complex with HSE sequence

Active trimer

Cellular stress

Trimerization via leucine zippers

Reversal to monomers following stress

Binding to HSEs

HSEs

Stress-inducible gene

Activation of transcription by HSF requires phosphorylation

Monomer-trimer transition, and activity while bound to DNA is regulated by molecular chaperones
Figure 2. Two fates for unfolded ER proteins controlled by the UPR. Unfolded proteins in the ER lumen or the ER membrane can either be folded (left branch) or degraded by ER-associated degradation (ERAD), by the appropriate proteins dedicated to these functions. An increased level of unfolded proteins increases the 'tone' of the UPR, causing concomitant increases in activity of each process. Importantly, the studies with null mutants indicate that these two fates both operate continuously in normal cells, such that loss of capacity to perform either branch results in measurable cell stress.