The Stress Response System—Organization, Physiology and Immunoregulation

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March 25, 2014
Advanced Topics in Immunology
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- Stress Response Proteins: Relationship to immune response?
HPA axis Activation Induced by Stressors

- Stress elicits an immune response resulting in bi-directional signaling between the immune and central nervous system (CNS)

- Cytokines from the periphery can initiate the cycle by crossing the blood–brain barrier

- Immune signaling of the CNS activates the hypothalamic-pituitary-adrenal (HPA) axis

- The HPA axis is responsible for the initiation of glucocorticoid (GC) stress responses in all vertibrate animals.

- The principal end products of the HPA axis are glucocorticoid hormones

Jankord et al., 2008 and Sternbergh et al. 2001
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<th>Hormone</th>
<th>Expression of receptors by immune cells</th>
<th>Examples of effects on cell function</th>
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<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&lt;sub&gt;H&lt;/sub&gt;1 to T&lt;sub&gt;H&lt;/sub&gt;2 cytokines</td>
<td>87,88</td>
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<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>
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<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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<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>
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<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>
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<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>
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<td>Catecholamines (adrenaline and noradrenaline)</td>
<td>T and B cells, NK cells, monocytes and macrophages</td>
<td>Induce a shift to a T&lt;sub&gt;H&lt;/sub&gt;2 response, involving antigen-presenting cells and T&lt;sub&gt;H&lt;/sub&gt;1 cells</td>
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<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>
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FN-γ, interferon-γ; IL, interleukin; NK, natural killer; T<sub>H</sub>, T helper; TNF, tumour-necrosis factor.
The immune and nervous systems communicate with and regulate each other.

CRV = corticotropin-releasing hormone
AVP = arginine vasopressin

CNS Cytokines

STRESSORS

Cytokines

Adrenal Glands

ACTH

Glucocorticoids

SNS

PNS

Immune System (Organs & Cells)

Thymus

Bone Marrow

Lymph Nodes

Spleen

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CNS Regulation of the Immune System?

• Glucocorticoid hormones— generally suppress immune function.

• The autonomic nervous system:
  – sympathetic and parasympathetic nerves and innervation by these nerves of immune organs including the spleen, lymph nodes, thymus and bone marrow.
  – At these sites immune cells including lymphocytes macrophages, dendritic cells and mast cells may be found in close apposition to neurons that secrete neurotransmitters, such as norepinephrine, acetylcholine and serotonin.

• Peripheral nervous system regulate immune responses at a local level at sites of inflammation.
  – Peripheral sensory nerves may be retrogradely activated to release neuropeptides into tissues, where these molecules also affect immune cell migration, activation and mediator release.
Norepinephrine Increases Tumor Growth and Metastasis

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

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

- Exposure to norepinephrine enhances prostate tumor survival by upregulating the expression of MAPK, and inactivating the apoptotic molecule, BAD. (Hassan et al, JCI 2012, Magnon et al Science 2013)
HPA axis and glucocorticoid hormones

- Hypothalamus receives and monitors information about the environment and coordinates responses through nerves and hormones.
- Vision, smell, hearing, temperature sensation and pain alert the hypothalamus to emergencies or hazards in the environment.
- Emotional portions of the brain also relay information to the hypothalamus.
- From this integrative center the brain controls hormone secretion from the pituitary gland and other tissues, such as the adrenal glands.

EX. Corticotrophin-releasing hormone is secreted from the paraventricular nucleus of the hypothalamus into the hypophyseal portal blood supply and subsequently stimulate the expression of adrenocorticotrophic hormone (ACTH) in the anterior pituitary gland

- ACTH then circulates in the blood stream to the adrenal glands where it induces the expression and release of GC hormones.
- These hormones affect cardiovascular and renal function and metabolism and act together with the nervous system to adjust our responses to the environment.
HPA axis and Glucocorticoid Secretion

http://speakingoffaith.publicradio.org/programs/stress/images/stressresponse.jpg
Both major and minor stressful events can have direct adverse effects on a variety of immunological mechanisms,

- To help demonstrate causal relations between psychosocial stressors and the development of infectious illness, investigators have inoculated subjects with several types of vaccines.

- Vaccine responses demonstrate clinically relevant alterations in an immunological response to challenge under well controlled conditions. Accordingly they act as a proxy for responses to an infectious agent.

- EX. Among medical students taking exams, stress and the degree of social support affect the virus specific antibody and T-cell responses to a hepatitis B vaccine.
- Chronic stress associated with caregiving for a spouse with Alzheimer’s disease was associated with a poorer antibody response to an influenza virus vaccine compared to well matched control subjects.

- Therefore, in individuals who produced delayed, weaker and shorter-lived immune responses to vaccines, it is reasonable to assume these same individuals would also be slower to develop immune responses.

Padgett and Glaser, 2003
Psychological stress can influence immune function, alter the pathophysiology of infection.

- Adults who show poorer responses to vaccines also experience higher rates of clinical illness as well as longer lasting infectious episodes.
- Therefore, from these vaccine studies, it is hypothesized that stress puts individuals at greater risk from more severe illness.
- Cohen et al. (1991) showed that human volunteers who were inoculated with 5 difference strains of respiratory viruses showed a dose dependent relationship between stress and clinical symptoms observed after infection.
Academic stress?

- Marshall et al., supports several published in vitro findings.
- In medical students taking exams, psychological stress produced a shift in the cytokine balance toward a Th2 profile.
- The data showed decreased synthesis of Th1 cytokines, including IFN-\(\gamma\) and increased production of Th2 cytokines, including IL-10.
- It is believed that this stress-induced decrease of Th1 cytokines results in dysregulation of cell mediated immune responses.
Stimulation of a TH2 System by Glucocorticoids

• Why the TH2 preference?

Hypothesis:

– During an immune and inflammatory response, the activation of the stress system, through induction of a TH2 shift may protect the organism from systemic “overshooting” with TH1 pro-inflammatory cytokines.

Elenkov et al, 2006 and Calcagni 2006
Animal studies confirm human findings:

- Stress diminishes vaccine responses
- Exacerbates viral and bacterial pathogenesis
- Slows wound healing
- Alters autoimmune disease
- Stress hormones inhibit:
  - the trafficking of neutrophils, macrophages, antigen presenting cells, NK cells, T and B cells,
  - Suppress the production of pro-inflammatory cytokines and chemokines,
  - Downregulate the production of cytokines necessary for the generation of adaptive immune responses and impair effector functions of macrophages, NK cells and lymphocytes.

Padgett and Glaser, 2003
How immune cells are affected

- 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 cytolytic activity.

- Example: treatment of peripheral blood leukocytes (PBLs) with catecholamines in vitro results in the suppression of IL-12 synthesis and an increase in IL-10 production.

- This can cause a shift in the phenotype of CD4 + T-helper cells, from a Th1 profile, which functions in cell-mediated immune activities, to a Th2 profile, which is involved in antibody production.
Glucocorticoids

- Immunomodulatory vs. Immunosuppressive and anti-inflammatory

- Most notable mechanism of action is the inhibition of cytokine production and action.

- Beneficial for short-term survival, but prolonged exposure can lead to serious metabolic, immune, and psychological dysfunction,

- Termination of the GC response is controlled by various feedback inhibition mechanisms

- Glucocorticoids are lipophilic molecules and so readily pass through the plasma membrane of all cells in the body.

Padgett et al., 2003 and Almawi et al., 1999
Glucocorticoids Regulate Cytokine Production

• Once a T cell response is engaged, glucocorticoids may now modulate its cytokine production pattern.

• The differentiation of CD4+ T cells into TH1 lymphocytes drive cellular immunity, while differentiation into TH2 lymphocytes drive humoral immunity.

• This differentiation depends on the type of antigen encountered and the type of cytokines produced during antigen presentation.

• Glucocorticoids block IL-12 secretion by monocytes and dendritic cells, but promote TH2 development by enhancing IL-10 secretion by macrophages.

Franchimont et al., 2004
Antigen-specific activation of T cells ensues upon recognition of processed antigen co-presented with MHC II proteins

Interleukin-12 is critical for Th1 lymphocyte differentiation and secretion of Th1 cytokines such as IFNγ and TNFα

It is the link between humoral and cellular immunity.

Glucocorticoids prevent IL-12 action by specifically inhibiting (Stat) 4 phosphorylation
Molecular Mechanisms of GC action

• Effects of GC on immunity occur through the glucocorticoid receptor (GR), an intracellular receptor that is part of the steroid hormone receptor family of ligand dependent transcription factors.
• Characterized by an N-terminal transactivation domain, and a ligand binding domain that includes the Tau 1 domain, a DNA binding domain, and a ligand binding domain.
• Once bound to ligand, the receptor dissociates from a heat shock protein complex, translocates to the nucleus, dimerizes, binds to DNA and activates transcription.
What is GR?

- A member of the steroid hormone-receptor superfamily
- Structurally it can be divided into three distinct regions.
  - N-terminal domain that is involved in transactivation
  - Middle section-DNA binding domain, is involved in DNA binding mediated by two zinc fingers
  - C terminal domain, or ligand binding domain of the GR is responsible for ligand binding and is also involved in transactivation, dimerization and heat shock protein 90 binding.

- In its unactivated state, the GR is located in the cytoplasm where it is part of an oligomeric complex containing HSP90 which is though to hold the GR in a conformation that is available to incoming cortisol or corticosterone.
- Upon ligand binding, GRs dissociate from this complex and translocate to the nucleus where they bind as a homodimer to target glucocorticoid response elements (GREs) through zinc fingers of the DNA binding domain.
- The bound GR-ligand complexes can then influence gene expression by modulating transcription through several proposed mechanisms.
Structure of the glucocorticoid receptor
Breakthrough….

• In 1995, two publications (Schenman et al., Science-v. 270, 1995 and Auphan et al., v. 270, 1995) presented data that GC could interfere with NF-kB activity….and showed that GC hormones could transactivate an inhibitor of NF-kB.

• Hypothesis: GC induces the transcription of IkBa which then sequesters NFkB in the cytoplasm and prevents it from translocating to the nucleus and inducing gene activation.

• This is a logical explanation for the broad spectrum of cytokines suppression mediated by GCs.
Figure 4  Schematic diagram of the mechanism(s) by which the glucocorticoid receptor inhibits the action of NFkB and AP-1.

Cytokines

GR = glucocorticoid receptor

hsp90

GR = glucocorticoid

\( \alpha \)

mRNAs

Proteins

Effects

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Glucocorticoids can inhibit innate immunity.
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

↑UCP1

The uncoupling protein in mitochondria drives adaptive thermogenesis

UCP1 Mediates Adaptive Thermogenesis by Uncoupling Oxidative Respiration

H^+ H^+ H^+
Complex I Complex III Complex IV
Outer Mitochondrial Space

H^+
Complex II

2e^-
ADP+ppi+O^2-+2H^+
Inner Mitochondrial Space

H^+ H^+ H^+ H^+ H^+ H^+ H^+
ATP Synthase

H^+ H^+ H^+
ATP

H^+
UCP1

HEAT
Stress and The Heat Shock Protein Systems: Chaperones and Antigen Processing
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

Inhibitors of glycosylation; hypoxia; calcium ionophores reductive stress

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

heat oxidizing agents cytoplasmic & mitochondrial damage
Heavy Metals

HSF
hsps

ER damage
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 with them peptide or protein elements that are related to the 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.
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.

From Lindquist, 2010