Objective: To describe recent findings on how occupational exposures alter the immune response leading to chronic diseases like CVD, autoimmune diseases and lung diseases.

Key points:

• Leading US causes of death involve inflammation
• Infections and chemicals activate innate immunity
• Infections and chemicals alter the adaptive immune response
• All major chronic diseases vary by sex
Global death by leading cause, 2002

54% Non-communicable diseases
14% Injury & other
6% Respiratory infections
5% HIV
3% Diarrheal diseases
3% TB
2% Malaria
2% Childhood diseases: pertussis, poliomyelitis, diphtheria, measles & tetanus
11% other infectious diseases

Leading causes of death for Males & Females in US: 2002 (in thousands)

A CVD, B Cancer, C Accidents D Chronic lower respiratory diseases, E Diabetes, F Alzheimer’s Disease

American Heart Assoc., Heart Disease & Stroke Statistics- 2006 Update

Source: CDC/ NCHS (National Center for Health Statistics)
All major causes of death in Western populations are due to “chronic inflammatory diseases”
Progression from Acute to Chronic inflammation

Resolution of Acute inflammation

Fairweather et al., 2001 J. Autoimm. 16: 175-186
Inflammation

• First described in Egyptian papyrus (3000 BC)
• Celsus, Roman writer from 1st century AD described 4 signs of inflammation:
  – rubor (redness)
  – tumor (swelling)
  – calor (heat)
  – dolor (pain)
  – Virchow later added functio laesa (loss of function)
• John Hunter, Scottish surgeon, 1793 “Inflammation is not a disease, but a nonspecific response of the host”
• Metchnikoff & Erlich (shared Nobel prize 1908) purpose of inflammation is to bring cells and soluble mediators to site of infection/damage.
What causes disease?

• Genetic- intrinsic
• Environment- acquired
• Epigenetic- intrinsic & acquired
The Exposome

Characterizing the exposome. The exposome represents the combined exposures from all sources that reach the internal chemical environment. Toxicologically important classes of exposome chemicals are shown. Signatures and biomarkers can detect these agents in blood or serum.
Causes of Cell Injury

• Oxygen deprivation (hypoxia) vs ischemia
• Physical agents i.e. mechanical trauma, extremes of temperature, radiation
• Chemicals and Drugs
• Infections
• Immune Response
• Genetic defects
• Nutritional imbalances i.e. starvation, vitamin deficiencies, obesity
Example of Apoptosis (programmed cell death)

When a cell undergoes apoptosis macrophages consume cell debris

Electron micrograph of a human GM3798 T-lymphoblastoid cell that has undergone apoptosis as a consequence of exposure to glucocorticoid hormone (dexamethasone). The dark "eye" and "smile" are regions of hypercondensed chromatin, a common feature of apoptosis. The study of chromatin condensation is a major focus of our apoptosis studies. Photo - Carol Cooke.

ghr.nlm.nih.gov/ghr/picture/apoptosis_macrophage

Fluorescence micrograph of an isolated HeLa cell nucleus that has undergone apoptosis in vitro in our cell free system. The bright regions are DNA stained with DAPI. One of the "eyes" looks suspiciously like the other one, but everything else is as it was. Photo - Kumiko Samejima. Data massage - Bill Earnshaw.

web.bio.ed.ac.uk/.../earnshaw/Apoptosis.htm
Examples of Necrosis

necrosis caused by downy mildew on a grape leaf

[Website 1]

Cell Type: Necrotic Marine P-815 tumour cell.
Treatment: Confluent necrosis due to ischaemia.

[Website 2]
Inflammatory Response

Steps of the Inflammatory Response

1. Damaged tissues release histamines, increasing blood flow to the area.
2. Histamines cause capillaries to leak, releasing phagocytes and clotting factors into the wound.
3. Phagocytes engulf bacteria, dead cells, and cellular debris.
4. Platelets move out of the capillary to seal the wounded area.

The inflammatory response is a body's second line of defense against invasion by pathogens. Why is it important that clotting factors from the circulatory system have access to the injured area?
Acute Inflammation
The inflammatory response is closely intertwined with the process of repair.

- **Protective** i.e. microbes, toxins, necrotic cells
- **Harmful** i.e. chronic inflammatory diseases
Chronic Inflammation

- Active inflammation
- Attempts at repair
  i.e. fibrosis
- Tissue destruction
Chronic Inflammation & Fibrosis (blue)
Outcomes of Acute Inflammation

Injury → Acute inflammation → Resolution → Healing: regeneration & scarring → Chronic inflammation
Acute inflammation leads to chronic disease in susceptible individuals.

Progression from Acute to Chronic inflammation

Resolution of Acute inflammation

Fairweather et al., 2001 J. Autoimm. 16: 175-186
Infections, injury & toxins/chemicals activate the Innate Immune Response using similar mechanisms.
What is the role of Innate Immunity in the pathogenesis of chronic diseases?

- Innate Immunity initiates inflammation i.e. Toll-like receptors (TLRs)
- Innate Immunity determines the nature/phenotype of the adaptive immune response i.e. T helper type (Th)1 vs. Th2 vs. Th17
- Innate Immunity initiates regulatory mechanisms i.e. T regulatory cells and regulatory cytokines
- The balance between a **proinflammatory** response and **regulation** of the response and **regulation of repair mechanisms** determines whether injury proceeds to chronic disease
Threats to the body

Protection requires being able to distinguish “self” from “non-self”
PRRs detect PAMPs

- PRRs recognize similar design patterns on microbes not present on host cells in order to destroy the pathogen
  - Ex. Toll-like receptors (TLRs) on immune & other host cells
- TLR’s important in cytokine production & inflammation
- PRRs can be found on “innate” or “adaptive” immune cells
Human TLR 1-11

Lipoprotéines
Gram+
HSV1
Hémagglutinine

LPS Gram-
Protéine F
Env
HSP
Toxol

Polyl-PolyC
dsRNA viral

ssRNA riche UG
Imidazoquinolines

CpG DNA
bactérienviral
HSV - mCMV

E coli
uropathogénique

Flagelline

TLR2
TLR4
TLR3
TLR7/8
TLR9
TLR11
TLR5

IRF3-P

NF-κb activation

Inflammation
External threats activate PRRs

- TLR1-13
- Dectin Rs
- Mannan Rs
- Inflammasome = NODs and NALPs
- Results in activation of NFκB
- Rarely do specific infections relate to chronic diseases

Karen M et al. 2006 Cell 124: 823
TLRs recognize “damaged self”

Particularly TLR2 & TLR4

**FIGURE 1.** HCM activates TLR2 and TLR8. A, HEK293-hTLR2 cells were stimulated with HCM or SKM. Supernatants harvested at 24 h were assayed for IL-8. LTA, Lipoteichoic acid (TLR2 ligand). B, HCM stimulated IL-8 secretion from HEK293-hTLR8 cells; p < 0.001. C, HCM S2-16 and S2-28 peptides activated TLR2 (p < 0.0001) and TLR8 (p < 0.0001). S2-16 is a cryptic pathogenic HCM peptide and S2-28 is the dominant epitope of cardiac myosin in experimental autoimmune myocarditis (13). CL075 is a TLR7/8 ligand and LPS is a TLR4 ligand. Values are means ± SEM. Representative results from three to six independent experiments are shown. The p value was obtained by Tukey’s test using one-way ANOVA.

**Cutting Edge: Cardiac Myosin Activates Innate Immune Responses through TLRs**

2009 *J Immunol* 183:27-31

Ping Zhang, Carol J. Cox, Kathy M. Alvarez, and Madeleine W. Cunningham
Endogenously derived ligands (self tissues) activate PRRs

- **Hsp** = heat shock proteins, induced by cell damage
- **ICs** = immune complex with DNA or RNA
- **AC** = apoptotic cells
- **SR** = scavenger Rs
- **HMG-B1** = chromatin component (DAMP)
- **IL-33** (DAMP)

Karen M et al. 2006 Cell 124: 823
Immune responses do not follow normal Dose-Response relationships.

Figure 2-4. Comparison of dose–response relationships for two different chemicals, plotted on a log dose-probit scale. Note that the slope of the dose–response relationship is steeper for chemical B than for chemical A. Dotted lines represent the confidence limits for chemical A.
A single stimuli can launch an “army” of immune cells
Low doses that appear to “do no harm” (i.e. do not result in death of cells and tissues) can potentially stimulate a harmful immune response
### Table 1 NLRP3 inflammasome activators

<table>
<thead>
<tr>
<th>Activator class</th>
<th>Activator</th>
<th>Disease associations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole pathogen</td>
<td><em>Candida albicans</em></td>
<td>Infection</td>
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<tr>
<td></td>
<td><em>Saccharomyces cerevisiae</em></td>
<td>Infection</td>
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<td></td>
<td><em>Staphylococcus aureus</em></td>
<td>Infection</td>
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<td><em>Listeria monocytogenes</em></td>
<td>Infection</td>
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<td></td>
<td>Influenza virus</td>
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<td></td>
<td>Sendai virus</td>
<td>Infection</td>
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<tr>
<td></td>
<td>Adenovirus</td>
<td>Infection</td>
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<tr>
<td>Pathogen-associated molecules</td>
<td>Bacterial pore-forming toxins</td>
<td>Infection</td>
</tr>
<tr>
<td></td>
<td><em>Hemozoin</em></td>
<td>Cerebral malaria, Silicosis</td>
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<tr>
<td>Environmental insults</td>
<td><em>Silica</em></td>
<td></td>
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<tr>
<td></td>
<td>Asbestos</td>
<td>Asbestosis</td>
</tr>
<tr>
<td></td>
<td>Skin irritants</td>
<td>Contact hypersensitivity reactions, Sunburn</td>
</tr>
<tr>
<td>Endogenous danger signals</td>
<td>Ultraviolet light</td>
<td>Injury or necrotic cell death, Metabolic syndrome, Gout</td>
</tr>
<tr>
<td></td>
<td><em>ATP</em></td>
<td></td>
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<tr>
<td></td>
<td>Glucose</td>
<td></td>
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<tr>
<td></td>
<td><em>MSU</em></td>
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<tr>
<td></td>
<td>Calcium pyrophosphate dihydrate (CPPD)</td>
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<tr>
<td></td>
<td><em>Amyloid β</em></td>
<td></td>
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<td></td>
<td><em>Hyaluronan</em></td>
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<td></td>
<td><em>Alum</em></td>
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<tr>
<td>Adjuvant</td>
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</table>

Infections, toxins, & environmental agents activate the inflammasome

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2010 Science 327:296

The NLRP3 Inflammasome: A Sensor for Metabolic Danger? Kate Schroder,¹² Rongbin Zhou,¹ Jurg Tschopp¹,*
Increased Th2 response

Alum (silica, asbestos, MSU)

Actin-mediated phagocytosis

Mφ, DC?

Acidification, maturation to lysosomes

Lysosome rupture

K+ efflux?

Activation of NLRP3/ASC-caspase 1

ROS?

Release of lysosomal contents

IL-1β, IL-18, IL-33

- Enhanced IgE, IgG1 production, T cell expansion.
- Recruitment, activation and mobilization of monocytes to draining lymph nodes.
- IL-4-expressing, Gr1+ cells prime splenic B cells.
- DC activation for T cell polarization to Th2 response?

Increased Th2 response

- Cathepsin B, lysosomal contents
- NADPH oxidase
- Mitochondria
- Inflammasome
Phagocytosed alum-containing lysosomes rupture and release their components to the cytosol by an unknown mechanism. The released contents and molecules generated during this process contribute to NLRP3/ASC/caspase-1 inflammasome activation, which in turn processes the proforms of IL-1 family members to active forms. Either IL-1 family member cytokines or the products from NLRP3 inflammasome activation may elicit various immunostimulatory effects in vivo. When and how potassium efflux and reactive oxygen species (ROS) play a role awaits further investigation. DC, dendritic cell; MSU, monosodium urate.
Th responses

J Neuroimmune Pharmacol. 2010 Jan 27
El-Behi M, Rostami A, Ciric B.
Low-Dose Inorganic Mercury Increases Severity and Frequency of Chronic Coxsackievirus-Induced Autoimmune Myocarditis in Mice

Jennifer F. Nyland, DeLisa Fairweather, Devon L. Shirley, Sarah E. Davis, Noel R. Rose, and Ellen K. Silbergeld

**FIG. 5.** Hg pretreatment increases fibrosis in the heart. PBS-treated (vehicle control) and Hg-treated mice were compared for the percent of the heart section with fibrosis on day 35 pi. Mice received 100 μl HgCl₂ at 200 μg/kg in PBS or vehicle alone every other day for 2 weeks followed by 10³ PFU of CVB3 ip on day 0. The group listed as “Hg alone” did not receive CVB3. On day 35 pi, (B) PBS and (C) Hg hearts were collected and stained with Masson’s trichrome to detect collagen deposition. (A) Data are presented as the mean percent of the heart section staining positive for fibrosis ± SEM of 8–10 mice per treatment group from one representative independent experiment of three. * indicates p < 0.01.
FIG. 6. Hg pretreatment increases the prevalence of DCM at day 35 pi. PBS-pretreated (vehicle control) and Hg-pretreated mice were compared for the prevalence of dilated phenotype on day 35 pi. (A) Data are expressed as the frequency of hearts per group with dilatation. H&E-stained sections from (B) PBS-pretreated and (C) Hg-pretreated mice, shown at ×1.25. * indicates significantly different by Fisher’s exact test.
2wk CS exposure 250mg/L, 2h/day, M-F

CVB3

Day 10pi

Myocarditis

Day 35-90pi

DCM

A

Day 10pi

% Myocarditis

Females

Males

0.0007

0.003

B

Day 10pi

PFU/g Heart

Females

Males

NSNV

Smoke

Virus

Smoke+Virus
CS increases acute Pericarditis, pericardial fibrosis & MC degranulation
CS alone induces DCM by d35
Sex differences exist for all major chronic inflammatory diseases
Annual age-adjusted cancer incidence rates US, 1975-2002

Gender Differences in Asthma Prevalence

NHANES III 1988-94 (SPAN=0.4)

Arbes and Zeldin, unpublished
Incidence of CVD that does not include hypertension by age and sex (FHS, 1980–2003). Source: NHLBI

Males develop DCM by day 35pi
Testosterone decreases heart function
Testosterone increases Inflammation
Testosterone increases inflammasome activation after infection
Synthesis of Vitamin D

Modulation of the immune system by UV radiation: more than just the effects of vitamin D?

Prue H. Hart*, Shelley Gorman* and John J. Finlay-Jones†

NATURE REVIEWS IMMUNOLOGY
Latitude correlates with CVD

**Fig. 2.** Associations between geographic latitude and IHD death rates in (a) females ($r = 0.49$, $P < 0.01$) and (b) males ($r = 0.51$, $P < 0.01$) of different European countries. A, Austria; AL, Albania; B, Belgium; BG, Bulgaria; BY, Belarus; CZ, Czech; D, Germany; DK, Denmark; E, Spain; EST, Estonia; F, France; FIN, Finland; GR, Greece; H, Hungary; I, Italy; L, Luxembourg; LT, Lithuania; LV, Latvia; M, Malta; N, Norway; NL, Netherlands; P, Portugal; PL, Poland; S, Sweden; SLO, Slovenia; RUS, Russia; UKR, Ukraine.

*British Journal of Nutrition (2005), 94, 483–492*
Vitamin D and Cardiovascular Disease

Will It Live Up to its Hype?

Carl J. Lavie, MD, John H. Lee, MD, Richard V. Milani, MD

New Orleans, Louisiana

<table>
<thead>
<tr>
<th>Serum 25-Hydroxyvitamin D, ng/ml</th>
<th>Vitamin D Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤10</td>
<td>Severe deficiency</td>
</tr>
<tr>
<td>10–20</td>
<td>Deficiency</td>
</tr>
<tr>
<td>20–30</td>
<td>Mild-moderate deficiency</td>
</tr>
<tr>
<td>≥30</td>
<td>Sufficient</td>
</tr>
<tr>
<td>40–50</td>
<td>Ideal</td>
</tr>
<tr>
<td>50–150</td>
<td>Indeterminate data*</td>
</tr>
<tr>
<td>&gt;150</td>
<td>Toxicity</td>
</tr>
</tbody>
</table>

Institute of Medicine Definitions†

<table>
<thead>
<tr>
<th>Serum 25-Hydroxyvitamin D, ng/ml</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;12</td>
<td>At risk of deficiency</td>
</tr>
<tr>
<td>12–19</td>
<td>At risk of inadequacy</td>
</tr>
<tr>
<td>20–50</td>
<td>Sufficient</td>
</tr>
<tr>
<td>&gt;50</td>
<td>Possibly harmful</td>
</tr>
</tbody>
</table>

*Some data suggest increased falls, fractures, certain cancers, and even cardiovascular risk at values >50 ng/ml. †Definitions adapted from Looker et al. (3).
• 75% GCM patients deficient or inadequate
• 85% GCM patients “deficient” (<30)
• 5% GCM patients sufficient

• 20% LM patients deficient or inadequate
• 45% LM patients “deficient” (<30)
• 55% LM patients sufficient
No apparent relationship between %EF and VitD in LM patients
Low VitD is associated with poor EF in women with LM, similar to GCM.

• Sex Difference in LM patients
• Higher VitD levels in men increase disease, like found in our animal model.

• Low VitD is associated with poor EF in women with LM, similar to GCM.
VDR increases Myocarditis in Males
VDR decreases Myocarditis in Females
Summary: Inflammation

- Inflammation progresses from an innate immune response to acute inflammation and then to either resolution of inflammation or chronic inflammation/pathology.
- Immune mediators (i.e. cytokines) are responsible for redness, swelling, heat & pain.
- Inflammation consists of immune cells infiltrating an organ/tissue.
- Inflammation is closely intertwined with the process of repair.
- Inflammation & repair can both protect or damage tissues.
- Inflammation is a critical component of heart disease (CVD), cancer, autoimmune diseases (ADs) like diabetes, and chronic respiratory diseases like COPD.
- Inflammation is also a critical component of chronic diseases that are not the top causes of death, such as allergies, asthma, obesity and ADs like lupus or multiple sclerosis.
Main Points

• Acute inflammation leads to chronic disease in susceptible individuals
• Low doses of toxins/chemicals & injury can potentially launch a damaging immune response
• Toxins and chemicals activate the Inflammasome driving a proinflammatory and profibrotic response (i.e. Th2 response)
• Sex hormones regulate inflammation and the inflammasome
Funding:
R01 HL087033
P30 ES03819