Stress-induced inflammation: Lessons from Familial Mediterranean Fever (FMF)

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I have no conflict of interest in relation to this presentation
Hans Selye, and the Birth of “Stress”

Stress: “the nonspecific response of the body to any demand for change”

The contribution of stress in the activation of inflammatory signaling pathways

Stress hormones can provoke changes in virtually every cell and tissue and alert them to the existence of a stressor.

- **Sympathetic nervous system activation:** ↑Catecholamines
- **Vagal withdrawal:** ↓Cholinergic activity
- **Hypothalamic pituitary adrenal axis (HPA) activation:** ↑Cortisol & glucocorticoid sensitivity

1. Stress-inflammation axis

   - Physical stress
   - Psychological stress
   - Metabolic stress
   - Environmental stress

2. Cellular stress
   - Cell repair
   - Temporary adaptation
   - Induction of autophagy
   - Cell death

3. Inflammatory response
   - Diseases & phenotypes

- Autoimmune
- Autoinflammatory
- Cardiovascular
- Metabolic
- Neurodegenerative
- Neoplastic/cancer
Neutrophil: major player in inflammation
Neutrophils

- the most abundant (50-70%) of circulating white blood cells
- arrive first at the site of inflammation
- migrate via chemotaxis (C5a, IL-8) toward site of inflammation
2004: “Neutrophil extracellular traps (NETs) is a new antimicrobial mechanism”

Dr. Volker Brinkmann  Prof. Arturo Zychlinsky

Neutrophil Extracellular Traps Kill Bacteria
Volker Brinkmann, et al.
Science 303, 1532 (2004);
DOI: 10.1126/science.1092385
• NETs formed of decondensed chromatin that is covered with antimicrobial components, bioactive proteins/cytokines and self-antigens

• NETs are released by neutrophils after activation by various inflammatory mediators

• Implicated in several disorders including infections/sepsis autoimmunity, thrombosis, cancer/metastasis


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Type of neutrophil death: balancing apoptosis versus NETosis

Stimuli:
- Inflammatory cytokines
- Opsonized bacteria
- Immune complexes
- Fungi/ECM components

NETosis:
- Pro-inflammatory effects
  - Release self-antigens
  - Stimulate interferon-α/β
  - Form a chromatin lattice
  - Present antimicrobial peptides

Apoptosis:
- Anti-inflammatory effects
  - Uptake by macrophages
  - Stimulate anti-inflammatory cytokines
  - Remove cellular debris

Mayadas et al Annu Rev Pathol. 2014
Plasticity of neutrophils can lead to functionally diverse NETs

Bone marrow → Peripheral neutrophils

Disease-specific environmental signals → NEUTROPHIL PLASTICITY

Phenotype-specific cytokines/mediators

NEUTROPHIL DIVERSITY

Pre-NETotic neutrophils → NETotic neutrophils

Phenotype-specific bioactive NETs

NET DIVERSITY

Skendros et al. PLANET project (submitted)
In the context of different diseases, neutrophils release NETs that are qualitatively different and deliver disease specific bioactive proteins, determined by the disease inflammatory environment.
Yoshinori Ohsumi

"Nobel Prize in Medicine or Physiology" in 2016

“for his discoveries of mechanisms for autophagy”
Regulation of the autophagic machinery in human neutrophils

Ioannis Mitroulis*1, Ioannis Kourtzelis*1,2, Konstantinos Kambas1, Stavros Rafail2, Akrivi Chrysanthopoulou1, Matthaios Speletas3 and Konstantinos Ritis1
Little are know about neutrophil response to stress upon inflammatory conditions

✓ How inflammatory stress is regulated?

✓ Can we decode the mechanisms translating stressful life events into neutrophil-mediated inflammation
Is there any clinical model system to study the role of stress in neutrophil-mediated inflammation???

1. Physical stress
2. Psychological stress
3. Environmental stress
4. Metabolic stress

Neutrophilic stress

- Cell repair
- Temporary adaptation
- Induction of autophagy
- Cell death

Inflammatory response

Disease phenotype

Relapsing Remitting
**Familial Mediterranean Fever (FMF)**

the most frequent periodic febrile syndrome among the autoinflammatory syndromes

*FMF: The prototype autoinflammatory disease*

- inherited autosomal recessive disorder
- **mutations in the MEFV gene**, encoding the **protein pyrin (TRIM20)**
- commonly found among individuals of Mediterranean descent
FMF & stress

- Unpredictable, recurrent and self limited inflammatory attacks of fever and serositis

- Several factors associated with emotional and physical stress are proposed to trigger FMF attacks

- Ben-Zvi I, Livneh A. Nat Rev Rheumatol. 2011
Preliminary Communication

**METARAMINOL PROVOCATIVE TEST: A SPECIFIC DIAGNOSTIC TEST FOR FAMILIAL MEDITERRANEAN FEVER**

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Fredrick F. Fenech

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**Summary**

The diagnosis of familial Mediterranean fever has been one of exclusion. In a placebo-controlled, double-blind, cross-over study a challenge with a 10 mg dose of metaraminol infusion was followed within 48 h by a typical disease-like attack in all of 21 patients with familial Mediterranean fever but in none of 21 control subjects. The induced attacks were milder and of shorter duration than the spontaneous ones. The metaraminol-induced symptoms were

**PATIENTS AND METHODS**

21 randomly selected patients fulfilling the diagnostic criteria of FMF and 21 control subjects matched for age and sex were tested. The study group included 13 males and 8 females. The mean age was 24·7 years (range 13 – 36 years), and the mean duration of illness was 8·3 years (range 3 – 15 years). In 5 patients the disease attacks were fairly well controlled with continuous colchicine therapy. The other 16 patients did not receive any specific treatment before this study.

Of the 21 control subjects, 10 were healthy volunteers, and the remaining 11 had chronic recurrent abdominal pains due to irritable bowel, peptic ulcer, chronic relapsing pancreatitis, or cholecystitis. All were tested during remission. 6 of these 21 controls were first-degree relatives of patients in the study group.

Informed consent was obtained from each subject and also from one or both parents of patients aged under 18. All forms of therapy were discontinued a week before the test. Untreated FMF patients were tested soon after recovery from the most recent attack.

The test was done in a double-blind cross-over fashion, once with an intravenous infusion of 500 ml of normal saline containing 10 mg of metaraminol and on another occasion with saline only. The two occasions were 1 – 2 weeks apart. Subjects with positive tests were rechallenged later with the metaraminol infusion after being treated with oral colchicine 0·5 mg twice daily for 1 – 2 weeks. The morning dose was given 1 – 2 h before the rechallenge.

**Metaraminol Provocative Test**
Aramine® (metaraminol)  
(stress analogue)

Metaraminol Provocative Test

FMF patient

Body temperature (°C)

FMF inflammatory attack
Recommendation 8

“Periods of physical or emotional stress can trigger FMF attacks, and it may be appropriate to increase the dose of colchicine temporarily”
Neutrophils: the main effector cells during acute inflammation in patients with FMF
<table>
<thead>
<tr>
<th>Factor</th>
<th>Evidence</th>
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<tbody>
<tr>
<td>Neutrophils</td>
<td>Presence of neutrophils in serosal fluid during acute FMF attacks</td>
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<tr>
<td>Colchicine</td>
<td>Beneficial effect on prevention of attacks</td>
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<td>Inhibition of chemotaxis and phagocytosis by neutrophils</td>
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<td>Pyrin/marenostin</td>
<td>Expression in the nucleus and cytoplasm in circulating neutrophils</td>
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<td>Lack of physiological inhibition of neutrophil activation; effect on neutrophil cytoskeleton</td>
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<td>Lack of control on the inflammatory process</td>
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<tr>
<td>C5a/IL-8 inhibitor(s)</td>
<td>Poor expression of protease(s) involved in inhibition of complement degradation (e.g., complement fragment C5a, IL-8) and neutrophil chemotaxis</td>
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</tbody>
</table>
Low basal autophagy levels in neutrophils of FMF patients in remission
Pyrin acts as a receptor for the selective autophagic degradation of inflammasome components, a function that is significantly impaired in mutated protein.
Pyrin modulates the production of IL-1β by interacting with proteins of the NLRP3 inflammasome complex.

FMF is a member of the IL-1β-dependent family of autoinflammatory disorders, characterised by their clinical response to IL-1β blockade.

Circulating (soluble) IL-1β is extremely low and undetectable by standard assays, even during the inflammatory attack.

FMF is an IL-1β-mediated disease.

- Dinarello CA et al Nat Rev Drug Discov 2012
WHERE?

IL-1β IL-1β IL-1β IL-1β
EXTENDED REPORT

Neutrophil extracellular traps regulate IL-1β-mediated inflammation in familial Mediterranean fever

Eirini Apostolidou,1,2 Panagiotis Skendros,1,2 Konstantinos Kambas,1 Ioannis Mitroulis,3 Theocharis Konstantinidis,1 Akrivi Chrysanthropoulou,1 Konstantinos Nakos,4 Victoria Tsironidou,1 Maria Koffa,4 Dimitrios T Boumpas,5,6 Konstantinos Ritis1,2
Neutrophils from FMF patients in remission are resistant to induction of NET release due to reduced basal autophagy levels

Colchicine do not inhibit NETosis
BUT reduces IL-1β production by neutrophils

**FMF:** clinical model of stress-induced IL-1β-associated inflammation that characterized by dysregulated autophagy & autophagy-mediated NETosis

How the stress/autophagy/NETs/IL-1β axis is activated and regulated leading in inflammatory attacks of FMF?
Stress-related protein REDD1 is overexpressed in neutrophils during FMF attacks
REDD1 is Involved in the Cellular Response to Different Kind of Stress
Induction of REDD1 is associated with autophagy induction and autophagy mediated NET-release

REDD1 colocalizes with pyrin & inflammasome in autolysosomes of neutrophils, but NOT when pyrin is mutated (FMF)

Adrenaline induce the expression of REDD1 in neutrophils leading to NET release

Aramine® (metaraminol) (stress analogue)

Metaraminol Provocative Test

FMF patient

Body temperature (°C)

FMF inflammatory attack
Could REDD1/autophagy/NETosis axis provide candidates diagnostic and therapeutic targets for IL-1-driven (auto)inflammation??
Targeting autophagy/NETosis axis in neutrophil-driven inflammation

neutrophil

Disease specific inflammatory environment (1st hit)

Induction of autophagy (2nd hit)

bioactive NETs

Positive feedback/enhancing

PBMCs & other bystander cells
Autophagy inhibition in adult-onset Still’s disease: still more space for hydroxychloroquine?

Autophagy & NETosis characterize stress-induced self-directed inflammation in neutrophilic diseases providing candidate diagnostic/therapeutic targets.
Inflammation Research Group of DUTH
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