Sepsis-omics
Can genomic approaches help us to understand sepsis and improve care for individual patients

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Overview of talk

1. **Sepsis: need for clarity in defining the disease**
2. Overview of ‘omics and omes’ and relevance to sepsis
3. Insights into the individual innate immune response

Hippocrates

Celsius
Sepsis: defining the phenotype

Sepsis - deleterious systemic host inflammatory response to infection in which some degree of organ dysfunction present

Sepsis: defining the phenotype

Sepsis - deleterious systemic host inflammatory response to infection in which some degree of organ dysfunction present

Pro- and anti-inflammatory responses coexist and can lead to immunosuppression.

Sepsis: defining the phenotype

The pro- or anti-inflammatory response that predominates varies:

**Between patients**

- Patient A
- Patient B

**Within a patient over time**

- Time 1
- Time 2
Sepsis: defining the phenotype

>$10 billion spent on developing effective adjuvant treatments in sepsis

>100 randomised clinical trials testing hypothesis that modulating septic response can improve survival: **none have resulted in new treatments currently in use**

Clinical trials of biologic response modifiers in sepsis

<table>
<thead>
<tr>
<th>Target</th>
<th>Strategies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endotoxin</td>
<td>Monoclonal Ab, TLR4 antagonist, anti-CD14, polymixin B</td>
</tr>
<tr>
<td>TNF</td>
<td>Antibodies, receptor constructs</td>
</tr>
<tr>
<td>IL-1</td>
<td>Receptor antagonist</td>
</tr>
<tr>
<td>Platelet activating factor</td>
<td>Small molecule inhibitors</td>
</tr>
<tr>
<td>Eicosanoids</td>
<td>Ibuprofen, sPAL2 inhibitor</td>
</tr>
<tr>
<td>Nitric oxide</td>
<td>L-NMMA, methylene blue</td>
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<tr>
<td>Hypercoagulability/DIC</td>
<td>APC, protein C concentrate, TFPI, anti-thrombin, anti-tissue factor, heparin</td>
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<tr>
<td>Immune suppression</td>
<td>iv Ig, G-CSF, GM-CSF</td>
</tr>
<tr>
<td>Endocrinopathy</td>
<td>corticosteroids, vasopressin</td>
</tr>
<tr>
<td>Others</td>
<td>selenium, extracorporeal hemoperfusion, lactoferrin, statins</td>
</tr>
</tbody>
</table>

Sepsis: defining the phenotype

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Appropriate effective intervention using anti- or pro-inflammatory modulators dependent on knowledge of prevailing immune state/disease phenotype: urgent need for biomarkers

“....the concept of sepsis embraces a host of disorders. Our challenge in developing effective adjuvant therapies is to better characterize this heterogeneity, and thus to determine discrete patient populations who might benefit from an equally heterogeneous group of interventions.”

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Omics and Omes

Sets of biological molecules

-OME: All constituents considered collectively

Genome: sum of genetic information (whole genome)

Methylome: DNA methylation

Epigenome: regulatory processes affecting chromatin and gene expression, not coded by DNA and interacting with environment

Transcriptome: coding and noncoding RNAs

Proteome: protein expression and isoforms

Metabolome: small molecules of metabolism

Metagenome: genomes of multiple organisms

Microbiome: microbial communities at body sites
Omics and Omes

- OME: ALL CONSTITUENTS CONSIDERED COLLECTIVELY
- OMICS: STUDY OF A BODY OF INFORMATION

INTEGRATION OF -OMIC DATA: SYSTEMS BIOLOGY
Omics and Omes

Sets of Biological Molecules: Structure and Function of Organism

High-Throughput, High-Dimensional, Big Data, New Technologies

-Ome: All Constituents Considered Collectively

-Omics: Study of a Body of Information

Integration of -Omics Data: Systems Biology

Made possible by technological advances enabling comprehensive resolution of biological processes, generating astronomical amounts of data.

High throughput genotyping

Microarrays: gene expression profiling

Sequencing nucleic acids

Mass spectrometry: resolving proteins and small molecules
Genomics and sepsis

High density low cost accurate genotyping
• genome-wide association studies
• pharmacogenomics (minimising adverse effects, enabling targeted therapy)

Currently no GWAS for sepsis reported

GWAS sepsis outcome: UK Genomic Advances in Sepsis study (GAinS)/Genetics of Sepsis and Septic Shock in Europe (GenOSept) consortium recruitment completed, analysis ongoing

GWAS for infectious disease including TB, malaria, bacteraemia, HIV, hepatitis B and C, leprosy, meningococcal disease
Genomics and sepsis: genetic association studies

Insight into disease pathogenesis

Drug discovery: new drug targets based on identified pathways

Drug repurposing opportunities: GWAS implicate gene encoding a known drug target but drug not currently used in that specific condition
DNA sequencing
2008 First complete human genome sequenced using ‘next generation sequencing’
- massively parallel sequencing by synthesis
- completed in two months at 1% of cost

High throughput ‘next generation’ sequencing

Highlights

The Road to the $1,000 Genome

Recent news articles marking the tenth anniversary of the announcement of the first draft sequence of the human genome also predicted the rise of DNA sequencing technologies that sequence a human genome for $1,000 or less in the next three to five years, a development that would change the face of biomedical research and clinical practice. Read more about recent sequencing breakthroughs toward achieving that goal.

Genomics England

Welcome to Genomics England

We are a new company set up by the Department of Health to help deliver the 100k Genome Project first announced by the Prime Minister, David Cameron, in December 2012.

This project will sequence the personal DNA code – known as a genome – of up to 100,000 patients over the next five years. This unrivalled knowledge will help doctors’ understanding, leading to better and earlier diagnosis and personalised care. Based on expert scientific advice, we will start by tackling cancer, rare diseases and infectious diseases.
Whole exome and whole genome sequencing and sepsis

Rare variants: primary immunodeficiencies (diagnostic utility, insights into immunity)

Number of OMIM phenotypes where molecular basis known

Rare variants and TLR signaling

**NGS and sepsis: epigenomics**

**Epigenomics:** Understanding how genome regulated - and dysregulated in disease

Chromatin interactions, transcription factor binding and enhancer function highly dynamic and context specific during endotoxin response

**Chromatin looping**
- IL1A, IL1B and IL1F7 regulatory regions localise to transcription factory in LPS stimulated human monocytes

**Chromatin regulators** remodel chromatin
- small molecule inhibitors modulate inflammation and endotoxin response
- inhibitor of BET regulator protective in a mouse model of sepsis

NGS and sepsis: epigenomics

Transcription factors networks involved in sepsis response
• priming genes for induction factors and regulation of specific gene programs
• PU.1, C/EBP, ATF3, STAT1, RUNX1, p50/p65 and IRF1

Regulatory RNAs
• extensive ‘non-protein coding’ transcription important in gene regulation
• bidirectional transcription at most gene promoters
• transcription at regulatory enhancers elements
• long noncoding RNAs and microRNAs shown to be epigenetic regulators of the endotoxin response, for example microRNAs induced by TLRs regulate initiation and termination of inflammatory responses

Transcriptomics and sepsis

**Genome-wide gene expression profiling** of patients
- gene sets and pathways differentially expressed in sepsis
- stratification and identification of biomarkers
- new insights into pathophysiology
- new therapeutic targets

**Microarray profiling**
Transcriptomics and sepsis: UK GAinS

Recruited >700 patients with sepsis admitted to ICU, ongoing analysis of individual transcriptomic response in sepsis

Effective rapid leukocyte isolation at bedside using leukodepletion filters on the ICU with high quality RNA obtained

Participating ICUs
**Microbiome and metagenomics**

**Microbiome**: ecological community of commensal, symbiotic and pathogenic microorganisms sharing different anatomical locations

**Targeted sequencing of 16S rRNA gene**: census of bacteria present

**Metagenomics**: shotgun sequencing of all nucleic acid present in a community
- all microbes including viruses, allows discovery and resolution of variation
- challenge of determining pathogenicity (correlation vs causation)

Next generation sequencing, microbiology and sepsis

Microbiology lab workflow based on whole-genome sequencing

- earlier microbiological diagnosis, higher resolution with definition of resistance and virulence
- understanding gut and lung microbiome and relevance to sepsis
- resolving cases where no pathogen identified by conventional microbiology
- determining extent and nature of viral co-infection (pneumonia)
- monitoring emergence and spread of bacterial pathogens
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Resolving functional genetic variants and modulated genes

GWAS
- association with disease phenotype
- at level of whole organism

Common SNP markers

Expression quantitative trait (eQTL) mapping
- association with gene expression
- intermediate heritable phenotype
Appropriate sensing by Toll-like receptor 4 (TLR4) results in epigenetic reprogramming and large-scale gene transcription with concomitant cytokine production, enhanced antigen processing and cell migration.

We analysed gene expression in CD14+ monocytes from 432 healthy volunteers (naïve or stimulated with LPS for 2h or 24h or IFNγ for 24h) and mapped gene expression as a quantitative trait (eQTL mapping).

This revealed that the individual response is modulated by genetic diversity involving canonical signaling pathways, the inflammasome, cytokines and receptors, and is informative for variants identified in common disease genome-wide association studies (GWAS).
Regulatory variant of LTA gene

Context specific eQTL involving lymphotoxin alpha gene in monocytes
NLRP3 inflammasome: activated by infectious stimuli and massively amplifies the activation of caspase-1, resulting in active IL-1β and IL18.
A common SNP is associated with **NLRP3 expression** in monocytes after 24h LPS (P 3.7x10^{-8}) and with **CRP levels** in large population cohorts (P 1x10^{-15})

Insights into inter-individual variation in response to infection and interpretation of widely used biomarker
Insights into modulated networks and pathways

Local association for *IFNB1* after 2h LPS (cis-eQTL)

Distant associations with a gene set at 24h LPS stimulation (trans-eQTL) reveals gene network
Regulatory genetic variants in endotoxin tolerance

Low dose endotoxin results in a transient hypo-responsive state, with epigenetic reprogramming and specific gene sets repressed/induced.

Endotoxin tolerance contributes to immunosuppression and mortality in sepsis.

We find inter-individual variation in endotoxin tolerance.
eQTL mapping reveals association with endotoxin tolerance involving *TNFRSF1B* encoding **TNF receptor 2**

Conclusions

Big data (omic approaches) are transforming biology and will change the way that we understand and practice medicine.

Urgent need to resolve heterogeneity in sepsis to better understand disease and effectively stratify patients, enabling informative clinical trials and targeted interventions.

Omic approaches may help establish informative biomarkers.

Significant inter-individual variation in the innate and adaptive immune response seen in healthy individuals and in response to disease.
Acknowledgements

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