Modelling Human Immunity in Health and Disease

Human Artificial Lymph Node Model (HuALN) for Biopharmaceuticals Testing and Disease Modelling in vitro

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Content

❖ Challenges of modelling human immunity \textit{in vitro}

❖ Recent status on relevant \textit{in vivo} models and current achievements for \textit{in vitro} modelling

❖ The HuALN model

❖ Conclusions
Modelling Autoimmune Diseases

Innovative human-specific investigational approaches to autoimmune disease (Anja van de Stolpe and Robert H. Kauffmann RSC Adv. 2015 (5))

Pathogenesis of autoimmune diseases: Breaking tolerance

Therapeutical intervention:
- “Old school” treatment: Immune suppression
- Innovative therapy: Inducing tolerance

Source: www.cytherapharm.com
Modelling the Human Immune System

Understanding immunity:
- Development and organogenesis
- Homeostasis, regeneration and aging
- Immune responses
- Immune system-related diseases

Treating immunity:
- Vaccination (Inducing immunity)
- Infection diseases (Breaking tolerance)
- Cancer (Lymphoma, leukemia; improving immunity)
- Inflammation diseases (Inducing tolerance)
- Allergy and sensitization (Inducing tolerance)
The Complexity of the Human Immune System

- Lymphoid recruitment
  - Bone marrow
  - Spleen
  - Thymus

- Blood system

- Lymphatic system
  - Lymph node

- Exposition
  - Dermal Circulation
  - Lungs
  - Intestines

Modelling Human Immunity in Health and Disease
Micro Physiological Systems (MPS) for the Human Immune System

**Biological challenges and technical solutions for MPS**

- Solid organs
  (Tissue complexity, cellular composition, compartmentalization)
- Mobile cells and fluidics
- Innate and adaptive, cellular and humoral responses
- Systemic effects
- Lifelong memory

**Challenges for MPS by biological heterogeneity**

- Population heterogeneity
- Seasonal changes, previous infections, hidden therapeutical treatments
- Non responders
- Diseases and disease status
- Maturation, age and senescence
Biological Scaling

Minaturization and „scalability of immunity“

What is the minimum functional unit of an organ?

Liver

- Organ
- Liver lobuli

Function
- Albumin production
- Cytochrome activity
- Bile production
- ...

Immune system

- Primary /secondary/tertiary immune organs
- Critical T cell and B cell repertoire?

Function
- Innate response
- Cellular responses
- Humoral responses
- Immunity/memory
- Cytokine release
- Antibody production
- Cellular reactivity
- ...

Technical environment
In vivo Testing: Customized Animal Models

- Immune deficient mice (e.g. SCID)
- Transgenic mice (Vector based; gene knock-out/knock-in)
- Humanized mice (with human immune cells)
- Xenograft models (with human tumor cells)
- Genome-edited animals (CRISPR/CAS9)?

Humanized animals as well as xenograft models show limitations in reproducibility and relevance to a certain extent.
Spheroid Tumor Models

The „cellular suspects“

The micro-enviroment

Multi-cell-type 3D tumor micro-spheroid co-culture models (hanging drop technology)

In combinations with cancer cells endothelial cells and stromal cells

Source: Volgin etal (2010)

Source: Kelm etal (ADDR, 2014)
Cell-based *in vitro* Methods for Immune Functional Testing at PBG

- ADCC, ADCP
- Peptide binding assays
- PMBC’s testing
- DC and DC/T cell assays
- HuALN Model

**Peptide binding assay**

MHC I/II human mDCs


**DC assay**

mAb exposition

iDCs

24 hrs

Analysis:
- Viability
- CD markers

**DC/T cell assay**

CD4+ T cell

monocyte separation

PBMCs

monocytes

iDCs

drug exposition

+24/+48 hrs

Analysis:
- Viability
- CD markers
- Proliferation
- Cytokine secretion

**FACS**

**PBMC co-culture**

iDCs

mDCs

24 hrs

PBMCs

monocytes

iDCs

mDCs

24 hrs

Analysis:
- Viability
- CD markers
- Proliferation
- Cytokine secretion

**ProBiogen**

Modelling Human Immunity in Health and Disease
The Human Artificial Lymph Node Model (HuALN)

Antigen-specific immune response

*in vitro*
The Human Artificial Lymph Node Model (HuALN)
Testing Services in Four Applications

IG: Understanding unwanted immunogenicity of biopharmaceuticals and formulations
IF: MoA and adverse effects of super-agonists and checkpoint modulators
VAC: Potency, candidates ranking, dosing and MoA of viral and peptide vaccines, adjuvants and formulations
ITOX: Assessing chemical immunotoxicology
Bioreactor Devices

HIRIS 3

2 mL culture volume (perfusion rate 1 mL/day) -
- large cell repertoire (10^8 DC/PBMC) -
- cell perfusion -

µALN (µALN 1.0)

- Miniaturized size (10-20 fold)
- 150-250 µL culture volume (perfusion rate 100 µL/day)
- Reduced cell repertoire (10^6-10^7 DC/PBMC)
- Parallelization for increased number of test samples and screening applications
- User friendly
- In-situ imaging

Basis for an autonomous device (µALN 2.0):
- Integrated fluidics, heating and gassing
- No incubator, no external fluidics, easy to handle
The HIRIS Bioreactor Device - Perfusion, Drug Exposition and Sampling

Antigen stimulation → Antigen/mDC restimulation → Antigen/mDC restimulation → Antigen/mDC restimulation

mDC and CD14neg co-culture → 28 days

Cell analysis and IHC

DC generation → Bioreactor culture → Sampling culture supernatants
HuALN: Analytical Parameters

- Cytokine profile
- Immunohistochemistry
- Marker expression
- Secreted antibodies
- Proliferation
Monitoring Cytokines to Immune Response Analysis

T cell activation for TH-1 pathway

Hubbell, Thomas, and Swartz; NATURE 2009
Monitoring Cytokines to Immune Response Analysis

T cell activation for TH-2 pathway

Hubbell, Thomas, and Swartz; NATURE 2009
Immune Responses Triggered by Cytokines

**Cytokine groups**

<table>
<thead>
<tr>
<th>Pro-inflammatory</th>
<th>Anti-inflammatory</th>
<th>( T_{H1} )</th>
<th>( T_{H2} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1β, TNFα, IL-6, GM-CSF</td>
<td>IL-6, IL-10</td>
<td>IL-2, IFNγ, TNFα</td>
<td>IL-4, IL-5, IL-6, IL-10</td>
</tr>
</tbody>
</table>

Normalized values of cytokines are added up complying the functional cytokine groups.

Modelling Human Immunity in Health and Disease
Graphical Cytokine Data Summary (Exemplified):
Immunogenicity of Induced Aggregates (Drug Substance: mAb)

Cytokine groups
(Classified by function)

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<tr>
<td>T(_H^2)</td>
<td></td>
</tr>
</tbody>
</table>

Normalized to HSA (control)

Normalized to tox-buffer (control)
Aim: Human Disease Models in vitro

- Rheumatoid Arthritis (RA)
- Multiple Sclerosis (MS)
- Morbus Crohn (MC)
- Atopic Dermatitis (AD)
- Allergy / Sensiziation (AL)
- Leukemia (LEU)
- Cancer (CA)
- HIV

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Modelling Autoimmune Diseases

*Innovative human-specific investigational approaches to autoimmune disease* (Anja van de Stolpe and Robert H. Kauffmann RSC Adv. 2015 (5))

Basic concept for modelling an inflammation disease on a miniaturized organ model (“patient-on-a chip”)

Important achievements:
- Miniaturized platform suitable for patient biopsy material
- Continuously perfused
- Long-term, organotypic culture
- Steady-state conditions
- Controlled drug exposition
- Dynamic response profiling
Conclusions

In general:

- Good bioreactor platforms available (Single organ and MOCs)
- Relevant human cell and tissue models available
- Miniaturized and multiplexed analytics available
- Advanced in situ-imaging technologies available

HuALN:

- The HuALN model is a useful tool for testing immune human responses in vitro
  Applications: IG, IF, Vac, Tox)
- The model is using “well trained” immune cells of adult and healthy donors
- GLP-like test procedures available
  (Medical devices, SOPs, fully documented, data/reports QA reviewed)
- The HuALN model is ready for service in Open for tech-transfer and licensing
Outlook

In general:

- Disease models with patient biopsy material
- Simulating disease models (Surrogates)
  - Drug induced inflammation
  - Leukemia/cancer (Co-cultivation models)
- Use of iPS technology and e.g. genome editing (CRISPR/CAS9)

HuALN:

- Spheroid model for the immune system
- Inflamed HuALN model
- HuALN with patient biopsy material
- HuALN and tumor co-culture mode (Leukemia model)