ANIMAL PROTEOMICS: IPG-DALT and more...

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ANIMAL PROTEOMICS:

- **animal science:**
  animal medicine (biomarker search)

- **food industry:**
  animal derived products

- **animal models:**
  study of diseases / expositions

- ...

ANIMAL PROTEOMICS:

- **animal science:**
  - animal medicine (biomarker search)

- **food industry:**
  - animal derived products *(COST - EuFAP)*

- **animal models:**
  - study of diseases / expositions

- **technical modifications**
A VARIETY OF SPECIES
A VARIETY OF SPECIES

- differences in protein properties (pI, Mr...)
- differences in protein concentrations (health, disease)
- species specific proteins
1 - serum albumin; 2 – transferrin; 3 – Ig γ-chain; 4 – haptoglobin β-; 5 – Ig L-chain
transferrin

(and other species)
transferrin

(and other species)

cow

rat

haptoglobin

cow

β-chain

α-chain

other species

β-chain

α-chain

apo A-I

apo A-I
Positive Acute Phase Proteins
(proteins up-regulated in inflammation)

<table>
<thead>
<tr>
<th></th>
<th>CRP</th>
<th>SAA</th>
<th>Hp</th>
<th>oroso</th>
<th>$\alpha_1$AT</th>
<th>other</th>
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</thead>
<tbody>
<tr>
<td>human</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>rat</td>
<td>±</td>
<td>x</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>dog</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>cow</td>
<td></td>
<td></td>
<td>++</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>pig</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>MAP (++)</td>
</tr>
</tbody>
</table>

x ... non existent
CRP cannot be detected in electrophoresis (except for rat)
EXAMPLES

- animal model of inflammation and shock (rat)
- transgenic models (mouse)
- physiological changes (cow)
- gammaglobulin disorders (dog)
- homologous proteins (fibrinogen)
- depletion
1. Animal model of inflammation and shock (rat)
proteome of control tissue / body fluid

pathological process

infectious process

proteome of pathological tissue / body fluid

mechanism of drug action

mechanism of drug resistance

mechanism of drug toxicity

proteome of tissue / body fluid after therapeutic treatment

proteome of tissue / biological fluid after sham treatment
Serum protein pattern in inflammation
Acute inflammation

Rat serum protein patterns

Serum (healthy)

Serum (inflamed, 48h)

male Sprague-Dawley rats
i.m. turpentine

Electrophoresis 1998, 19, 1484-1492 + 1493-1500.
Protein concentration changes

**Positive acute phase reactants**
- $\alpha_2$-M
- $\alpha_1$-AG
- clusterin
- Hp
- $\alpha_1$-MAP
- SPI3
- CRP
- $\alpha_1$-AT
- Cp
- Hpx

**Negative acute phase reactants**
- $\alpha_1$-l3
- $\alpha_2$-HS
- KBP
- RBP
- TTR
- albumin

pink: female, blue: male rats

48 hours

Time-course

Acute inflammation

Effect of adjuvant arthritis on serum protein levels

Chronic inflammation


female Lewis rats
heat-inactivated M. tuberculosis
Acute / Chronic inflammation

Differentially regulated proteins

% change

-100 0 100 200 300 400 500

Hp a-
Hp a1-AG
Hp b-
a2-M
SPI-3
a1-MAP
a1-AT
CRP
Gc
Cp
transferrin
TTR
apo A-IV
albumin
a2-HS
apo Al
a1-I3
KBP

AA (48 h)
CA (14 d)
Effect of NSAID treatment on serum protein levels in adjuvant arthritis

- haptoglobin chain α
- α₁-acid glycoprotein
- haptoglobin chain β
- α₂-macroglobulin
- serine protease inhibitor-3
- thiostatin (1)
- thiostatin (2)
- α₁-antitrypsin
- C-reactive protein
- transferrin
- transthyretin
- apolipoprotein A-IV
- albumin
- α₂-HS-glycoprotein
- apolipoprotein A-I
- α₁-inhibitor III
- α₁B-glycoprotein
- kallikrein binding protein

Effect of NSAID treatment per se on serum protein levels

Treatment of healthy animals
Liver proteome and endotoxic shock
Experimental shock model:

- male Sprague Dawley rats
- 8 mg/kg LPS i.p. / i.v.
- 16 hours after LPS challenge

Liver failure

Increased levels of ALT

Lipopolysaccharide (LPS)

[Graph showing increased ALT levels in LPS group compared to control]
Liver

Homogenization

10 min 600 g → nuclear fraction

10 min 9 000 g → mitochondrial fraction

10 min 100 000 g → microsomal fraction (ER)

cytoplasmic fraction

Fractionation
(differential centrifugation)

2D-DIGE
Mitochondria

green: control
red: LPS

ATP-Synthase

more fragments of carbamoylphosphate synthase
Mitochondrial superoxide dismutase (SOD [Mn])

aldehyde dehydrogenase polymorphism?

protection against toxic radicals
Endoplasmic reticulum

*green*: control
*red*: LPS

Kozlov et al., *BBA* 2009, 1792, 521-530.
Differentially regulated ER protein spots

- 78 kDa glucose-regulated protein
- Protein disulfide-isomerase
- Transthyretin
- Gamma-actin
- Transitional ER ATPase
- Serum albumin
- Argininosuccinate synthase
- Peroxiredoxin-1

**[kD]**

67 67
63 63
57 57
43 43
30 30
20 20
14 14

**pI**

4 4  pl  pl  10 10

*Example functions: transport, folding, oxidation, acute phase*
Testing of function:
function of mitochondria improved,
function of ER decreased.

ROS production

histological changes

Kozlov et al., Am. J. Physiol. 2006, 290, G543.
Kozlov et al., BBRC 2007, 352, 91-96.
More (pronounced) changes in endoplasmic reticulum (ER)

ER is much more susceptible to endotoxic shock than mitochondria
2. Transgenic animals (mouse)
Apolipoproteins

knock-out: murine Apo A-I and/or II

knock-in: human Apo A-I and/or II
Additional changes of:
- orosomucoid + clusterin,
- $\alpha_1$-macroglobulin,
- contrapsin,
- carboxylesterase,
- haptoglobin.

Improved resolution of relevant proteins by varying running conditions

contrapsin
haptoglobin
$\alpha_2$-HS-glycoprotein

3. Physiological changes (cow pregnancy)
Cow serum protein map

Wait et al., Electrophoresis 2002, 23, 3418-3427.
Cow pregnancy

acidic zoom gels, non-reducing conditions

Elisabetta Gianazza et al.
Università di Milano

SDS-PAGE

time course

healthy animal

endometritis

Elisabetta Gianazza et al.
Università di Milano

SDS-PAGE

time course

healthy animal           endometritis

Cairola et al., Electrophoresis 2006, 27, 1617-1625.
healthy animal

endometritis

orosomucoid

haptoglobin $\beta$-
4. Disorders (gammopathy, dog)
Normal immunoglobulin pattern

dog serum
(healthy dog)

gammopathies: polyclonal, monoclonal
Multiple myeloma with monoclonal IgA

SERUM

in late stage of the disease / severe cases also detectable in urine (kidney damage)
Bence Jones Proteins (free Ig L-chains)

**URINE**

**Dog 1:**
chronic lymphatic leukemia; 
mlgA and mlgG in serum

**Dog 2:**
plasmacytoma; 
dimeric BJP + 
mlgA in serum

Bence Jones Proteins (free Ig L-chains)

**URINE**

**Dog 1:** chronic lymphatic leukemia; mIgA and mIgG in serum

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Bence Jones Proteins (free Ig L-chains)

Dog 1: chronic lymphatic leukemia; mIgA and mIgG in serum

Dog 2: plasmacytoma; dimeric BJP + mIgA in serum

non-reducing 2-DE: for the detection of single subunits, incomplete molecules

in serum

Different combinations

- native (non-denaturing) IEF / native PAGE
- native IEF (non-denaturing) / SDS-PAGE
- native IEF (non-denaturing) / red. SDS-PAGE
- denaturing IEF / SDS-PAGE
- red./denat.IEF / red. SDS-PAGE

2-DE
Rabbit milk

non-denaturing IPG native PAGE

non-denaturing IPG SDS-PAGE

IPG (urea)
SDS-PAGE

IPG (urea, DTT)
SDS-PAGE (DTT)
5. Homologous proteins (fibrinogen)
Structure of fibrinogen

340 kD plasma glycoprotein
Structure of fibrinogen

340 kD plasma glycoprotein

3 pairs of polypeptide chains:

\( \alpha-, \beta-, \gamma- \)

linked by disulfide bonds and stabilized by \( \text{Ca}^{2+} \)
Approaches for identification:

a) comparison serum/plasma

b) immunoblotting
Approaches for identification:

c) heat precipitation

dissolve and analyse precipitate in 2-DE

<table>
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<th></th>
<th>Plasma</th>
<th>Serum</th>
<th>Heat Precipitate</th>
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Physicochemical data of fibrinogen chains of different species

![Graph showing the physicochemical data of fibrinogen chains of different species. The graph plots pI on the x-axis and Mr on the y-axis. Different symbols represent different chains: α-chain (purple diamonds), β-chain (blue circles), and γ-chain (red triangles).]
6. Depletion of high-abundance proteins (albumin)
Albumin binding to Cibacron Blue F3G A

albumins of different species bound on a "blue column" as a function of pH

better binding and higher capacity for HSA

Albumin binding to Cibacron Blue F3G A


binding of all serum proteins increases at lower pH
University of Veterinary Medicine, Vienna
LBI for Exp. & Clin. Traumatology, Vienna
Medical University, Vienna
University of Graz
Università degli Studi, Milano
Kennedy Institute, London
and all the others...

Thank you!
...and you!

Thank you!