INFLAMMATORY PROTEIN PROFILE DURING SYSTEMIC HIGH DOSE INTERLEUKIN-2 ADMINISTRATION

Leonardo Rossi, PhD
Clinical Center Department of Transfusion Medicine
Section of Immunogenetics
NIH

ALEXANDRIA
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Systemic IL-2 can effectively treat metastatic renal cell cancer and melanoma and plays an essential role during active-specific immunization against cancer by increasing the frequency of tumor regressions.

Modulatory effects of IL-2 on various pathways of cellular immune responses have been extensively described.

The use of high dose IL-2 treatment is limited by the occurrence of several side effects and severe toxicity.

The mechanisms of IL-2 mediated cancer regression remain today largely unknown. Recent data suggest that IL-2 acts through the activation of mononuclear phagocytes at the tumor site.
IL-2

PBM C

Enhancement of innate effector mechanisms
Chemo-atraction
M1-immune stimulation

MCP-3
MIP1-β
MIP1-α
MCP-1
PARC
MCP1
IL-8
GRO-1
MIG
We characterized the protein profile of sera obtained from ten patients with RCC undergoing high dose (720,000 IU/kg intravenously every 8 hours) IL-2 therapy.
PROTEOMIC ANALYSIS OF SERUM IN PATIENTS UNDERGOING IL-2 THERAPY

SERUM

Multiprotein array platforms

- SELDI
- Immuno-nephelometry

H4
SAX2
WCX2
IMAC
IL-2 treatment is able to induce a very complex cytokine storm

Panelli et al., Journal of Translational Medicine 2004, 2:17
Storm of chemokines, cytokines and soluble factors increasing in the circulation in response to IL-2

Many soluble factors that increased during IL-2 administration are also increased in inflammatory conditions:

- Soluble forms of adhesion molecules
- Chemoattractant factors
- Matrix metalloproteinases and their inhibitors
- TNF-alpha and soluble TNFR1
FROM PROTEIN ARRAYS TO SELDI ANALYSIS OF IL-2 INDUCED PROTEINS

Multiplexed protein array platforms

Sensitive tools which allows the detection of proteins at very low concentration in serum

Discovery limited by the number of capture antibody selected for protein detection.


Serum samples

Different matrix are available to allow the preferential binding of proteins on the basis of their specific chemical or biological characteristics.

**SELDI ADVANTAGES**

- Fast and easy
- Very reduced amount of samples are necessary to perform the analysis
- Accurate information about the molecular weight
- Possibility to discriminate between isoforms (phosphorilation, glycosilation, truncated forms)
- Possible capture of co-precipitating proteins
SELDI ANALYSIS OF RCC SERUM

SAX2

Apo C-I 6629
Apo A-II 8679
Apo C-II 8909
TTR 13859
Apo A-I 17959
Alpha-globin 15113
Beta-globin 15699
RBP 21017
C3dg 25380

Apo C-I 6632
Apo A-II 8688
Apo C-II 8911
TTR 13860
Apo A-I 17969
Alpha-globin 15125
Beta-globin 15863
RBP 21024
C3dg 25384

Apo C-I 6630
Apo A-II b 17233
Apo A-II b 17250
Apo A-II b 17304
Apo A-II b 17383
C3dg 25382
C3dg 25380

Apo A-I 28036
Apo A-I 28071
Apo A-I 28164
RBP 21011
CRP 23108

Apo A-I 28066
Apo A-I 28114
RBP 21011
CRP 23108

C3dg 25382
C3dg 25384

Rossi L. et al., Proteomics 2005 in press
SELDI IMMUNOAFFINITY CAPTURE OF SAA AND CRP

Rossi L. et al., Proteomics 2005 in press
UNSUPERVISED HIERARCHICAL CLUSTERING OF RCC SERUM SAMPLES OBTAINED BEFORE AND AFTER 1 AND 4 DOSES OF IL-2 (SELDI M/Z AREA).

- Increase in SELDI m/z
- Decrease in SELDI m/z
- No change in SELDI m/z

Rossi L. et al., Proteomics 2005 in press
QUANTIFICATION OF SERUM FACTORS CONCENTRATION BY IMMUNONEPHELOMETRY

Rossi L. et al., Proteomics, 2005 in press
SEQUENTIAL DILUTION OF ONE POST 4 SAMPLE WITH THE CORRESPONDING PRE SAMPLE.

Intensity

1 POST4
2 PRE
0.3% of tot prot

1 POST4
4 PRE
0.17% of tot prot

1 POST4
9 PRE
0.08% of tot prot

1 POST4
14 PRE
0.06% of tot prot

m/z

SAA

0.08% of tot prot
0.06% of tot prot
CORRELATION BETWEEN % SERUM SAA IN TOTAL SERUM PROTEIN AND THE RELATIVE M/Z AREA IN PATIENT SERA

Rossi L. et al., Proteomics, 2005 in press
The interplay between different platforms could provide a variety of information

Critical aspect: collection of good quality material

1) Simultaneous detection of the expression levels of several factors

2) Bio-functional suggestions

3) Discovery of new factors
SUMMARY

1. We observed an outburst of a multitude of proteins increasing/reducing in the circulation in response to IL-2:

2. Changes became more dramatic with increasing doses

3. Subclasses of soluble factors displayed different kinetics

4. All these data well correlate with a systemic inflammatory profile
PROTEIN INTERPLAY FOLLOWING HIGH DOSE IL-2 THERAPY

Act as an inflammatory stimulus

IL-2

IL-1  IL-6  TNF α  sTNFR1

After the 1° dose

ICAM, VCAM E-SELECTIN
CHEMOATTRACTANT
ACTIVE RECRUITMENT
OF MONONUCLEAR CELLS
TO INFLAMMATORY SITES

MCP-1

ACTIVATION OF MONOCYTES
AND RELEASE OF TNFR

CRP  SAA

After the 4° dose

ECM DEGRADING ENZYMES

MMP-1  MMP-2,3,8,9,10,13

Capillary leak syndrome
and hypotension

MPs activation
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