POSSIBLE ROLE OF PTX3 ON LEPROSY:

*M. leprae* RECOGNITION, PHAGOCYTOSIS MODULATOR OR VESSEL INFLAMMATION MARKER?

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Pentraxins constitute a superfamily of multifunctional, multimeric proteins that are phylogenetically conserved from arachnids to mammals. All members of the family contain, in their carboxy-terminal, the “pentraxin domain”, which is characterized by a conserved 8-amino-acid long sequence (HxCxS/TWxS, where x is any amino acid) called the “pentraxin signature” (Garlanda et al. 2005). Based on the primary structure of the protomer, pentraxins are divided into two groups: short pentraxins and long pentraxins.
How important PTX3 is??

Long pentraxin 3: a marker of inflammation in untreated psoriatic patients.


Dermatology Unit, AORNAS, Ganzabbi Hospital, Catania, Italy.


Persistent high levels of plasma pentraxin 3 over the first days after severe sepsis and septic shock onset are associated with mortality.


Dipartimento di Medicina, Poliambulanza e Terapia Intensiva, Azienda Ospedaliera San Gerardo di Monza, Via Perugia 33, 20052, Monza, Italy.


Pentraxin 3, a new marker for vascular inflammation, predicts adverse clinical outcomes in patients with heart failure.


Department of Cardiology, Pulmonology, and Nephrology, Yamagata University School of Medicine, Yamagata, Japan.


PTX3 function as an opsonin for the dectin-1-dependent internalization of zymosan by macrophages.

Dimz SN, Nomizo R, Cisalpino PS, Telha weekend BM, Brown GD, Mantovani A, Gordon S, Reis LF, Dils AA.

Ludwig Institute for Cancer Research, São Paulo, Brazil.


Exogenous pentraxin 3 restores antifungal resistance and restrains inflammation in murine chronic granulomatous disease.


Department of Experimental Medicine and Biochemical Sciences, University of Perugia, Perugia, Italy.


DC-SIGN (CD209), pentraxin 3 and vitamin D receptor gene variants associate with pulmonary tuberculosis risk in West Africans.


A framework to identify gene expression profiles in a model of inflammation induced by lipopolysaccharide after treatment with thalidomide

Why PTX3??

PTX3

Fold induction of PTX3

FOLD CHANGE PTX3

FOLD CHANGE PTX3

PTX3

PTX3
ENL X Thal 100mg/day (7days)

- Lesion Biopsies
- RNA extraction

Microarray
- 35k Human Genes
- Analysis

894 downregulated
412 Upregulated

Microarrays under Analysis/Validation

Integrative genomics approach

ENL X Thal + THAL
692 Donwregulated genes

LIxENL up regulated genes

202 PTX3

Lee DJ et al. J Infect Dis. 2010

PTX3 is the #4 on the rank of the most reduced expression genes
(Fold change 16.89)

Hidden challenges
Skin biopsies were collected at leprosy diagnosis (non reactional), during ENL onset and after one week of thalidomide treatment (300 mg/day). All patients showed improvement on ENL lesional and sistemic related symptoms. PTX3 expression levels were accessed by Taqman Real-Time PCR.

<table>
<thead>
<tr>
<th>Patient</th>
<th>% inhibition PTX3 after treatment</th>
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<tbody>
<tr>
<td>1</td>
<td>85.54</td>
</tr>
<tr>
<td>2</td>
<td>61.31</td>
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<tr>
<td>3</td>
<td>78.83</td>
</tr>
<tr>
<td>4</td>
<td>97.85</td>
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<tr>
<td>Avg ± SE</td>
<td>80.88±7.62</td>
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PTX3 is reduced on ENL lesions after thalidomide treatment.
What do we already know about ENL and PTX3?

“When lesions in either type 1 or type 2 reaction were compared to lesions not in reaction, a significant increase in apoptosis was found only in lesions with type 2 reaction…”

“Our observations indicate that increased apoptosis is present in leprosy lesions and that in leprosy it progressively increases with anti-leprosy treatment up to 6 months.”

“A neutralizing anti-PTX3 monoclonal Ab (mAb) inhibits the capture of late apoptotic neutrophils by macrophages.”
The role of opsonins in the handling of apoptotic cells. Appropriate opsonization of apoptotic cells leads to their rapid uptake by macrophages (Mφ) or iDC, and to a modulation of these phagocytic cells, leading to an anti-inflammatory response and maintenance of tolerance. In contrast, quantitatively or qualitatively inappropriate opsonization may disturb the apoptotic cell uptake, leading to a loss of tolerance, as illustrated in the lower panel.

Neutrophils of ENL patients have higher spontaneous apoptosis

Neutrophils of ENL patient

Neutrophils of Health Donor

Neutrophils were obtained from Ficoll gradient followed by decantation in Dextran 3%. Cells were labeled by Annexin- FITC and PI during 15min and read on accuri C6 flow cytometer.
ENL isolated neutrophils seems to express more PTX3 at membrane

Neutrophils were obtained from Ficoll gradient followed by decantation in Dextran 3%. The neutrophils were labeled by ant-PTX3 (MNB1) followed by an secondary anti-rat FITC and read on accuri C6 flow cytometer.
Apoptotic Neutrophils shows PTX3 in the surface

Apoptosis of human neutrophils was induced by UV irradiation followed by 2h of incubation in no serum media RPMI 1640. The neutrophils were labeled by anit-PTX3 (MNB1) followed by an secondary anti-rat FITC.
Anti-PTX3 (MNB1) and excesso of soluble PTX3 decreases apoptotic neutrophils phagocytosis by macrophages.

<table>
<thead>
<tr>
<th>Sample</th>
<th>%M1</th>
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<tbody>
<tr>
<td>MO</td>
<td>0,2</td>
</tr>
<tr>
<td>MO+NO</td>
<td>46,7</td>
</tr>
<tr>
<td>MO+NO+PTX3</td>
<td>24,5</td>
</tr>
<tr>
<td>MO+NO+MNB1</td>
<td>20,7</td>
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Neutrophils were labeled by PKH67 (green fluorescente) and added to monocyte differentiated macrophage culture in the proportion of 2:1 followed by addition of rh-PTX3 (100 ng/mL) and MNB1 (2ug/mL). After 2 hours of incubation cells were washed and the attached cells harvested and analysed by flow cytometry.
PTX3 seems to bind *M. leprae*
*M. leprae* pre-incubation with PTX3 100ng/mL increases its phagocytosis rate
*M. leprae* pre-incubation with PTX3 increases chemokines production

**IL-8**

<table>
<thead>
<tr>
<th></th>
<th>ML</th>
<th>ML+PTX</th>
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<tr>
<td>0 pg/ml</td>
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**MIP1β**

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<td>200000 pg/ml</td>
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PTX3 facilitates phagocytosis/association of Mycobacteria

M. Intracellulare - PTX3 100ng/ml – 1 hour incubation
Zymosan coated beads – 30 min incubation
Human in vitro differentiated macrophages by 5 days of incubation without any additional cytokines.

Myc

Myc+PTX3

Zymosan Coated beads

Zym+PTX3
Evidences

PTX3 is downmodulated by thalidomide – directly and indirectly
PTX3 is raised on ENL lesions and is downmodulated after thalidomide treatment/symptoms remission
PTX3 is expressed by neutrophils (is the neutrophils the source of PTX3 in ENL?)

Neutrophils are the main leukocytes on ENL infiltrate
Microarray assays indicate cellular migration genes are prominent on ENL lesions
Neutrophils and monocytes when in apoptosis express PTX3 in membrane
There are accumulation of apoptotic cells on ENL lesions
Neutrophils of ENL patients shows increased rate of spontaneous apoptosis
PTX3 is important for phagocitosis of apoptotic cells

Usually in systemic inflammation PTX3 is increased on serum
Soluble PTX3 inhibit phagocytosis of apoptotic cells
Problems on clearance of apoptotic cells trigger inflammation

Considerations

Probably other kind of programmed cellular death, eg. pyroptosis, could be involved on ENL.

IL-1b is raised in ENL, and seems to be important for its development, cell death mediated events could be responsible by the increases in the cytokines levels on ENL and its persistance.
Next Steps

Identify the cells expressing PTX3 on lesions.

Confirm the increased membrane expression of PTX3 on circulating neutrophils of ENL patients.

Identify if the subset of neutrophils more susceptible to spontaneous apoptosis are the same expressing PTX3 on membrane.

Identify possible alterations in the clearance of apoptotic cells on ENL lesions.

Identify if PTX3 could be used as an early marker of ENL onset.

Confirm by other techniques the binding of PTX3 to *M.leprae* and how it affects the immune system functions.
Obrigado!
Thank You!

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