

Modulation of immune function and foetal exposure.

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PLUTOCRACY project

One of the objectives of the PLUTOCRACY project was to investigate the epidemiological relationship between exposures to pollutants which occur in utero with alterations in the immune responses and the early childhood allergy.

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    graph TD
      A[Environmental pollutants in placenta] --> B[Allergy in childhood]
      C[Immune status: mother, placenta, cord blood, child] --> B
      A <--> C
  
```

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Background

- Xenobiotics → immunotoxic
- Early markers of atopy in cordblood (Allam, Allergy, 2005)
 - Cord blood IgE levels
 - soluble mediators: chemokines ,cytokines, Ig receptors
 - Hygiene hypothesis : sCD14
 - Polyunsaturated fatty acids (PUFA)
 - Cytokine production by MNC
 - Antigen presenting cells
 - Genetics

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Objectives

To study if alterations in the selected immune functions

- are linked to environmental exposure during pregnancy
- have a predictive value (risk factor) for the allergic outcome of the child (18 months)

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Methods

- Cord blood total IgE
 - Serum
 - 100 samples from each of the 5 regions
 - Pharmacia CAP RAST FEIA system (Fluoro Enzyme Immuno Assay)
- Oxidative enzyme status
 - placenta and erythrocyte lysate from maternal peripheral blood and cord blood
 - 100 samples from each of the 5 regions
 - spectrophotometric measurement
 - Superoxide dismutase (SOD) activity
 - Glutathione peroxidase activity (GPx)
 - Glutathione transferase activity (GST)
 - Glutathione reductase activity (GR)
 - Total thiol levels assay: (proteic and nonproteic) SH-groups

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Methods

- Th1/Th2 cytokine balance
 - Cord blood
 - Isolation mononuclear cells, storage liquid N₂
 - Stimulation:
 - Mitogen: PHA
 - Inhalant allergen: HDM
 - Food allergens: ovalbumin, β-lactoglobulin
 - Elisa: IFN-γ, il-4, il-5, il-10, il-12
 - 100 samples from each of the 5 regions
 - Child peripheral blood (18 months)
 - Isolation mononuclear cells, storage liquid N₂
 - Stimulation: PHA
 - Multiplex analyses (Luminex): IFN-γ, il-4, il-5, il-10, il-2

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Methods

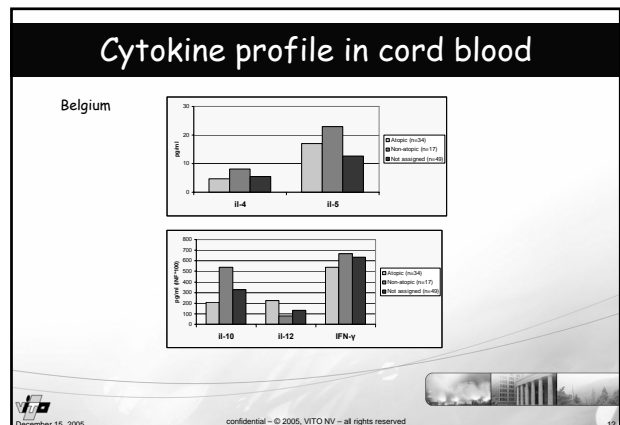
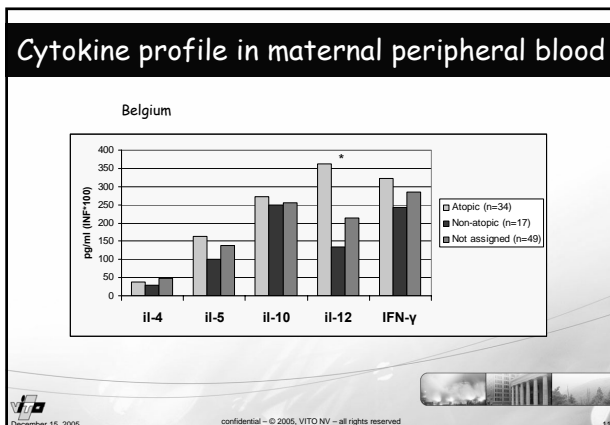
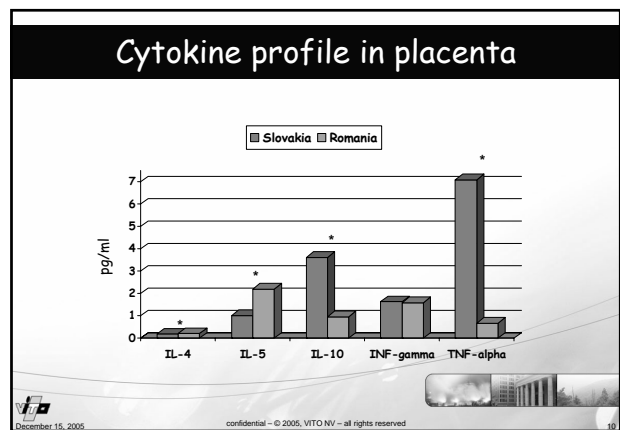
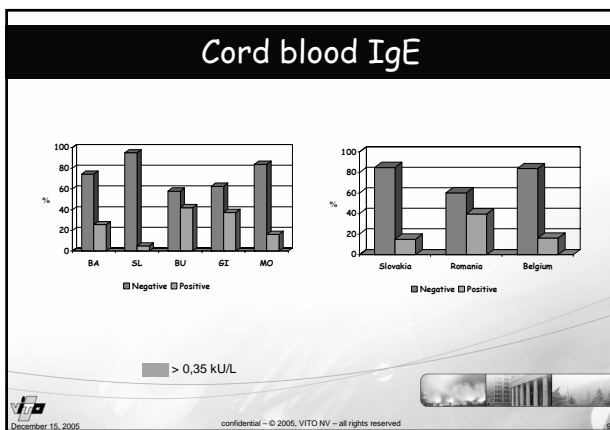
- Th1/Th2 cytokine balance
 - Maternal peripheral blood
 - Isolation mononuclear cells, storage liquid N₂
 - Stimulation:
 - Bacterial: PHA ;
 - Inhalant allergen: HDM;
 - Food allergens: ovalbumin, β-lactoglobulin
 - Elisa: IFN-γ, il-4, il-5, il-10, il-12
 - 100 samples from each of the 5 regions
 - Placenta
 - Trophoblast cultures from fresh tissue
 - Bratislava (S), Bucharest (R)
 - Elisa: IL-4, il-5, il-10, TNF-α, IFN- γ

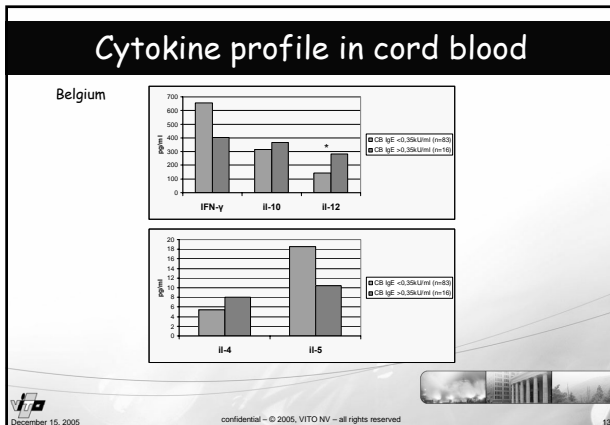
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Results

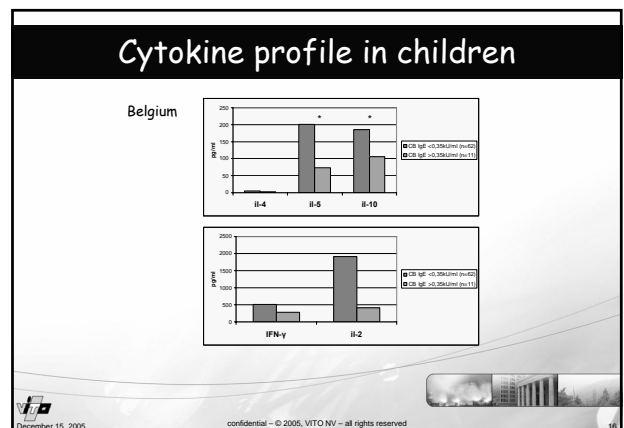
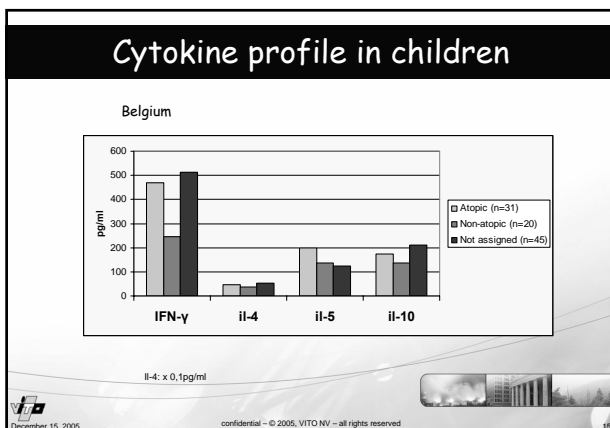
- Milestones
 - M8: evaluation of effects of in utero exposure to xenobiotics on the function of the neonatal immune system
 - M9: evaluation of immune response as a predictor of subsequent clinical development of allergic disease
 - M10: evaluation of placental function with respect to xenobiotic contamination and clinical manifestation of allergy during childhood
- State of affairs
 - Most analyses are finished
 - Data entry into database
- Preliminary results

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- ### Cytokine profile in cord blood
- Correlations between CB cytokines
 - Positive
 - CB il-12 / CB IFN- γ ($p < 0,01$)
 - CB il-4 / CB il-5 ($p < 0,01$)
 - Negative
 - CB il-4 / CB IFN- γ and il-12 ($p < 0,01$)
 - CB il-5 / CB IFN- γ and il-12 ($p < 0,01$)
 - Correlation with cytokine profile peripheral blood mother
 - Statistical significant positive correlation between il-4 levels ($p = 0,04$)
 - Correlation with xenobiotics in placenta
 - No significant correlations
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- ### Cytokine profile in children
- Correlations between cytokines
 - Positive
 - IFN- γ / il-2 and il-10 ($p < 0,01$)
 - il-4 / il-5 ($p < 0,01$)
 - Negative
 - il-4 / IFN- γ and il-2 ($p < 0,01$)
 - il-5 / IFN- γ and il-2 ($p < 0,01$)
 - Correlations with cord blood cytokines
 - Positive
 - Child IFN- γ / CB IFN- γ ($p = 0,02$)
 - Child IFN- γ / CB il-12 ($p = 0,03$)
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- ### Cytokine profile in children
- Correlations with xenobiotics
 - Placenta
 - No sign correlations with Pb and Cd in placenta
 - No sign correlations with chlorinated benzenes, insecticides or PCBs
 - Breastmilk
 - No significant correlations
 - Maternal serum
 - No significant correlations
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Cytokine profile in children


- Reduced production of IFN- γ and il-12 associated with increased risk for atopy
(Prescott, 1999; Tang, 1994; Warner, 1994; Liao, 1996; Kondo, 1998)
- Conflicting results
IL-13, IFN- γ , il-4
(Rowe, 2004; Maccaubas, 2003; Miceli Sopo, 2003; Lang, 2003)
→methodology (cytokine detection, stimulation), study design, outcome measures, definitions



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Conclusion


- Preliminary results
- Further statistical analyses needed
- Relation between cytokine profiles and allergic outcome measures in the child at the age of 18 months.



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