Tuberculosis immunology in children

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Plan

- Reactivation rate of Tuberculosis in infancy

- Immune modifications in infancy
  - Changes in antigen presenting cells profile
  - Th2 bias

- Predisposing factors in host for active tuberculosis

- Interféron γ assays in infancy

- Conclusions
Exposition

Infection

Tuberculose latente

Maladie

Anamnèse

Primoinfection

Avec conversion:
- du test de Mantoux
- Des tests Gamma interferons

Manifestations Pulmonaires et extra-Pulmonaires

Stades de la tuberculose
INCIDENCE OF TUBERCULOSIS AMONG INITIAL REACTORS TO TUBERCULIN BY AGE WHEN TUBERCULOSIS WAS FIRST DIAGNOSED

Comstock; Am. J. Epidemiol; 1974; 99:131
AGE-SPECIFIC RISK TO PROGRESS TO DISEASE AFTER PRIMARY INFECTION WITH *Mycobacterium tuberculosis* IN IMMUNOCOMPETENT CHILDREN

<table>
<thead>
<tr>
<th>Age at Primary Infection (yr)</th>
<th>Risk to Progress to Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 1</td>
<td>No disease, 50%</td>
</tr>
<tr>
<td></td>
<td>Pulmonary disease, 30–40%</td>
</tr>
<tr>
<td></td>
<td>Disseminated (miliary) disease or TBM, 10–20%</td>
</tr>
<tr>
<td>1–2</td>
<td>No disease, 75–80%</td>
</tr>
<tr>
<td></td>
<td>Pulmonary disease, 10–20%</td>
</tr>
<tr>
<td></td>
<td>Disseminated (miliary) disease or TBM, 2–5%</td>
</tr>
<tr>
<td>2–5</td>
<td>No disease, 95%</td>
</tr>
<tr>
<td></td>
<td>Pulmonary disease, 5%</td>
</tr>
<tr>
<td></td>
<td>Disseminated (miliary) disease or TBM, 0.5%</td>
</tr>
<tr>
<td>5–10</td>
<td>No disease, 98%</td>
</tr>
<tr>
<td></td>
<td>Pulmonary disease, 2%</td>
</tr>
<tr>
<td></td>
<td>Disseminated (miliary) disease or TBM, &lt; 0.5%</td>
</tr>
<tr>
<td>&gt; 10</td>
<td>No disease, 80–90%</td>
</tr>
<tr>
<td></td>
<td>Pulmonary disease, 10–20%</td>
</tr>
<tr>
<td></td>
<td>Disseminated (miliary) disease or TBM, &lt; 0.5%</td>
</tr>
</tbody>
</table>

*Definition of abbreviation: TBM = tuberculous meningitis.*

Adapted from Reference 8.

Ben J Marais et al AJRCCM 2006;173:1078
Defence mechanisms against M. Tuberculosis

1) Bactericidy in monocytes / macrophages

2) Sensitisation and activation of cytotoxic lymphocytes CD8 or CD4(Th1; Th17) by dendritic cells

3) (Necrosis)/Apoptosis of infected macrophages by
   - Fas –Fas ligand / TNF
   - by the cytotoxicity of lymphocytes
Bactericidy of M. tuberculosis by macrophages in their phagosomes after «maturation / activation»

Maturation of phagosome:
- decrease of pH
- activation of proteases, Lipases, DNAses, hydrolases
- production of NO-

Various mechanisms used by mycobacteria for their survival:
- inhibition proton pump for the acidification of phagosomes
- recruitment of coronin from host to prevent the fusion of phagolysosomes
- production of serine/threonine protein kinase G (PknG)
Phagocytosis and activation of T cell immunity by DCs
Pathogens recognition receptors on resting immature (i)DC

Lung DC like immature blood derived DC have many Mannose receptors to capture bacteria and viruses

L. Cochand et al AJRCMB 1999

Human lung DC have other pathogen recognition receptors such as DC-SIGN a major receptor for Mycobacterium tuberculosis

Dual phenotype of dendritic cells:

A) High level of accessory molecules and production of cytokines inducing high level of IFN-Gamma (IL-12; IL-18…) for bactericidy

B) Low level of accessory molecules and production of regulatory factors (IL-10) leading to bacterial persistance
Compared efficacy of antigen presenting cells to activate T cell proliferation in the presence of PPD(A) or M. Tuberculosis

**Figure 2.** DC are potent accessory cells for antimycobacterial responses. T cells (4 x 10^5) from primed CBA mice were cultured with graded numbers of DC (C), FcR^+ cells (Δ), or PEC (◯) in presence of 50 μg/ml PPD (A) or 10^5/ml M. tuberculosis H37RA (B). Data are calculated as in legend to Figure 1. In absence of presenting cells, T cell response was 1173 ± 670 (A) and 221 ± 231 (B).

- Dendritic cells (o)
- Splenic monocytes: FcR^+ (Δ)
- Peritoneal macrophages (◯)

Neonate lungs are severely impaired in the amount of cDCs and pDCs

CD 4 (Th1;Th17), CD8, NK cells and gamma delta (Th17) T cells involvement in tuberculosis immunity
Immunology against tuberculosis

Adapted from LP Nicod SMW 2007;137:357
Not only CD4 lymphocytes are required, but also CD8 T cells activated classically by MHC class I to allow the survival of mice against M. tuberculosis (β/-/- = MHC class I « knock out »)

β2 -/-  ● control mice
a) mice infected with M. tuberculosis
b) mice immunized with BCG and challenge with M. Tb
CD8⁺ T Cells Provide an Immunologic Signature of Tuberculosis in Young Children

Christina Lancioni¹,²**, Melissa Nyendak³*, Sarah Kiguli⁴, Sarah Zalwango²,⁵, Tomi Mori⁶, Harriet Mayanja-Kizza²,⁵,⁷, Stephen Balyejusa⁴, Megan Null⁸, Joy Baseke²,⁵, Deo Mulindwa²,⁵, Laura Byrd⁸, Gwendolyn Swarbrick⁹, Christine Scott², Denise F. Johnson², LaShaunda Malone², Philipa Mudido-Musoke⁴, W. Henry Boom²,⁵, David M. Lewinsohn²,⁹, and Deborah A. Lewinsohn²,⁸; for the Tuberculosis Research Unit

An Mtb specific CD8+ T cell response is generated in response to high bacillary load and may be able to distinguish children with TB disease from those with Mtb exposure!

(to be confirmed!)
Essential role of interferon-γ for the resistance of mice against Mycobacteria tuberculosis

Survival curves of mice infected with M. Tb.

■ = knock out mice  ● = control mice  Δ = knock out mice given INFγ

INTERLEUKIN-12 (IL-12) IS CRUCIAL TO THE DEVELOPMENT OF PROTECTIVE IMMUNITY AGAINST M. TUBERCULOSIS

Method: - mice were infected via the lateral vein with $10^5$ M. Tb.
- data represent mean bacterial number in the organs of 4 mice.

Log 10 viable bacteria in target organs

Liver      spleen        Lung

K/O IL-12

● = Control    ○ = IL-12 p40 knockout mice

A. Cooper et al. J. Exp Med 1997; 186:39
IMMUNO REGULATORY FUNCTION OF INFγ

- Enhance MHC I and II expression

- Enhance processing of antigens (proteasomes; peptides transporters)

- Enhance Th1 response: - \( \Rightarrow \) IL-12 production
  - \( \Rightarrow \) IgG2 a production

- Inhibit Th2 response

- Activation of microbicidal activities of Mo/Mø

- Increase cytotoxic effector cells (CD4, CD8, NK Cells)
Biased Th2 responses in neonatal lungs

(IFN-γ) with or without addition of anti-CD3 mAb

Neonatal CD4 T cells seemed to have an inherent Th2 bias.

Adult BCG-lung-DCs were co-cultured with neonatal naïve CD4 T cells, the production of IL-5 was predominant over IFN-γ whereas they did prime adult naïve CD4 T cells to produce IFN-γ but not IL-5.

Transcription factors of CD3 T cells show a higher ratio of GATA-3 over T-bet cells confirming the TH2 transcription bias in neonates.

The Likelihood of an Indeterminate Test Result from a Whole-Blood Interferon-Release Assay for the Diagnosis of Mycobacterium tuberculosis Infection in Children

Data derived analyzed from 237 patients
With proven active TBC or suspected latent TB

875 results of all QFT assays from children attending the Royal Children’s Hospital Melbourne over a 5-year period (November 2003 to November 2008) With overall 16% indeterminate results
Development of the respiratory mucosal immune system and role of the microbiome

Harald Renz et al
Nature Immun. review 2012
TLR9-Notch ligand (delta like 4), and IL-17 for *Mycobacterium-dependent* granuloma

In the absence of TLR-9 and of IL-17 they are less DCs and effector Tcells, but more macrophages in granuloma with poor control of Mtb.
Essential Role of IL-17A in the Formation of a Mycobacterial Infection-Induced Granuloma in the Lung

A

\[ M. \textit{tuberculosis} \text{ infection} \]

\( P = 0.022 \)

B

WT

IL-17A KO

NB: IL-17 was mostly from Gamma delta T cells!
IL-17 Increased chemokines and adherence molecules

Yoshida et al. Journal of Immunology, 2010, 184: 4414
# Lymphocytes and cytotoxicity pathways

## Cytotoxicity Pathways

<table>
<thead>
<tr>
<th>Cell types</th>
<th>Granules exocytosis</th>
<th>Fas</th>
<th>TNFs</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD8 CTL</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CD4 Th1 Cells</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CD4 Th2 Cells</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>NK Cells</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

Granules contain:
- Cytolisin / Perforin
- Granzymes (granule serine proteases)
Granulysin and lesions induced in Mycobacteria.
Perforins and holes produced in membranes
Defence mechanisms against M. Tuberculosis

1) Bactericidy in monocytes / macrophages

2) Sensitisation and activation of cytotoxic lymphocytes CD8 ou CD4(Th1; Th17) by dendritic cells

3) (Necrosis) / Apoptosis of infected macrophages by
   - Fas – Fas ligand / TNF
   - by the cytotoxicity of lymphocytes
THE TWO PATHWAYS OF LYMPHOCYTE-MEDIATED CYTOTOXICITY

Activated T cells

Killing of infected Macrophages: Apoptosis/necrosis

**TNF in innate and adaptive immunity**

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>T Cells</td>
<td>➤ T Cell proliferation</td>
</tr>
<tr>
<td></td>
<td>➤ IL-2 receptor</td>
</tr>
<tr>
<td>Macrophages</td>
<td>➤ IL-1 : ➤ PAF ...</td>
</tr>
<tr>
<td></td>
<td>➤ ( O_2^- ) (synergy with : IL-2 ; INF(\gamma))</td>
</tr>
<tr>
<td></td>
<td>➤ bactericidy (Mo human)</td>
</tr>
<tr>
<td>Dendritic Cells</td>
<td>➤ maturation</td>
</tr>
<tr>
<td>B Cells</td>
<td>proliferation ; differentiation</td>
</tr>
<tr>
<td>NK Cells</td>
<td>➤ Cytotoxicity</td>
</tr>
</tbody>
</table>

**NB**: Blocking TNF prevents granuloma formation.

No direct effect on M. Tuberculosis

Differential effect of total and partial neutralization of TNF on cell mediated immunity to M. Bovis BCG infections

Control

High sTNF R prevent Granuloma formation and low sTNFR increase (loose but efficient) Granuloma formation!

R. Guler et al, Infection and immunity 2005; 3668
Genetic susceptibility leading to active tuberculosis

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interferon γ receptor 1</td>
<td>Severe atypical mycobacterial infection</td>
</tr>
<tr>
<td>Point mutation at nucleotide 395, which introduces a stop codon</td>
<td></td>
</tr>
<tr>
<td>Interferon γ receptor 2</td>
<td>Infection of <em>M. fortuitum</em> and <em>M. avium</em></td>
</tr>
<tr>
<td>Homozygous dinucleotide deletion at nucleotides 278 and 279, resulting in a premature stop codon</td>
<td></td>
</tr>
<tr>
<td>Interleukin 12p40</td>
<td>BCG infection</td>
</tr>
<tr>
<td>Large homozygous deletion</td>
<td></td>
</tr>
<tr>
<td>Interleukin 12β1 receptor subunit</td>
<td>Severe mycobacterial and salmonella infections</td>
</tr>
<tr>
<td>Various missense mutations and deletions</td>
<td></td>
</tr>
<tr>
<td>STAT1</td>
<td>Disseminated BCG or <em>M. avium</em> infection</td>
</tr>
<tr>
<td>Point mutation at nucleotide 2116</td>
<td></td>
</tr>
</tbody>
</table>

STAT1 = signal transducer and activator of transcription 1. BCG = Bacille Calmette Guérin.
Facteurs immuns à considérer dans la réactivation de la tuberculose

VIH
Immunosuppresseurs
(infliximab, …)

-  

Primo-infection  
Défenses de l’hôte  
Variables selon l’âge et la génétique  
(polymorphismes IFN.R1-2; IL-12 βR1; …)

+  

Nouveaux vaccins  
(MVA85A, …)

Immunostimulants :
IFN-γ, …

+  

Génétique des mycobactéries  
(virulence, résistances, …)

Mycobactéries  
Maladie active  
LP Nicod  SMW 2007;137:357
Conclusions

Defences against tuberculosis depend on:
1) Innate and adaptive immunity of the host influenced by:
   - host genetics
   - age of the subject with a Th2 bias in young children
   - nutritional status
   - concomitant infections or tumors
   - immunomodulating treatments
2) Virulence factors of the mycobacteria

All these points need to be kept in mind for the clinical presentations and for the diagnostic tools used