Immunomodulation in Osteoarticular Tuberculosis

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Of all “Individuals Infected”
Only 5% develop Clinical Disease:
another 5% develop Post primary TB
( American Thoracic Society, 1990 )

Rest 90-95% go on to contain the infection and
develop Delayed Type Hypersensitivity.
Montoux +ve
Ever Wondered?

Why 90% individuals infected with Mycobacterium Tuberculosis............

“Do not” develop “Clinical Disease”?

Before ATT drugs were available..........

“30%-50% patients of TB made Spontaneous Recovery”? 
Immunity
Immunity against Mycobacterium Tub.

↓

Cell Mediated

• Macrophages and Lymphocytes play a major role.
Lymphocytes are of two types - T (Thymic) and B Lymphocytes. T lymphocytes play a main role.
Formation of CD4 cells from T lymphocytes
CD4 and CD8 T-Lymphocytes

Central mediators of this cellular Immunity.

(Janeway 1992, Kaufman 1993)

The Mean number of CD4 cells in healthy volunteers has been found to be 968 cells cu/mm of blood.

(Tripathi et al 2000)
Can we harness this “Force” and utilise it to our benefit in treating this disease?
Clinical and serological studies of tuberculosis patients in Argentina receiving immunotherapy with *Mycobacterium vaccae* (SRL 172)

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Two small, placebo-controlled studies of immunotherapy with heat killed *Mycobacterium vaccae* added to routine chemotherapy for pulmonary tuberculosis, together involving 40 HIV seronegative patients, were carried out in Argentina. The immunotherapy was associated with reduced sputum smear positivity of AFB at 1 month and a
<table>
<thead>
<tr>
<th></th>
<th>Immunotherapy (n = 34)</th>
<th>Placebo (n = 47)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mortality</strong></td>
<td>0 / 34 (0%)</td>
<td>19 / 47 (40%)</td>
<td>P&lt;0.00001</td>
</tr>
<tr>
<td><strong>Mean increase in body weight</strong></td>
<td>7.91 kg (n = 33)</td>
<td>2.04 kg (n = 26)</td>
<td>P&lt;0.003</td>
</tr>
<tr>
<td><strong>Mean fall in ESR</strong></td>
<td>42 mm (n = 33)</td>
<td>15 mm (n = 26)</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td><strong>Sputum still positive for AFB</strong></td>
<td>11 / 33 (33%)</td>
<td>22 / 26 (85%)</td>
<td>P&lt;0.00002</td>
</tr>
</tbody>
</table>
The Adjunctive Immunotherapy

Immunotherapy of Lepromin-Negative Borderline Leprosy Patients with Low-Dose Convit Vaccine as an Adjunct to Multidrug Therapy; a Six-Year Follow-Up Study in Calcutta

LEVAMISOLE AS ADJUNCT TO DAPSONE IN LEPROSY

Str,—Levamisole has a stimulatory effect on some aspects of the cellular immunity mechanism of the host. The mechanism of this effect is not well understood, but it is thought to involve enhancement of T-cell function and inhibition of macrophage activation.

Adjuvant Modulation of Immune Responses to Tuberculosis Subunit Vaccines
Modalities of adjunctive immunotherapy

• Cytokines (especially Th-1 and Th-1-like cytokines such as IFN-gamma, IL-2, IL-12, IL-18)

• Inhibitors of
  – immunosuppressive cytokines (TGF-beta)
  – proinflammatory tissue-damaging cytokines (TNF-alpha), and

• Immunomodulatory agents such as ATP and its analogs.
Adjunctive Immunotherapy…

- Levamisole
- Heat-killed Mycobacterium vaccae
- IL-12
- L-arginine
- Vitamin D
- Chinese traditional medicines
- Synthesized mycobacterial oligoDNA
- DNA vaccine expressing mycobacterial HSP65
- Imidazoquinoline, diethyldithiocarbamate, poloxamer, dibenzopyran, ………….
Non Responsiveness

• Nonresponsiveness to standard chemotherapy $\approx 5\text{-}10\%$

• IIInd line ATT drugs too toxic.

• Focus now shifting to “Immunepotentiation”

  Improve the Immunity........
Our Experience
Iliac bone OM
18 Yr Female
H/o 8 mo.

ATT......
• Pain
• Constitutional symptoms
• Sinus Discharge

Continued for 4 months..

CD4 Count - 453/cu mm

Immunotherapy
1 month after Immunotherapy

CD4 Count - 1710/cu mm
Radiological Healing at 18 months
• 6 mo F/U Pain
• Constitutional symptoms
• Destruction continued

Immunotherapy

718/cu mm
After 3 months......

1134/ cu mm
After 4 months of treatment, immunotherapy achieved a white blood cell count of 670/cu mm.
The Immunotherapy Regime

1. **Tab. Levamisole** - 2 mg/kg/day for 3 days, followed by an interval of 7 days, >>>>>>> 6 such cycles repeated.

2. **Inj. BCG** - 0.1 ml intradermal at the start…

3. **Inj. DT…** - 0.5 ml Intramuscular after one month of Inj. BCG.

4. **Inj. BCG** – again, one month after DT.
Material and Methods

61 patients suffering from Osteoarticular TB......

Group I
41 cases
Virgin Fresh

Group II
20 cases
Non-Responders
“Fresh Patients”

Recently diagnosed, and were to be started on antitubercular therapy.
Group I (Virgin Fresh Cases)

Group I: 41 cases of freshly diagnosed Osteoarticular Tuberculosis...

- CD4 and CD8 cell count
- Haemogram with ESR
- Blood Sugar Estimation
- Serum Proteins
- X-Ray of Chest and Affected part

→ ATT and indicated orthopaedic management
Fresh - First Line ATT

- INH
- Rifampicin
- Ethambutol
- Pyrazinamide
Group I (Virgin Fresh Cases)

After 3 months of Treatment

- Clinic radiological Response documented
- All Investigations Repeated

Pretreatment CD4 and CD8 cell counts were compared with counts after 3 months of treatment
“Non Responders”

- Patients of OA-TB already on uninterrupted ATT for a minimum of 3 months and......

  - Not showing clinicoradiologic response or
  - Showing deterioration of disease or
  - Appearance of fresh lesion(s).

- Patients showing recurrence of a lesion, which had previously healed under the influence of ATT.
Group II (Non Responders)

Investigations done

• CD4 and CD8 cell counts
• Haemogram with ESR
• S. Protein
• Blood Sugar Estimation
• X-Ray chest (PA)
• ELISA for HIV
Group II (Non Responders)

**Immunotherapy** added as an adjunct to ATT drugs in an attempt to improve Immune Status of these patients

- Indicated Orthopaedic Management
Group II (Non Responders)

- CD4 and CD8 cell counts along with other relevant investigations repeated after 3 months of initiation of immunomotherapy and compared.
Results (Group II – Non Responders)

- 16/20 patients responded
- Constitutional symptoms resolved in 8 wks.
- Sinuses healed in 7/8 patients.
- No HIV+ve (no+ve history too) or diabetic
### Results (Group I – Virgin Fresh Cases)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>At Start of Therapy</th>
<th>At Three Month therapy</th>
<th>‘p’ value (paired ‘t’ test)</th>
<th>Significance (p&lt;0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>S.D.</td>
<td>Mean</td>
<td>S.D.</td>
</tr>
<tr>
<td>Total Lymphocyte count/cu mm</td>
<td>2607</td>
<td>±126</td>
<td>2245</td>
<td>±750</td>
</tr>
<tr>
<td>CD 4 Cells /cu mm</td>
<td>803</td>
<td>±426</td>
<td>1174</td>
<td>±460</td>
</tr>
<tr>
<td>CD 8 Cells /cu mm</td>
<td>850</td>
<td>±414</td>
<td>802</td>
<td>±272</td>
</tr>
<tr>
<td>CD4:CD8 Ratio</td>
<td>.9633</td>
<td>±.271</td>
<td>1.47</td>
<td>±.327</td>
</tr>
</tbody>
</table>
## Results (Group II—Non Responders)

<table>
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<tr>
<th>Parameter</th>
<th>At Start of Therapy</th>
<th>At Three Month therapy</th>
<th>‘p’ value (paired ‘t’ test)</th>
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<td>S.D.</td>
<td>Mean</td>
<td>S.D.</td>
</tr>
<tr>
<td>Total Lymphocyte count/cu mm</td>
<td>2078</td>
<td>±650</td>
<td>1829</td>
<td>±614</td>
</tr>
<tr>
<td>CD 4 Cells /cu mm</td>
<td>630</td>
<td>±288</td>
<td>969</td>
<td>±348</td>
</tr>
<tr>
<td>CD 8 Cells /cu mm</td>
<td>496</td>
<td>±181</td>
<td>491</td>
<td>±200</td>
</tr>
<tr>
<td>CD4:CD8 Ratio</td>
<td>1.27</td>
<td>±.274</td>
<td>2.10</td>
<td>±.693</td>
</tr>
</tbody>
</table>
The use of immunomodulators as an adjunct to antituberculous chemotherapy in non-responsive patients with osteo-articular tuberculosis

We studied 51 patients with osteo-articular tuberculosis who were divided into two groups. Group I comprised 31 newly-diagnosed patients who were given first-line antituberculous treatment consisting of isoniazid, rifampicin, ethambutol and pyrazinamide. Group II (non-responders) consisted of 20 patients with a history of clinical non-responsiveness to supervised uninterrupted antituberculous treatment for a minimum of three months or a recurrence of a previous lesion which on clinical observation had healed. No patient in either group was HIV-positive. Group II were treated with an immunomodulation regime of intradermal BCG, oral levamisole and intramuscular diphtheria and tetanus vaccines as an adjunct for eight weeks in addition to antituberculous treatment. We gave antituberculous treatment for a total of 12 to 18 months in both groups and they were followed up for a mean of 30.2 months (24 to 49). A series of 20 healthy blood donors served as a control group.

Twenty-nine (93.6%) of the 31 patients in group I and 14 of the 20 (70%) in group II had a clinicoradiological healing response to treatment by five months.

The CD4 cell count in both groups was depressed at the time of enrolment, with a greater degree of depression in the group-II patients (686 cells/mm$^3$ (SD 261) and 545 cells/mm$^3$ (SD 137), respectively; p < 0.05). After treatment for three months both groups showed significant elevation of the CD4 cell count, reaching a level comparable with the control
**Immunotherapy**

- If immunotherapy is so beneficial, then why not to give it to all fresh cases of osteoarticular Tuberculosis and...

- Start Immunomodulation at the time of starting Antitubercular treatment, instead of waiting for nonresponsiveness and then giving Immunotherapy.
…..and if we start Immunotherapy from very beginning, along with ATT, will there be an actual benefit or Immunotherapy works only for nonresponders (who might be having some correctible cause/ deficiency) by correcting that cause (Paracetamol for fever)
Two categories of 42 patients of Osteoarticular Tuberculosis

30 Fresh Patients...

12 Non responder patients
30 “Fresh Cases”

15 patients
ATT alone
“CONTROLS”

15 patients
ATT
plus…
Immunotherapy
from the
beginning of
treatment.

In 12 Nonresponders

Immunotherapy
was added to
ATT
42 patients in Three groups

- Interleukin levels in peripheral blood were measured at different stages.

- The clinical picture was compared with change in Interleukin Profile.

- ATT was continued for 12-18 months.
9 months on ATT

PCR – Typical Mycobacterium Tuberculosis
ATT for 18 months
26 MONTHS LATER..........

PCR – Typical Mycobacterium Tuberculosis
Immunotherapy + ATT
Immunotherapy added to ATT
Immunotherapy added to ATT
Response to Therapy

- 29 out of 30 Fresh patients responded.
- 10 out of 12 Nonresponder patients responded to addition of Immunotherapy to ATT.
Mean Interleukinin Levels (pg/ml)

- At Presentation
- After Treatment
Interleukin-1: Helps controlling disease
Interleukin-2: **Prime** IL for Immunity

ATT alone | ATT + Immod | Non Responder
---|---|---
10.6 | 8.2 | 5.4
55.4 | 74.1 | 131.0
5X | 9X | 24X
Interleukin-10: Inhibit Immune response

ATT alone

ATT + Immod

Non Responder
IL - 6
IL -12
IL -18
Inflammatory Cytokines
Interleukin-18: Inflammatory Cytokine

ATT alone

ATT + Immod

Non Responder
Tumor Necrosis Factor: “Cachexin”

ATT alone

ATT + Immod

Non Responder
Interleukin-6: Inflammatory Cytokine
Interleukin-12: Inflammatory Cytokine

- ATT alone: 346.1
- ATT + Immod: 482.8
- Non Responder: 561.0
Inferences from this study

• Improvement in clinical picture = Improvement in Beneficial Interleukins and reduction in Inhibitory ILs.

• Addition of Immunotherapy to ATT induces manifold improvement in interleukin profile as compared to ATT alone.

• Proposed Immunotherapy does improve the immunological status as manifested by clinical and interleukin response in Fresh cases and Non responders.
Cytokine profiles of HIV patients with pulmonary tuberculosis resulting from adjunct immunotherapy with herbal phytoconcentrates Dzherelo and Anemin

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b Ekomed LLC, 80-A Prospect Pravdy, Kiev 04208, Ukraine
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d Luhansk Regional AIDS Center and Luhansk State Medical University, 50-Years of Defense of Luhansk Street, Luhansk 91045, Ukraine
Effect of ATT alone or ATT + Dzherelo or ATT + Dzherelo + Anemin on cytokine production in TB/HIV patients shown in percentage values relative to baseline levels.
**Immunotherapy**

**BCG**

- Induces significant increase in Mycobacterial specific T-cell proliferative response (*Lowry et al 1998, Hoft et al-double blind placebo controlled study-1999*)

- BCG is capable of inducing beneficial TH1 type immunological response in standard or high doses (*Ishibashi et al 1998*)

- Stimulates natural killer cells.

- Generalised systemic activation of lymphoid tissue and long lasting effect of immunepotentiation (*Ishibashi et al 1978*)
**Immunotherapy**

**DPT**

- Causes non specific augmentation of immune status by stimulating Reticuloendothelial System. *(Tuli 1999)*
Immunotherapy

Levamisole

- Increases T-Cell immunity and delayed hypersensitivity (Diasio and Obuglio 1997)

- Has been used in – Leprosy with Dapsone
  - Hodgkins Disease
  - Colorectal Carcinoma
  - Rheumatoid Arthritis

- No side effect related to Levamisole noted
Immunity and Tuberculosis

Pathogen  Host Resistance
Immunity and Tuberculosis

Pathogen

Host Resistance

CLINICAL DISEASE
Pathogen

Immuno therapy

Host Resistance
Pathogen

Immunotherapy

Host Resistance

DISEASE CONTROL
Long Duration - Drug Defaulters

- After starting ATT - Extracellular Mycobacteria are killed within few weeks of chemotherapy
- Intracellular persistors – need prolonged therapy
- Once extracellular bacteria killed symptoms rapidly improve

“Drug Defaulters” main cause multidrug resistant mycobacteria in the community.
• Helping in treatment of “CLINICALLY” Nonresponder Cases.

• Reducing the Total duration of Antitubercular Chemotherapy...
  >> Less of “Drug Defaulters”
  >> Less Resistant Cases
Thank you