Lymphocyte transformation test – a practical approach - short

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When skin and in vitro tests?

- **Not in acute stage:**
  - IgE: mast cells degranulated: effect of acute treatments
  - T-cells: T cells still activated, effect of treatments

- **Better in remission**
  - IgE: >1 week and within 6 (-12) months
  - T cells: > 4 weeks after event, best within 6 - 12 months; it is possible later !

- **Persistence:** variable: 1yrs to >20yrs
LTT-procedure I

Anti-coagulated blood (heparin, EDTA, citrate)

Send at room temperature, max. 24 hrs, processing <36hrs = lab needs to be informed

Cell separation - ca. $30 \times 10^6$ lymphocytes from 30ml blood

Culture with drug: pure drug substance (Sigma, companies,... = powder solved in NaCl, PBS, DMSO, NaOH... , injection fluid)

NOT tablets or sirups or suspensions...
Delayed reaction

1 2 3 4 5 6 7 8 9 10 days

No symptoms

Few precursor cells

... Expansion....

Symptoms arise if a certain amount of specific T-cells exerts effector function (in tissue): homing
**LTT procedure II**

2x10^5 lymphocytes / ml in AB serum or autologous plasma

Quadruplicate cultures

3-5 drug concentrations
often 0.1 -1 -10 -100mg/ml

c. 150 pure substances:
1. Each drug concentration tested for toxicity in vitro
2. Lack of spontaneously inducing proliferation
Stimulation Index (SI)

- No (moderate, spontaneous) proliferation in control cultures (without antigen): background control
- Enhanced proliferation in positive control (TT)
- \[ SI: \frac{\text{cpm with drug}}{\text{cpm without drug}} \]

- \[ SI: \frac{8000 \text{ cpm}}{4000 \text{ cpm}} = \frac{\Delta = 4000 \text{ cpm}}{\text{or} \frac{1000 \text{ cpm}}{500 \text{ cpm}}; \Delta = 500 \text{ cpm}} \]
Positive LTT for Phenytoin and Lamotrigine (DRESS) dose dependence, reaction in both, AB serum and autolog. plasma

But Pantoprazole: ???   INTERPRETATION NEEDED
LTT – technical aspects

Fresh heparinized (citrated, EDTA) blood, Moon EXPRESS (< 24-36hrs)

LTT in AB-Serum and autologous Plasma (may differ)

Dosis-urve (1, 10, 100µg/ml)

Interpretation: the better, the more information calling, e-mail....
When is a LTT positive?

**Easy:**
SI >4 (mostly relevant...)
clear dose dependence

**Difficult:**
SI >2-4: in more than one concentration,
in both AB-serum and autol. plasma

Consider: quadruplicate values (CV)
positive control (TT)
background proliferation
experience with drug in LTT

It requires careful interpretation! The more data are available, the better the interpretation
SI reflects the intensity of T cell proliferation, \textit{but}

- The SI does not correlate to severity of symptoms
- SI may be marginal in fatal SJS/TEN, but very high in «harmless» MPE
- Type of drug, way of stimulation (p-i vs hapten) may also influence T cell proliferation
### Sensitivity & Specificity of the lymphocyte transformation test (LTT)

<table>
<thead>
<tr>
<th>Author</th>
<th>n</th>
<th>sensitivity</th>
<th>specificity</th>
<th>disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nyfeler &amp; Pichler, 1997</td>
<td>100</td>
<td>74</td>
<td>85*</td>
<td>All</td>
</tr>
<tr>
<td>Luque E et al., 2001</td>
<td>50</td>
<td>62</td>
<td>93</td>
<td>Penicillins (imm. &amp; non-immediate)</td>
</tr>
<tr>
<td>Hari Y et al, 2001</td>
<td>21</td>
<td>67</td>
<td>98</td>
<td>MPE &amp; bullous E.</td>
</tr>
<tr>
<td>Naisbitt D et al, 2003</td>
<td>36</td>
<td>94</td>
<td>100</td>
<td>DRESS/ DiHS</td>
</tr>
</tbody>
</table>

The sensitivity of LTT is dependent on the type of reaction!!
Specificity of LTT

- Excellent 85, 93, 95, 98 & 100% = ca. 95%

- Unspecific expansion of cells in vitro to drugs is rare and limited to a few drugs only (paracetamol, vancomycin, .... )

- penicillin G causes a moderate increase in some donors (cut off SI 2,5 – 3) cut off SI=3

If you have a positive LTT, it is meaningful !!
The **specificity** of LTT is acceptable

the **sensitivity** is 60-70% in studies*

*) ca. 10-20% of LTT analysed in routine lab are positive
max. 50% if drug allergy considered highly likely....

A negative result does **not** rule an drug hypersensitivity
1 2 3 4 5 6 7 8 9 10 days
No symptoms
precursor cells proliferation 2,5fold
but strong cytotoxicity

1 2 3 4 5 6 7 8 9 10 days
No symptoms
precursor cells proliferation 5fold

1 2 3 4 5 6 7 8 9 10 days
No symptoms
precursor cells proliferation 2,5fold
but strong cytotoxicity

SI: 5
Moderate Exanthema

SI: 2,0
Dangerous bullous skin reactions
**In vitro** drug causality assessment in Stevens-Johnson syndrome – alternatives for lymphocyte transformation test.

*G Porebski et al, CEA 2013*

- 15 patients with SJS/TEN (ALDEN score ≥6)
- 18 drug-exposed controls

<table>
<thead>
<tr>
<th>Assay</th>
<th>Positive</th>
<th>Sensitivity</th>
<th>CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>LTT</td>
<td>4/15</td>
<td>27%,</td>
<td>8-55%</td>
</tr>
<tr>
<td>granzyme B-ELISpot</td>
<td>5/15</td>
<td>33%,</td>
<td>12-62%</td>
</tr>
<tr>
<td>granulysin (NKp46⁺)</td>
<td>6/15</td>
<td>40%,</td>
<td>16-68%</td>
</tr>
<tr>
<td>granulysin (CD3⁺CD4⁺)</td>
<td>8/15</td>
<td>53%,</td>
<td>27-79%</td>
</tr>
<tr>
<td>IL-2 + IL-5</td>
<td>6/14</td>
<td>43%,</td>
<td>18-71%</td>
</tr>
<tr>
<td>IFNγ</td>
<td>6/11</td>
<td>55%,</td>
<td>23-83%</td>
</tr>
<tr>
<td>IFNg, GranzB, GrlyCD4</td>
<td>12/15</td>
<td>80%,</td>
<td>52-96%</td>
</tr>
</tbody>
</table>

Specificities of tested assays were in the range of 95 (CI:80-99%)-100% (CI:90-100%).
Punktescore zur Bewertung der LTT Resultate, ohne Berücksichtigung von Zusatzinformationen

**Bewertung SI:**
- < 2: keine Sensibilisierung nachgewiesen
- 2-3: Sensibilisierung möglich
- >2,5-4: schwache Sensibilisierung
- > 4: Sensibilisierung sehr wahrscheinlich (falls mehr als ein Wert)

| Schwankungen der Quadruplikate: > 50% | -1 /betroffenes Quadruplikat |
| „Ausreisser“: nur ein Wert hoch (meist Aggregate?) | -1 |
| Werte mit SI > 2.5 nur im autologen Serum oder AB Serum | -1 |
| SI Wert >2.5 (>3 bei Penizillin) | +1/Wert |
| SI Wert > 4 | +2/Wert |
| Dosisabhängigkeit über mindestens 2 Dosen | 4 |
Common mistakes

- Drug mixtures: cough sirup, mixtures....
- too many drugs
- wrong ideas: an in vitro proliferation assay is not suitable to explain pruritus to a drug headache, diarrhoe,.....
- Macular exanthema, rash, delayed urticaria, minimal exanthema...: not enough T cells in circulation
- Tablets: doubtful results ...
Value of LTT with different diseases

**often positive:**
- DHS/DRESS (35/36 = 97%)
- generalised maculo-papular exanthema
- bullous exanthema
- AGEP
- anaphylaxis (IgE-mediated, generalised, severe symptoms)+

**occasionally positive:**
- hepatitis*
- pancreatitis°
- nephritis (dependent on type of drug)
- interstitial lung disease°
- urticaria, angioedema+
- SJS/TEN

°) seldom examined
*) strongly dependent on drug
+) positive in IgE-penicillins
Value of LTT with different diseases

seldom or never positive (<10% or less):

• Vasculitis°
• macular exanthema (without cell infiltration)
• Guillain-Barré°
• blood diseases as ITP°, haemolytic anaemia°
• fixed drug exanthema

°) seldom examined
The main caused for a negative LTT in spite of very likely sensitization is

- Disease became clinically manifest due to cofactors
- It was **no** strong T cell reaction
  - No more detectable in peripheral blood cells
  - (But may remain detectable in skin (homing))
- It was more a cytotoxic reaction and thus LTT / proliferation was not the correct choice (see example SJS or abacavir)
Suitable drugs for LTT

- **antibiotics**: b-lactams, quinolones, macrolids, sulfonamides, tetracycline, vancomycin+
- **antiepileptics**: phenytoin, carbamazepin, lamotrigine, gabapentin
- **ACE-inhibitors**: enalapril, ...
- **antituberculous drugs**: isoniazid, rifampicin
- **diuretics**: hydrochlorothiazide, furosemide, indapamid, ...
- **NSAID** (Cox 1 and Cox 2 inhibitors): diclofenac, celecoxib, mefenaminic acid,
- **pyrazolones**: propyphenazone
- **HMG-CoA-reductase inhibitors**: acrivastatin
- **morphin-derivatives**: pethidin, codein, ...
- **radio-contrast media+**: iohexol, iopamiro, ...
- **muscle relaxants**: suxamethoniumchlorid, ...
- **vitamins**: cyancobalamin (Vit. B12), folic acid, ...
- **contact allergens**: p-phenylendiamine
- **Metals**: NiCl₂, CrSO₄
- **varia**: .....
lymphocyte transformation test

+ in vitro, not dangerous
positive result
meaningful as good
specificity
sensitivity +/-
cross-reactivity
research tool

- laborious, time consuming
complicated logistic,
need of fresh cells
sensitivity +/-

(limited availability,
radioactivity, expensive)