TCE and T Cell Activation

Neil R. Pumford, Ph.D.
University of Arkansas
Kathleen G. Gilbert, Ph.D.
University of Arkansas for Medical Sciences
Autoimmunity

• Loss of self-tolerance that results in immune reactions against one’s own or self antigens.

• Etiology appears to multifactorial:
  – Genetic Factors
  – Environmental Factors
    • Chemicals
    • Microbial infections
Autoimmunity

- Over 80 separate autoimmune diseases including:
  - Hashimoto’s thyroiditis
  - Type 1 diabetes mellitus
  - Rheumatoid arthritis
  - Multiple sclerosis
  - Systemic sclerosis (scleroderma)
  - Systemic lupus erythematosus
Autoimmunity

• Autoimmune diseases effects over 9 million Americans (1 in 5)
Trichloroethylene

? Does trichloroethylene cause or exacerbate an autoimmune response?

- Mechanism?

Autoimmune Disease
Inflammation and Fibrosis
Trichloroethylene Case Reports

- Over 100 case reports associating trichloroethylene with autoimmune diseases
- Systemic sclerosis (scleroderma) and systemic lupus erythematosus
Trichloroethylene and Autoimmune Disease

• Byers et al., 1988
  – Altered ratios of T lymphocyte subpopulations
  – Increased incidence of auto-antibodies

• Kilburn and Warshaw, 1992
  – Associated with lupus erythematosus and ANA

• Clark et al., 1994
  – Association of perceived exposure to solvents including trichloroethylene with ANA
Trichloroethylene and Autoimmune Disease

• Nietert et al., 1998
  – Occupational exposure was associated with an increased risk of systemic sclerosis

• Garabrant et al., 2003
  – Associated with systemic sclerosis but not significant
Experimental Design

Autoimmune-Prone MRL+/+ Mice

Trichloroethylene (TCE) in drinking water

Serum
Spleen & lymph nodes
Liver & lungs

Toxicity
Autoimmune markers

T-cell Activation
Cytokines (IFNγ & IL-4)

Metabolic Activation
Markers of Oxidative stress
Low-Dose Chronic Study

Trichloroethylene Levels in the Drinking Water (mg/ml)
Immunohistochemistry for Trichloroethylene-Protein Adducts

Control

Trichloroethylene
Antinuclear Antibodies

4 Weeks

Relative Absorbance

Trichloroethylene (mg/ml)

0 0.1 0.5 2.5

0.35

0.3

0.25

0.2

0.15

0.1

0.05

0
Activation of CD4+ T Cells

- CD44 expression on CD4+ T cells has been used to monitor the transition from naïve to effector state.

- CD45RB is often used as a second marker of T cell activation.
Expression of CD44 and CD45RB from Mice Treated for 4 Weeks with Trichloroethylene

[Graph showing the expression levels of CD44 and CD45RB in control and TCE 2.5 treated samples.]
CD4$^+$ T Cell Activation in Spleens following treatment with Trichloroethylene

<table>
<thead>
<tr>
<th></th>
<th>CD44$^{hi}$</th>
<th>CD45RB$^{low}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% Total Cells</td>
<td>% Total Cells</td>
</tr>
<tr>
<td>Control</td>
<td>39</td>
<td>58</td>
</tr>
<tr>
<td>0.1 TCE</td>
<td>39</td>
<td>59</td>
</tr>
<tr>
<td>0.5 TCE</td>
<td>44</td>
<td>64</td>
</tr>
<tr>
<td>2.5 TCE</td>
<td>53</td>
<td>69</td>
</tr>
</tbody>
</table>
CD4⁺ T Cell Maturation

Naïve CD4⁺ T Cell

PreTH

TH₁

TH₂

IFN-γ

Inflammatory

IL-4

Humoral
Interferon-\(\gamma\) Levels in MRL\(+/+\) Mice
(4 weeks)
IL-4 Levels in MRL+/- Mice (4 weeks)

Trichloroethylene (mg/ml)

IL-4 (ng/ml)
Cytokine Levels
(32 weeks)

IFN-γ (ng/ml)

IL-4 (ng/ml)

Trichloroethylene (mg/ml)

**Legend:**
- *: Statistical significance
Toxicity Testing

• Mild, but significant increase in serum ALT levels indicating hepatic damage.
Liver Histology

Control

TCE
Liver Histopathological Score

Portal Inflammation

Trichloroethylene (mg/ml)

0 0.1 0.5 2.5
Liver Histopathological Score

Reactive Changes

Trichloroethylene (mg/ml)

0 0.1 0.5 2.5

0 0.4 0.8 1.2 1.6
Mechanism of Trichloroethylene-Induced Autoimmunity

- Trichloroethylene
- Chloroacetylated-Protein
- Oxidative Stress
- Metabolite(s)
- Activation of T cells
  - Th1 Cytokines
    - Indirect Factors
      - Cytokines
      - Endothelial Cell Injury
    - Cytokines
    - ROS
    - RNS
    - Activation of Fibroblast
    - Inflammation and Autoimmune Hepatitis (No Fibrosis)
- B cells
  - Anti-Chemical or Autoantibodies
  - Activation of Macrophages
    - Cytokines
Western Blot of CYP2E1 Following TCE Treatment of MRL+/-+ Mice

![Western Blot Image]

**Treatment Groups:**
- Control
- Diallyl Sulfide
- TCE
- TCE+ Diallyl Sulfide

**Relative Intensity (ADU):**
- 50 kDa

Bars labeled with:
- a
- b
- c

End of Document
Liver Trichloroethylene Adducts

Treatment Groups

- Control
- TCE
- Diallyl Sulfide
- TCE+ Diallyl Sulfide

Molecular Weights:
- 200 kDa
- 116 kDa
- 98 kDa
- 66 kDa
- 44 kDa
CD4+ T Cells Mitogenic Response to Con A

Counts Per Minute

Treatment Groups

Control | Diallyl Sulfide | TCE | TCE+ Diallyl Sulfide

a

b

Legend:
- Control
- Diallyl Sulfide
- TCE
- TCE+ Diallyl Sulfide
T Cell Activation by Forming a Schiff-Base

Antigen-Presenting Cell

B7

MHC Class II

B7

CD28

CD28 T Cell Receptor

T Cell
T Cell Activation by Forming a Schiff-Base

Antigen-Presenting Cell

B7
MHC Class II

CD28

T Cell

Costimulatory Signal

B7 → CD28

NH₂

C = N

H
Trichloroethylene Metabolite Activation of T Cells by Forming a Schiff-Base

TCE

P-450

Trichloroacetaldehyde (Chloral)

Trichloroacetaldehyde Hydrate (TCAH) (Chloral hydrate)

Antigen-Presenting Cell

B7

MHC Class II

CD28

T Cell Receptor

CD28

Costimulatory Signal
Trichloroacetaldehyde Binding to Th1 Cell Surface Protein

Gilbert et al., Int. Immunopharmacol., 4, 2004
Trichloroacetaldehyde Hydrate (TCAH) Costimulates T-Cell Proliferation \textit{in vitro} (0.01 $\mu$g/ml anti-CD3)

Gilbert et al., Int. Immunopharmacol., 4, 2004
TCAH Stimulates Th1 Cells Activation

No Anti-CD3

2.8%

Anti-CD3

26.3

No TCAH

TCAH (1 mM)

CD28

Forward scatter

Gilbert et al., Int. Immunopharmacol., 4, 2004
Trichloroacetaldehyde Hydrate (TCAH) Stimulates Activation (CD62Llo) in CD4⁺ T Cells

Gilbert et al., Int. Immunopharmacol., 4, 2004
Experimental Design

Autoimmune-Prone MRL+/+ Mice

Trichloroacetaldehyde Hydrate

Serum
Spleen & lymph nodes
Liver & lungs

Toxicity
Autoimmune markers

T-cell Activation
Cytokines (IFNγ & IL-4)

Metabolic Activation
Markers of Oxidative stress
Expression of CD62L^{lo} on T cells
(MRL+/+ mice 4 weeks \textit{in vivo} treatment)
T cells Cytokine Secretion
(MRL+/+ mice 4 weeks *in vivo* treatment)

**INF-γ**

- 0
- 0.1
- 0.9

**IL-4**

- 0
- 0.1
- 0.9

Trichloroacetaldehyde Hydrate mg/ml
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References


