Vitamin D and autoimmune disease

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Cytokines that regulate Th cell differentiation.

- **Th0**: NKT CD4+, VDR
  - IL-2, IL-4, IFN-γ
  - TGF-β1

- **Th17**: IL-17
  - IL-6, IL-12, IFN-γ, TGF-β1

- **Th1**: IL-12
  - IFN-γ, IL-4, TGF-β1

- **Th3/Treg**: CD25+, CD45RBlow
  - IL-2, TGF-β1, IL-10

- **Th2**: IL-4
  - IL-4, IFN-γ, IL-10

VDR VDR

**Self-tolerance**: CD25+, CD45RBlow TGF-β1, IL10

**Bacterial immunity, asthma and allergies**: IL-4, IL-5, IL-10

**Autoimmune disease**: Cytokines secreted
- IFN-γ
- IL-2
- TNF-α

**Viral/TB immunity, cancer immunity**

1,25D3

**Self-tolerance**: CD25+, CD45RBlow TGF-β1, IL10

**Bacterial immunity, asthma and allergies**: IL-4, IL-5, IL-10

**Autoimmune disease**: Cytokines secreted
- IFN-γ
- IL-2
- TNF-α
Autoimmunity

Multiple sclerosis
Lupus
Arthritis
Type I Diabetes
Inflammatory Bowel Disease
Biological relatives of IBD patients show 10 fold increased risk.

Sisters/brothers show a 30 fold increased risk.

However, monozygotic twins show a 18% (ulcerative colitis) and 50% (Crohn’s) concordance rate.
Inflammatory Bowel Disease

Environment:
Higher: urban than rural
    northern than southern
(Europe and North America)
developed than underdeveloped

Sunlight?
Bacterial flora

When measured vitamin D status low/bone
diseases!
Does vitamin D status affect the development of autoimmune diseases?
Experimental Inflammatory Bowel Disease

Spontaneous colitis - as a consequence of targeted mutations

IL-10 KO mice spontaneously develop IBD symptoms in the ileum and colon because of a defect in regulatory T cells.

Disease develops sporadically beginning at 9-10 weeks of age. Some mice may not show symptoms after much longer.

Wasting, diarrhea, rectal prolapse and bleeding which can lead to premature mortality.
Vitamin D and VDR deficiency exacerbates Inflammatory Bowel Disease

Cantorna et. al 2000 Journal of Nutrition, Froicu et. al 2003 Molecular Endocrinology
Dextran sodium sulfate induced colitis

DSS in drinking water
5 days/5 days regular drinking water

WT VDR KO

- colonic length
- Histopathology colon
- body weight changes
VDR KO mice are highly susceptible to dextran sulfate induced colitis

[Graph showing BW (% original) and Survival% over Days after DSS po.]
Wild type bone marrow transplantation rescues VDR KO mice from DSS colitis.
Vitamin D or VDR deficiency increased the mortality rate in IBD susceptible strains of mice.

$1,25(\text{OH})_2\text{D}_3$ reduced inflammation in IL10 KO mice. The reduction in inflammation correlated with the decreased expression of TNF$\alpha$ related genes.

VDR/IL10 double KO mice develop a fulminating form of IBD. IBD transferred via splenocytes.

VDR KO mice are highly susceptible to DSS colitis. WT bone marrow protects VDR KO mice from DSS. $1,25(\text{OH})_2\text{D}_3$ treatment reduced symptoms of colitis.
Model of defect in VDR KO CD4+ T cells increase IBD

VDR KO - vitamin D deficient

Normal T cell function
Less IBD

Vitamin D and VDR $\rightarrow$ $1,25(OH)_{2}D_{3}$
**IBD: Following CD4/CD45RB^{high} T Cell Transfer into RAG KO mice.**

**CD4 naive (CD25-)**

![C57BL/6 Rag KO mice](image)

**Donor Cells**

<table>
<thead>
<tr>
<th></th>
<th>Body Weight (g)</th>
<th>SI/BW (%)</th>
<th>LI/BW (%)</th>
<th>Colitis</th>
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<tbody>
<tr>
<td>WT naive</td>
<td>18.8 ± 0.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.8 ± 0.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.6 ± 0.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.9 ± 0.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>VDR KO naive</td>
<td>17.4 ± 0.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.0 ± 0.9&lt;sup&gt;d&lt;/sup&gt;</td>
<td>6.4 ± 1.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.8 ± 0.5&lt;sup&gt;c&lt;/sup&gt;</td>
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</table>

VDR KO CD4+ T cells contain highly pathogenic T cells

Froicu et al. 2003 Molecular Endocrinology
More Th17 and Th1 cells in VDR KO mice.

Th17 driven

CD3 & CD28
IL-6 and TGF-β1

Naive

CD3 & CD28

Th17 driven

WT

VDR KO

Th17

IFN-γ/IL-17

Naive

IFN-γ/IL-17

Th1

IL-17

IFN-γ

12.01 ± 0.15

13.12 ± 0.25

20.40 ± 0.19

15.07 ± 0.47

10.77 ± 0.55

20.41 ± 0.63
Unconventional T cells as regulatory cells

Classical T cells CD4+, CD8+ etc. are present in normal numbers in the VDR KO mice.
In vitro and in vivo T reg function

Numbers of T reg (FoxP3+) cells are not different in VDR KO and WT mice.

T reg from VDR KO mice are functionally normal.

Yu & Bruce et. al 2008 PNAS
NKT cells are regulatory cells providing early cytokine secretion.
Yu et al. 2008 PNAS

TCRβ CD1d αGalCer tetramer

WT VDR KO

thymus

spleen

liver

% of IL-4 producing NKT cells

% of IFN-γ producing NKT cells

Liver WT VDR KO

Spleen 15 3 51 22

Liver 46 25 71 16

Liver 0.5 0.15 28 5.9
Yu et al. 2008 PNAS

Stage 1

Stage 2

Stage 3

Conventional T cells

DN

CD4+CD8+

Fyn

NF-κB

CD44-

CD44+

CD44+NK1.1+

T-bet

NF-kB

VαiNKT

Fig 3

A

WT

VDR KO

88±3

28±4

CD44 (Gated on TCRβ+tetramer+)

B

CD44lowNK1.1-

CD44highNK1.1-

p<0.05

p<0.05

WT

VDR KO

WT

VDR KO

CD44highNK1.1+

p<0.001

p<0.05

WT

VDR KO

Thymocytes

iNKT

T-bet

GAPDH

Stage 1

Stage 2

Stage 3

T bet
Gated on CD8αα

CD8αα VDR KO IEL

<table>
<thead>
<tr>
<th></th>
<th>Total CD4</th>
<th>Total CD8αβ</th>
<th>Total CD8αα</th>
<th>CD8αα TCRαβ</th>
<th>CD4+/CD8αα TCRαβ</th>
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</thead>
<tbody>
<tr>
<td>WT</td>
<td>4.64 ± 0.22</td>
<td>23.44 ± 0.56</td>
<td>49.03 ± 3.09</td>
<td>36.73 ± 4.16</td>
<td>3.7 ± 0.28</td>
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<tr>
<td>VDR KO</td>
<td>4.49 ± 1.01</td>
<td>24.67 ± 3.3</td>
<td>29.01 ± 1.05</td>
<td>16.86 ± 1.39</td>
<td>0.47 ± 0.096</td>
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<tr>
<td>p value</td>
<td>0.89 not sig.</td>
<td>0.7 not sig.</td>
<td>0.0036 **</td>
<td>0.0106 *</td>
<td>0.0004 ***</td>
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Yu & Bruce et. al 2008 PNAS
Homing and IL-10 secretion of VDR KO T cells

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<tr>
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<th>IL-10</th>
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<tr>
<td></td>
<td>% IEL</td>
</tr>
<tr>
<td>WT</td>
<td>13.6 ± 2.1</td>
</tr>
<tr>
<td>VDR KO</td>
<td>0.8 ± 0.4*</td>
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Yu & Bruce et. al 2008 PNAS
**IBD targets**

CD4+CD45RB$^{\text{high}}$ T cells from VDR KO mice induce greater pathology than WT counterparts.

More IL-17, and IFN-γ less IL-10 in the VDR KO host.

Expression of the VDR is not required for T reg cell development or function.

NKT cell development and function require the VDR.

T cell homing and expression of CD8$^{\alpha\alpha}$ in the IEL require the VDR.
What is the effect of changing levels of vitamin D on immunity?

Cyp27B1 KO mice: unable to use the vitamin D in the diet to make 1,25(OH)2D3.
Cyp27B1 ko/+ breeders: compare WT and KO fed the same diets.
Vitamin D deficient Cyp27B1 KO and WT mice: VERY FEW iNKT cells.
Conclusions

There is a block in the development of iNKT cells following vitamin D deficiency in utero.

D- iNKT cells fail to increase to +D WT levels with either vitamin D supplementation or 1,25(OH)2D3 treatment beginning at the day of birth.

Epigenetic changes in iNKT cells following in utero vitamin D deficiency.
Revised model

DC
Naïve CD4+ T cell
Activation
Th 0
Th 1
Th 2
Treg

VDR KO
CD8αα
IEL

IBD

Vitamin D and VDR
1,25(OH)₂D₃