Vitamin D: Should a regular dose be part in immunomodulation of regulatory T cells in immunomediaded diseases?

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The role of regulatory T-cells in autoimmunity

Buckner, Nat Rev Immunol, 2010
Effects of Vitamin D in the immune system

Bouillon, Endocrine Reviews, 2008
Vitamin D supplementation and regulatory T cells in healthy humans

Pilot study 1x 140,000 IU Cholecalciferol

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<tr>
<td>n</td>
<td>50</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>31±8</td>
</tr>
<tr>
<td>Females (%)</td>
<td>64</td>
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<td>BMI (kg/m²)</td>
<td>23.3±4.3</td>
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%CD4+CD25hiFoxp3+CD127dim

Prietl B, Pilz S et al IMAJ 2010
High-dose cholecalciferol supplementation significantly increases peripheral CD4^+ Tregs in healthy adults without negatively affecting the frequency of other immune cells.

140,000 IU Vit D3

Baseline

Prietl B, Treiber G et al Eur J Nutr 2014
Significant increase in % Treg in CD4\textsuperscript{pos} T cells

Unchanged Treg suppression function in healthy humans

Other immune cells not effected

No treatment related side effects

Prietl B, Treiber G et al Eur J Nutr 2014
Regulatory T cells in Typ 1 Diabetes

- **T1D:** no global deficiency in Treg cell numbers, but functional capacity like suppression of Treg cells is impaired. *(reviewed in Bruckner Nat Immun 2010)*

  ➢ **Targeting the Treg population directly in vivo to increase frequency and/or function of Treg**

  - Analog of active form of vitamin D \((1,25(\text{OH})_2\text{D}_3)\) increased CD4\(^+\)CD25\(^+\)Treg in pancreatic lymph nodes in NOD mice. *(Gregori 2002)*

  - VitD3 **increased** CD4\(^+\)CD25\(^+\)FoxP3\(^+\) T cells in pancreatic lymph nodes and reduced diabetes development in NOD mice. *(Takiishi Diabetes 2014)*

  - 2000 IU VitD3 **increased** regulatory CD4\(^+\)CD25\(^+\)FoxP3\(^+\) T cells and slower decline of residual β-cell function in new onset T1D. *(Gabbay APDM 2012)*
RCT: Vit D3 in patients with T1D

- 30 Patients with new-onset T1D (<12 weeks)
- Intervention 12 month:
  - oral therapy of cholecalciferol (70IU/kg bodyweight/day)
  - or placebo
- Immunologic Assessment at month 0, 3, 6 and 12:
  - Immune phenotyping: FACS-analysis
    - CD4^{pos}CD25^{hi}Foxp3^{pos}CD127^{dim} Treg
    - Th-Subtypes (Th1, Th2, Th17)
    - DC , B-cells, NK, NKT-cells
  - functional tests: FACS sorted Treg and Teff
    - ex vivo suppression co-cultures ([^{3}H]-thymidine incorporation)
    - Apoptosis (AnnexinV/7-AAD)
RCT: Vit D3 in T1D

Regulatory T cells

Cholecalciferol

Placebo

Treiber G et al in preparation
RCT: Vit D3 in T1D

- No significant effect on the frequency of other peripheral immune cells
  - Th1 /Th2 / Th17 cells
  - B cells
  - Cells from the innate immune system
RCT: Vit D3 in T1D

**Fasting C-peptide**
- Cholecalciferol
- Placebo
- p=0.078

**Stimulated C-Peptide**
- Cholecalciferol
- Placebo
- p=n.s.

**Insulindose**
- Cholecalciferol
- Placebo
- p=0.004

**Serum calcium**
- Cholecalciferol
- Placebo
Treg origin: Thymus and Gut associated lymphoid tissue

Li L et al Clin Immun 2011
GALT: interface for maintenance of immune homostasis

- **T1D**: *Increased* intestinal permeability precedes clinical onset of type 1 diabetes. *(Bosi Diabetologia 2006)*
- Cow milk, early cereal exposure and enterovirus infection risk factors for *islet autoimmunity*. *(Lempainen DMRR 2012)*
- **T1D**: *reduced* regulatory Foxp3$^+$ T cells in duodenal biopsies *(Badami Diabetes 2011)*
- **IBD**: *Decreased* frequency of CD4$^+$Foxp3$^+$ Treg in PBMC and increased Foxp3$^+$ cells in inflamed mucosa *(Wang JDD 2011).*
  Increased apoptosis of regulatory T lymphocytes in peripheral blood and in mucosa. *(Veltkamp C Gut 2011)*

- Vit D3 effect on Treg in gastrointestinal mucosa in humans unknown
Vit D3 effect on Treg in gastrointestinal mucosa in humans

Screening
16 healthy volunteers

Gastro

Colo

BL V2/V3 V4 V5/V6 V7

Safety

Follow-up

Cholecalciferol
70IU/kg BW/d Loading dose

blood biopsy

blood biopsy

blood biopsy

blood
systematic assessment of upper and lower GI tract

- Biopsy regions

<table>
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<tr>
<td>Age (yrs)</td>
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<tr>
<td>Females (%)</td>
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</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23±3</td>
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Gating strategy
FACS analyses
Distribution of CD4<sup>pos</sup>-, CD8<sup>pos</sup> – and Regulatory T Cells in the Upper and Lower Gastrointestinal Tract in Healthy Young Subjects

Martin Tauschmann<sup>1</sup>, Barbara Prietl<sup>1</sup>, Gerlies Treiber<sup>1</sup>, Gregor Gorkiewicz<sup>2</sup>, Patrizia Kump<sup>3</sup>, Christoph Högenauer<sup>3</sup>, Thomas R. Pieber<sup>1</sup>

*Plos one 2013*
Vit D3: on blood Treg

Serum 25 (OH) D

- BL
- 4 weeks
- 8 weeks
- follow-up

Peripheral blood regulatory T cells

- CD4⁺CD25⁺Foxp3⁺CD127⁻
- BL
- 8 weeks
- 10 weeks

Helios⁺ Treg

- BL
- 8 weeks
- 10 weeks
Vit D3 on GI CD4⁺ and CD8⁺

CD4⁺ T cells

CD8⁺ T cells

- Corpus
- Antrum
- Duodenum
- Ilium
- Appendix
- re Colon
- li Colon

%CD4⁺ in lymphocytes

%CD8⁺ in lymphocytes

- BL
- 8 weeks

* Statistical significance
Vit D3 on GI Treg

**Baseline**

% Treg in CD4

**8 weeks Vit D3**

% Treg in CD4

**regulatory T cells**

- **BL**
- **8 weeks**

**Helios+ Treg**

% Helios+ Treg

- **BL**
- **8 weeks**
Vit D3 and Mikrobiom

- Vit D alters upper GI community structure
- Reduction of Proteobacteria
- Increase of richness in upper GI
Summary and Conclusion

• Vitamin D3 – Cholecalciferol - increases peripheral regulatory T cells in healthy humans

• Impaired suppressive capacity in type 1 diabetes improved with Cholecalciferol supplementation along with preservation of fasting C-peptide

• 8 weeks of VitD3 did not alter total number of Treg in gastrointestinal mucosa in healthy humans but showed an distinct pattern in Helios⁺ Treg compartment

• Vitamin D3 elevates CD8⁺ cytotoxic T cell numbers, accompanied by modulation of the gut microbiome with marked reduction of Gammaproteobacteria

➤ Potential as adjunctive immunomodulatory therapy of immunomediated diseases like T1D in combination with other future immune therapies
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