Title
Differential responses of human dendritic cells to metabolites from the oral/airway microbiome.

Permalink
https://escholarship.org/uc/item/78f9t9w5

Journal
Clinical and experimental immunology, 188(3)

ISSN
0009-9104

Authors
Whiteson, K
Agrawal, S
Agrawal, A

Publication Date
2017-06-01

DOI
10.1111/cei.12943

Peer reviewed
Biotin Deficiency Enhances the Inflammatory Response of Human Dendritic Cells

Sudhanshu Agrawal#, Anshu Agrawal†#, Hamid Said†*

# Division of Basic and Clinical Immunology,
†#Department of Medicine
University of California,
Irvine, CA, 92697, USA.

† &VA Medical Center-151
Long Beach, CA 90822

*Corresponding author
Tel. (949)824-7706
aagrawal@uci.edu
Tel. (562) 826-5811
Fax. (562) 826-5731
hmsaid@uci.edu

Keywords: human, dendritic cells, biotin, inflammation.

Running Title: Biotin deficiency, DCs and inflammation
ABSTRACT

The water-soluble, biotin (vitamin B7), is indispensable for normal human health. The vitamin acts as a co-factor for five carboxylases that are critical for fatty acid, glucose and amino acid metabolism. Biotin deficiency is associated with various diseases, and mice deficient in this vitamin display enhanced inflammation. Previous studies have shown that biotin affects the functions of adaptive immune T and NK cells, but its effect(s) on innate immune cells is not known. Because of that and because vitamins such as vitamins A and D have a profound effect on dendritic cell (DC) function, we investigated the effect of biotin levels on the functions of human monocyte derived DCs. Culture of DCs in a biotin deficient medium (BDM) and subsequent activation with LPS resulted in enhanced secretion of pro-inflammatory cytokines, TNF-α, IL-12p40, IL-23 and IL-1β compared to LPS-activated DCs cultured in biotin sufficient (control) and biotin over-supplemented media. Furthermore, LPS-activated DCs cultured in BDM displayed a significantly higher induction of IFN-γ and IL-17 indicating Th1/Th17 bias in T cells compared to cells maintained in biotin control or over-supplemented media. Investigations into the mechanisms suggested that impaired activation of AMP kinase in DCs cultured in BDM may be responsible for the observed increase in inflammatory responses. In summary, these results demonstrate for the first time that biotin deficiency enhances the inflammatory responses of DCs. This may therefore be one of the mechanism(s) that mediates the observed inflammation that occurs in biotin deficiency.
INTRODUCTION

Biotin, a member of the family of water-soluble vitamins (also known as vitamin B7) is an indispensable micronutrient for normal human health due to its essentiality for cellular metabolism, proliferation, and survival. Marginal and severe degrees of biotin deficiency lead to a variety of clinical abnormalities that include neurological disorders and dermal abnormalities (40, 45). Such deficiency/sub-optimal levels occur in a variety of conditions including inflammatory bowel disease (IBD) (1, 12), inborn errors in biotin metabolism (multiple carboxylase deficiency); (10), and chronic alcoholism (6) among others. At the metabolic level, biotin acts as a co-factor for five carboxylases that are critical for fatty acid, glucose and amino acid metabolism (27, 40, 45). Important roles for this vitamin in cellular energy metabolism (i.e., ATP production) and in regulation of cellular oxidative stress (24), as well as in gene expression (where expression of over 2,000 human genes appears to be affected by biotin status; (36, 38, 40) have also been reported recently. Emerging evidence has also been accumulating showing a role for biotin in the functions of immune cells (20). In reference to the latter, biotin was shown to be important for the activity of human natural killer (NK) lymphocytes (32), for the generation of cytotoxic T lymphocytes (CTLs) (19), and for the maturation and responsiveness of immune cells (4). Defects in T cell and B-cell immunity have been reported in patients with multiple carboxylase deficiency, a condition associated with biotin deficiency (10). Increase in the levels of pro-inflammatory cytokines, (TNF-α) and interleukin-1b (IL-1β) has also been observed in biotin deficiency (20-22). Our recent studies in mice with a conditional (intestinal-specific) knockout (KO) of the biotin transporter SMVT (product of the SLC5A6 gene) have shown that these animals also develop chronic spontaneous intestinal inflammation, especially in the cecum (13), presumably in response to the moderate degree of biotin deficiency.
uniformly induced by defective biotin transport. From the above, we infer that biotin deficiency leads to significant metabolic disturbances and to immune dysfunction.

The majority of the previous studies have examined the effect of biotin deficiency on the functions of adaptive immune T, B and NK cells (14, 37). Virtually nothing is known about the effect of biotin deficiency on innate immune cells such as dendritic cell (DCs). DCs are the primary antigen presenting cells and key to initiating and regulating an immune response (5). DCs are distributed throughout the body including below the epithelial cells lining the gut and are amongst the primary responders to infections (5, 29). DCs sense and capture pathogens via various pathogen recognition receptors (PRRs). Subsequently DCs become activated by upregulating the expression of costimulatory and antigen-presenting molecules as well as secreting pro-inflammatory cytokines. During activation, DCs migrate to the draining lymph nodes to prime and activate naïve T cells. The activation molecules and cytokines secreted by DCs have a major influence on T cell responses (16, 25). Exposure of DCs to ligands of all these PRRs results in production of cytokines that modulate the type of T cell response and functions. Upon interaction with DCs, CD4\(^+\) T cells can differentiate into a variety of effector and regulatory subsets, including classical Th1 cells and Th2 cells, follicular helper T cells, induced regulatory T cells and the more recently defined Th17 cells (16, 17, 25). The nature of the cytokines produced by DCs in response to various ligands dictates the type of Th cell responses. For example, IL-12p70 secretion by DCs polarizes towards Th1 cells while the production of IL-23 along with IL-1β from DCs leads to the generation of Th17 cells (2, 3, 23). The cytokines secreted by DCs thus have a major influence on downstream inflammatory responses. Aberrant inflammatory cytokine secretion by DCs has been observed in many diseases including Crohn’s disease and rheumatoid arthritis (26, 44) among others. Accordingly, we speculate that
understanding the factors which can modulate the DC responses is likely to important in understanding the immune dysregulation in biotin deficiency.

Vitamins have a profound effect on DC responses. For example, Vitamin A metabolite, retinoic acid as well as Vitamin D have been reported to induce tolerance in DCs (9, 35). Almost all studies have investigated the effect of fat soluble vitamins on DC functions and there is a scarcity of information regarding the effect of water soluble vitamins like biotin on DC function. Here we examined the effect of biotin status on DC functions.
MATERIALS AND METHOD

**Blood donors**

Blood samples were obtained from healthy volunteers via Institute for Clinical and Translational Science (ICTS), UC Irvine. This study was approved by the Institutional Review Board of the University of California (Irvine, CA).

**Preparation of biotin deficient medium**

DMEM deficient in B vitamins was obtained from Sigma. The media was supplemented with all the B vitamins except biotin. Fetal bovine serum (FBS; obtained from Hyclone), treated with streptavidin beads to remove any traces of biotin, was then added to the culture medium at a concentration of 5%. Finally, biotin deficient, sufficient (control) and over-supplemented culture media were then prepared by adding 0, 10 and 100 \( \mu \)M biotin, respectively.

**Culture and stimulation of human monocyte-derived DCs**

Monocyte derived DCs were prepared as described before by culturing the purified monocytes with GMCSF and IL-4. DCs (CD14\(^{-}\)HLA-DR\(^{+}\)CD11c\(^{+}\) cells) were collected after 6 days (3). The purity of the DCs was > 95% as determined by the expression of CD14, CD11c and HLA-DR. DCs collected were washed and cultured in biotin deficient, control and over supplemented media for 72h. For the last 24h, the cells were stimulated with LPS (100 ng/ml) and supernatant was collected and stored at -70°C until analyzed. Multiplex cytokine/chemokine detection was performed using Magpix kit (eBioscience) as per the manufacturer’s protocol.
Control and LPS-stimulated DCs were stained for the expression of CD80, CD86, and HLADR (BD PharMingen) using specific antibodies (3). Analysis was performed with Flow jo (Treestar Inc).

**DC-T cell co-cultures**

LPS-stimulated and unstimulated DCs were cultured with magnetic bead purified (StemCell, Vancouver, Canada), allogeneic CD4 T cells at a ratio of 1:10. After 6 days of incubation, the supernatant was collected and the secretion of IFN-γ, IL-10, IL-17 and IL-22 was assessed using multiplex (eBioscience). IL-22 was assayed using ELISA (RnD systems).

**Phospho AMPK and Total AMPK detection**

DCs cultured in Biotin deficient, control and over supplemented media for 72h were stimulated with AMPK activator, 5-Aminoimidazole-4-carboxamide 1-β-D-ribofuranoside, Acadesine, N¹-(β-D-Ribofuranosyl)-5-aminoimidazole-4-carboxamide (AICAR) (1mM) for 45 min. Subsequently the cells were lysed. Phospho AMPK and total AMPK in the lysates was determined using specific ELISA kit as per the manufacturer’s instructions (RnD systems).

**Statistical analysis**

Statistical analysis was performed using Graph Pad Prism. Within group differences between unstimulated and stimulated conditions were tested using paired t-tests. Values of $p < 0.05$ were considered significant.
RESULTS

Biotin deficiency has no significant effect on DC phenotype

Biotin deficiency may alter the activation of DCs. Therefore, we investigated whether the up-regulation of DC activation markers in response to LPS were altered in biotin deficient (0 µM) or over-supplemented (100 µM) DCs as compared to DCs cultured in control biotin (10 µM) media. Stimulation with the LPS resulted in substantial activation DCs cultured in all biotin media (Figure 1). DCs cultured in all three media displayed significantly enhanced (p < 0.05) expression of co-stimulatory marker CD80 (Figure 1A), CD86 (Figure 1B) and HLADR (Figure 1C) in response to LPS compared to un-stimulated DCs. However, the expression of CD80, CD86 and HLADR was comparable between DCs cultured at various concentrations of biotin both at the level of MFI as well as percent positive cells (Figures 1A-C). These data suggest that biotin levels (deficiency or over supplementation) have no significant effect on DC phenotype.

Biotin deficiency enhances pro-inflammatory cytokine secretion from LPS-stimulated DCs

Next, we investigated the cytokines secreted by stimulated DCs. After stimulation with LPS for 24h, supernatants were collected and assayed with multiplex to quantify cytokine secretion. In keeping with activation markers, stimulation of DCs with LPS resulted in the production of significantly enhanced (p < 0.05) levels of several pro-inflammatory cytokines including IL-6, TNF-α, IL-1β, IL-1α, IL-23, IL-12, IL-10 and chemokines such as CXCL-10, CCL-3, CCL-4 (Figure 2) in all groups. However, the level of the pro-inflammatory mediators was substantially different between LPS-stimulated DCs cultured in biotin deficient medium (BDM) compared to control medium. Compared to DCs cultured in control medium DCs cultured in BDM secreted significantly (p < 0.05) increased levels of TNF-α (BDM ~500pg/ml
vs. control ~345pg/ml), IL-1β (biotin deficient ~53pg/ml vs. control ~27pg/ml), IL-23 (BDM ~181pg/ml vs. control ~100pg/ml) and IL-12p40 (BDM ~4080pg/ml vs. control ~1842pg/ml) after stimulation with LPS (Figure 2A). LPS stimulated DCs cultured in biotin over-supplemented medium displayed comparable level of these cytokines to controls except for IL-12p40 which was significantly reduced in this group (p=0.02). IL-23 secretion was also reduced although not to a significant level (p = 0.7).

In addition to the above cytokines, LPS-stimulated DCs cultured in BDM also secreted significantly (p < 0.05) higher levels of IL-1α, IL-6, CXCL-10 and IL-10 (Figure 2B) compared to un-stimulated DCs. Though there was no significant difference in the level of these cytokines between DCs cultured in BDM verses the control medium, nevertheless DCs cultured in BDM displayed higher secretion and increased significance levels for all these cytokines compared to control and biotin over supplemented DCs. For example, IL-1α levels were significantly (p < 0.022) increased after LPS stimulation only in DCs cultured in BDM and not in control or biotin over-supplemented DCs (Figure 2B). CXCL-10 secretion was also significant (p <0.005) in LPS-stimulated biotin deficient DCs verses biotin over supplemented DCs. The secretion of chemokines CCL-3 and CCL-4 was comparable between the three groups (Figure 2C). Chemokines, CCL-2 and CXCL-8 were not induced at significant levels (p > 0.05) after LPS stimulation in all groups (Figure 2C). In summary these data demonstrate that biotin deficiency enhances the capacity of LPS-stimulated DCs to secrete pro-inflammatory and Th1, Th17 promoting cytokines and chemokines.

**Biotin deficient DCs bias the Th cell response towards Th1/Th17**
Our own studies (3) as well as evidence from the literature indicate a key role for the type of cytokine secreted by DCs in controlling the polarization of Th cell responses towards Th1, Th2, Treg or Th17. High IL-23 and IL-1β favor IL-17 production from Th cells, while high IL-12p70 favors IFN-γ production (16, 25). Therefore, given the distinct profile of cytokine secretion by biotin deficient DCs, we explored its effect on Th cell responses. DCs were cultured in medium with various concentrations of biotin and stimulated with LPS as described in Figure 1. Subsequently, the DCs were washed and cultured together with purified, CD4 T cells for five days to allow differentiation of T cells towards Th17 or Th1. The results showed (Figure 3) that LPS-stimulated biotin deficient DCs induced significantly higher (p < 0.05) levels of IFN-γ, IL-17 and IL-22 from CD4 T cells compared to biotin control DCs. Biotin over-supplemented DCs were comparable to control DCs. The secretion of IL-10 was also comparable between the 3 groups. Altogether, these data demonstrate that biotin deficiency enhances the secretion of TNF-α, IL-1β, IL-23 and IL-12p40 from DCs which biases the Th cell responses towards Th1/Th17. Biotin deficiency thus favors inflammation since these are all highly pro-inflammatory responses.

**Biotin deficiency impairs the activation of AMP Kinase (AMPK) signaling pathway in DCs**

The maintenance of cellular defense systems and removal of pathogens is an energetically demanding process that requires integration of multiple checkpoints to maintain immune cell energy homeostasis (28). AMP-activated protein kinase (AMPK) has emerged as an important regulator of inflammatory responses in immune cells including DCs (31). Given that biotin deficient DCs display increased secretion of inflammatory cytokines, we compared the phosphorylation of AMPK-α in un-stimulated and AICAR stimulated DCs from the three different biotin level groups using ELISA. AICAR is an activator of AMPK and was used as a
positive control. As evident from the results shown in Figure 4A, phospho AMPK levels were significantly reduced ($p=0.025$) in biotin deficient DCs compared to control DCs before activation with AICAR. Furthermore, activation with AICAR enhanced the pAMPK levels significantly in ($p < 0.05$) in both control and biotin over-supplemented DCs but had no significant effect on biotin deficient DCs (Figure 4A). The levels of total AMPK were comparable in all 3 groups both before and after activation AICAR. These results suggest that biotin deficiency impairs the activation of AMPK in DCs which in turn enhances inflammation.
DISCUSSION

Previous studies have shown that biotin deficiency impacts the functions of immune function particularly those of NK and T cells (20). Our investigations into the effect of biotin on DC functions revealed that deficiency of this vitamin results in enhanced pro-inflammatory cytokine secretion from DCs. The DCs produce significantly high levels of TNF-α, IL-1β, IL-23 and IL-12p40 which prime the Th cell responses towards IFN-γ and IL-17 producing Th1/Th17 inflammatory cells (Figures 1 & 2). This is in keeping with previous studies in which an enhanced secretion of TNF-α was also observed in murine macrophages cultured in biotin deficient (21). Moreover, biotin starvation is also reported to enhance the production of reactive oxygen species (ROS) (24). Our own results with SMVT KO mice (all of which develop biotin deficiency) (13) and with mice made biotin deficient via dietary means (39) also demonstrate the association between biotin deficiency and intestinal inflammation. Furthermore, both the IL-12/Th1 as well as IL-23/Th17 responses though essential for generating immunity against pathogens have also been shown to play a major role in numerous inflammatory diseases. For example, excessive Th1 responses are associated with multiple sclerosis, Crohn’s disease, rheumatoid arthritis, and crescentic glomerulonephritis (15). A distinctive positive clinical response to very high dose biotin supplementation has been reported in multiple sclerosis (34, 41). This reversal of clinical impairment has not been achieved with any other therapy to date. Similarly, increased levels of IL-23/Th17 have been demonstrated to be of pathogenic relevance in a growing number of chronic inflammatory diseases (7). GWAS studies in humans suggest that Th17 cells have a major role in inflammatory diseases of the mucosal tissues including the gut, lung and skin (33). In this regard increased activity of IL-23/Th17 axis has been implicated in Crohn’s disease, ulcerative colitis and colon cancer in the gut (42, 46). Asthma, chronic
obstructive pulmonary disease (COPD) and autoimmune diseases of the lung also display enhanced activation of the Th17 pathway. IL-23/Th17 pathway is also considered a major perpetuator of skin disorders such as Psoriasis and atopic dermatitis(46). Recent studies also suggest that in each of the Th17-associated chronic inflammatory diseases both Th17 and Th1-like cells are found in the involved tissues (11). Thus the enhanced induction of Th1/Th17 cells by biotin deficient DCs may be one of the mechanisms of increased inflammation associated with biotin deficiency

Biotin has a major role in cellular energy homeostasis because it functions as a key cofactor in various carboxylases which are essential for the mitochondrial metabolism of glucose, fatty acids and amino acids (19, 24, 27, 40). A recent study in yeasts has also shown that biotin starvation alters cellular respiration. Emerging evidence indicates a major role of AMPK as a metabolic and energy sensor of DC activation (8, 31). AMPK is a serine/threonine kinase composed of three subunits, α,β,γ, where the α subunit is the one involved in phosphorylation. It phosphorylates targets that switch off ATP-depleting processes and turns on ATP-generating pathways(43). Recent reports suggest an important role for AMPK in modulating inflammatory responses in DCs (8, 18). APCs from mice lacking AMPK-α1, promote pro-inflammatory cytokine production in response to LPS while the presence of AMPK-α1 attenuated these responses (8). Furthermore, activation of AMPK has been shown to reduce NFκB activation via sirtuin 1 (SIRT1) - mediated deacetylation of p65 at Lys310 in macrophages (47). AMPK becomes activated when the ATP levels in the cells decrease. AMPK activation enhances mitochondrial respiration and fatty acid synthesis(43). The process of activation of DCs depletes the energy reserves of the cell to synthesize/process proteins required for the response. This creates a state of starvation in DCs and instead of obtaining energy from mitochondrial
respiration and activating AMPK, DCs shift to glycolysis to meet their demands of the energy (30). Therefore, in DCs decreased AMPK activation is associated with increased TLR induced activation (18, 30). Our results suggest that the enhanced inflammatory responses of biotin deficient DCs are a consequence of decreased AMPK activation (Figure 4) are in keeping with the role of AMPK in DC inflammatory responses.

In conclusion, these data demonstrate for the first time that biotin deficiency can enhance the pro-inflammatory cytokine responses of DCs. The increased production of pro-inflammatory cytokines, TNF-α, IL-12, IL-23 and IL-1β by DCs in turn leads to the induction of pro-inflammatory Th1/Th17 responses. We also find that the activation of AMPK, a major regulator of inflammation, is reduced in biotin deficient DCs. Our studies thus highlight a possible mechanism of inflammation induced by biotin deficiency.
Grants: NIH grants AG045216 (to AA) and DK58057 (to HMS), a grant from the DVA (HMS) and a Grant UL1 TR000153 to National Center for Research Resources and the National Center for Advancing Translational Sciences. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

Disclosure: The authors declare no conflict of interest.
REFERENCES


FIGURE LEGENDS

Figure 1: Biotin deficiency has no significant effect on DC phenotype. DCs were cultured in biotin deficient (0µM), control (10µM) and biotin over-supplemented (100µM) media for 48 and subsequently stimulated with LPS for another 24h. Bar graphs depict the MFI and % positive DCs of the expression of activation molecules on LPS stimulated aged and young DCs. A. CD80; B. CD86; C. HLADR. Data is mean +/-S.E. of 3 experiments.

Figure 2: Biotin deficiency enhances pro-inflammatory cytokine secretion from LPS-stimulated DCs. Bar graphs depict the levels of cytokine and chemokines secreted by LPS-stimulated biotin deficient, control and over supplemented DCs. A. TNF-α, IL-1β, IL-23, IL-12p40; B. IL-1α, IL-6, IL-10, CXCL-10; C. CCL-3, CCL-4, CCL-2, CXCL-8. Data is mean +/-S.E. of 8 experiments.

Figure 3: Biotin deficient DCs bias the Th cell response towards Th1/Th17. Bar graphs depict the level of cytokines secreted by T cells after 5 days of co-culture with LPS-stimulated biotin deficient, control and over supplemented DCs. IFN-γ; IL-17; IL-22 and IL-10. Data is mean +/-S.E. of 8 experiments.

Figure 4: Biotin deficiency impairs the activation of AMPKinase signaling pathway in DCs. pAMPK and total AMPK levels were determined in biotin deficient, control and biotin over-supplemented DCs before and after AICAR stimulation by ELISA. Bar graphs depict the levels of A. pAMPK; B. AMPK in DCs. Data is mean +/-S.E. of 6 experiments.