The Effects of Aldehydehydrogenase1A1 on Immunoglobulin Production in Mice

Thesis

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By

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Abstract

Vitamin A and its metabolites, retinoic acid (RA) and retinal, regulate multiple life-sustaining processes including vision, haematopoiesis, skeletal growth, fertility (male and female), embryogenesis, epithelial cell integrity against infections, and immunity \cite{1,7,9-19}. Deficiency in dietary vitamin A in animals leads to severe malfunction of the intestinal enzymes associated with mucosal immunity and decreased immunoglobulin A (IgA) production, or impaired IgA function \cite{1,10-19}. In HIV-infected children, supplementation with vitamin A decreases diarrhea and mortality \cite{1,11}. At present, the mechanisms by which vitamin A exerts its immune function remain poorly understood. Recent studies in animals supplemented with the vitamin A metabolite RA showed increases in IgA levels in mice \cite{12-13,18-19}. RA is generated from retinaldehyde by the aldehyde dehydrogenase-1 family of enzymes (Aldh1) that is comprised of three members: Aldh1A1, A2, and A3 \cite{2-7}. Aldh1A1 is the major cytosolic enzyme involved in RA production. However, the role of Aldh1A1 in the regulation of IgA production has not been investigated. We hypothesize that Aldh1A1 affects immunoglobulin production in mice.

We studied the effect of Aldh1A1 on IgA production in Aldh1A1/-/- and WT mice on either a normal chow diet or a high-fat diet. Enzyme-linked immunosorbent assay
(ELISA) was used to measure IgA and IgG levels in the plasma, spleen, and feces. Western blot was used to estimate IgA heavy chain and light kappa and lambda chains in spleen. Using ELISA, we demonstrated that IgA levels in plasma were significantly higher (292%) in Aldh1A1-/-(n=12) than WT (n=8) mice on regular chow. Plasma IgG levels were moderately higher in Aldh1A1-/-(127%) than in WT. Next we examined whether Aldh1A1 deficiency influences immunoglobulin production in major sites of immunoglobulin production by B cells. We found several abnormalities in the spleen of Aldh1A1 deficient mice. Aldh1A1-/ spleens were enlarged 120% as compared to WT mice. There were higher levels of spleen IgA (162%) in Aldh1A1-/ than in WT with a higher expression of light IgA kappa chains. Spleens had (307%) higher IgG levels in Aldh1A1-/ than WT mice. Fecal samples were used to investigate the effects of Aldh1A1 on immunoglobulin production at mucosal sites. Fecal IgA levels were the same in both groups. Significantly higher amounts of fecal IgG (4000%) were found in Aldh1A1-/ as compared to WT mice.

A high fat diet appeared to additional influence immunoglobulin production. The Aldh1A1 dependent changes in immunoglobulin production were augmented by a high-fat diet. In high fat diet fed animals, plasma had 575% and 741% higher levels of IgA and IgG in Aldh1A1-/ than WT respectively. Spleens had (297%) higher IgG levels in Aldh1A1-/ mice as compared to WT. Both plasma and spleens expressed higher protein levels of light kappa chain in Aldh1A1 -/- than in WT mice on a high-fat diet. Our data reveal that the Aldh1A1 enzyme suppresses immunoglobulin A and G production and
plays a significant role in the regulation of immunoglobulins light chain levels in response to diet.
Dedication

Dedicated to my parents Gabriel and Florence Mayeku
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I express my gratitude to my family-mom and dad, my siblings and my extended family--for their moral support and for believing in me. Appreciation to Dr Ziouzenkova’s laboratory members--Mr. Fangping Yang, Ms Rumana Yasmeen, Ms Barbra Reichert and Ms Colleen Miller--and Dr Harrison laboratory members--Ms Vanessa Reed, Ms Emily Brown, Ms Shiva Singh, Mr. Mathew Fleschman, Mr. AbdulKerim Eroglu and Mr. Carlo Dela Sena--for your continuous support in and out of the laboratory. Special thanks to Ms. Astrid Bonnegarde and Dr. Prosper N Boyaka for their contribution and Dr Papenfuss for her guidance on this research project. To my advisors Dr Harrison and Ziouzenkova: thanks for giving me a chance to start my career. Thank you for your guidance, support and patience. You made it more than just school. I am very grateful to work under your mentorship.

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List of Figures

**Figure 1.** Increased IgA, but not IgG Production in Plasma in *Aldh1A1-/-* Compared to WT Mice on Regular Chow.................................................................24

**Figure 2.** Increased IgG production and Similar IgA Levels in Spleen in *Aldh1A1-/-* Compared to WT Mice on Regular Chow.................................................................25

**Figure 3.** Body Weights and Increased Spleen Weights in *Aldh1A1-/-* Mice Compared to WT on regular chow.................................................................26

**Figure 4.** Decreased CD 19, CD 68 and CD8a mRNA Expression in the Spleen of *Aldh1A1-/-* Compared to WT Mice on Regular Chow .................................................................27

**Figure 5.** Increased IgG Production and Similar IgA Levels in Fecal in *Aldh1A1-/-* Compared to WT Mice on Regular Chow .................................................................28

**Figure 6.** Increased IgA, IgG and kappa Light Chains Concentration in Plasma in *Aldh1A1-/-* Compared to WT Mice on a High Fat Diet.................................................................29

**Figure 7.** Increased IgA, IgG production, and Kappa Light Chain Concentration in Spleen in *Aldh1A1-/-* Compared to WT Mice on a High Fat Diet.................................................................30
Table of Contents

Abstract.................................................................................................ii

Dedication...........................................................................................iii

Acknowledgments..................................................................................iv

Vita..........................................................................................................v

List of Figures........................................................................................vi

Chapter 1: Introduction and Literature Review.................................1

Chapter 2: Methodology.........................................................................5

Chapter 3: Results..................................................................................8

Chapter 4: Discussion and Conclusion.................................................11

References..............................................................................................19

Appendix: Data figures..........................................................................24
Chapter 1: Introduction And Literature Review

Mucosal immunity and immunoglobulin production

Even in the era of remarkable strides in medicine, mucosal disorders still remain a challenge. For instance, diarrheal diseases remain the second leading cause of child death worldwide killing nearly 1.7 million children annually. Rotaviruses claim 527,000 lives, while cholera causes 4,288 deaths annually. At least 1.4 million Americans suffer from inflammatory bowel diseases and stress-related mucosal disorders yearly [1, 8& 11]. The challenge of either finding a reliable vaccine or a treatment for mucosal diseases is still overwhelming.

Immunoglobulins are proteins that are secreted by B lymphocytes and function in adaptive immunity of the body through the humoral pathway. The immunoglobulin super family is comprised of A, D, M, G and E immunoglobulin classes. Immunoglobulin A (IgA), the most abundant immunoglobulin produced in the body (≈3 g/day), is the most abundant in mucosal secretions, has been linked to mucosal immunity and may be related to mucosal diseases [9, 12, 14-19]. In conjunction with innate mucosal defenses, IgA is the main antibody associated with providing protection against microbial
Vitamin A and mucosal immunity

Dietary vitamin A has been shown to play a role in mucosal immunity. Deficiency of dietary vitamin A in rats leads to severe functional disturbance of the enterocytes and enzymes associated with diarrhea\textsuperscript{[10, 12]}. For instance, in the vitamin A deficient rats, enterocyte brush-border enzyme activities revealed that lactase, sucrase, \( \gamma \)-glutamyl-transpeptidase (GGT), and dipeptidyl peptidase IV (DPP IV) were significantly reduced as compared to the pair-fed controls\textsuperscript{[9-10]}. Dietary vitamin A deficiency also increases severe diarrhea and bacterial translocation to extra-intestinal sites such as the kidneys, in particular. In these studies, there was also a greater susceptibility to intestinal
infections and toxicity in the vitamin A deficient rats in comparison to controls \[10, 13\]. Similarly, supplementation of dietary vitamin A in HIV-infected children decreased diarrhea and mortality in this population \[1, 11\].

Various studies investigating effects of dietary vitamin A on IgA have shown that its depletion in rats decreased levels of total intestinal IgA and mucosal antigen-specific IgA responses, while in mice it led to impaired IgA secretion and protection at mucosal sites \[10, 12-16\]. An impaired IgA response to cholera oral toxins in vaccinated dietary vitamin A deficient rats has also been reported \[13\]. Given these studies demonstrating a link between dietary vitamin A on both mucosal diseases and IgA production, it is still unclear how dietary vitamin A and its metabolites influence immunoglobulin production.

**Vitamin A metabolism and Aldh1 enzymes**

The effects of vitamin A are predominantly mediated by its metabolites, retinal and retinoic acid (RA). These metabolites are tightly regulated by distinct retinoid-generating enzymes, retinoid-binding proteins, and retinoid-activated nuclear receptors. For instance, retinol is reversibly converted to retinal by alcohol dehydrogenases (Adh). Retinal is then irreversibly converted to retinoic acid (RA) by a family of aldehyde dehydrogenases: Aldh1A1, Aldh1A2 and Aldh1A3. Of these enzymes, Aldh1A1 is the major enzyme associated with RA production \[2-5\]; however, Aldh1A1 can utilize many different substrates including lipid aldehydes \[4-6\].
Recent studies have focused on investigating the effects of these metabolites, especially RA, on mucosal immunity. RA, a widely studied vitamin A metabolite, is involved in gene regulation and has been associated with mucosal immunity \cite{12, 14-19}. Exposing B cells to RA increases their class switching to IgA and increases production of IL-6 by B cells which induces IgA secretion \cite{12}. Additionally, RA has also been shown to induce gut-homing receptors on B cells and IgA-antibody secreting cells (ASCs) \cite{14-117}.

Despite the associations of vitamin A and its metabolite RA with IgA production and mucosal immunity, the effects of Aldh1A1, a major enzyme producing RA, on IgA production have not been investigated. We hypothesized that Aldh1A1 regulates immunoglobulin production and studied immunoglobulin levels in plasma, spleen and feces of Aldh1A1-/- and WT mice.
Chapter 2: Methodology

Materials and Methods:

Animal Studies:

Aldh1A1<sup>−/−</sup> mice used in the study were generated and characterized by Duester and colleagues [2, 5].

STUDY 1: Twelve Aldh1A1<sup>−/−</sup> (7 females and 5 males) and eight WT (5 females and 3 males) mice were used. The mice were 3 months old and fed on a regular chow.

STUDY 2: Seven Aldh1A1<sup>−/−</sup> were used. They were fed on a high-fat diet containing 45% fat/kcal with a standard vitamin A content of 4 IU/g (Research Diet Inc., Canada) for 180 days. Spleens, plasma, and feces from the mice were collected and used for IgA and IgG protein analysis. All experimental protocols were approved by the Institutional Animal Care and Use Committee.

Reagents:

All reagents were from Sigma unless otherwise indicated.
Protein analysis:
Tissue homogenates were prepared in radioimmunoprecipitation assay buffer (RIPA) containing complete protease and phosphatase inhibitors with EDTA (Hoffman-LaRoche, Pharma Genentech, USA). Protein content was measured by Bicinchoninic acid protein assay (Thermo Fisher Scientific, USA).

Western blot:
For western blot analysis, spleen tissue homogenates and plasma were separated on a gradient (4-20%) or 10% acrylamide gel under reducing conditions. After transfer to a polyvinylidene fluoride membrane (Immobilon-P, Millipore, USA), proteins were analyzed using Odyssey Infrared Imaging System (LI-COR, USA). Antibodies to β-actin and tubulin were from Abcam while infrared labeled secondary antibodies were from LI-COR, USA. Anti-mouse IgA was from Sigma and the purified mouse IgA was purchased from BD Pharmingen.

Analysis of mRNA:
mRNA was isolated from the spleen using RNeasy Min Kit (Qiagen Sciences, USA) according to the manufacturer’s instructions. For semi-quantitative analysis of expression, cDNA was prepared from purified mRNA and analyzed using 7900HT Fast Real-Time PCR System and TaqMan fluorogenic detection system from Applied Biosystems (California, USA). Validated primers were also purchased from Applied Biosystems. Comparative real-time PCR was performed in triplicates. We analyzed the
following markers: Macrophages (CD68, Mm03047343_m1), B cells (Cd19, Mm00515420_m1), T cells (Cd4, Mm00442754_m1 and CD8a Mm01182108_ml), B-1 cells (CD5, Mm01143417_m1), molecule epsilon (Cd3e, Mm005999683 m1), and Itgax (Mm00498698_m1) were the analyzed in the spleen. Mouse housekeeping gene ornithine decarboxylase antizyme 1 (OAZ1) was used as a normalization control for a Ct method of quantification.

ELISA:
ELISA kits for IgA and IgG were purchased from Alcop and Genway companies. They were used to measure and quantify IgA and IgG protein levels in spleen, plasma and fecal samples according to the manufacturer’s instructions.

Histology:
Spleen tissue was embedded in paraffin before sectioning and staining with hematoxylin and eosin (H&E) which was followed by examination of morphological differences.

Statistical analysis:
Data are shown as mean±SD in each animal group. Group comparison was performed using T-test for statistical analysis between WT and Aldh1A1<sup>−/−</sup> male and female groups.
IgA and IgG production in *Aldh1A1*+/-. than WT mice on regular chow.

ELISA was used to measured IgA and IgG levels in plasma of *Aldh1A1*+/-. and WT mice on regular chow. Data show that plasma IgA was significantly increased (292%) in *Aldh1A1*+/-. compared to WT mice (Fig 1A). Plasma IgG was also 127% higher in *Aldh1A1*+/-. mice as compared to WT, but the differences were not statistically significant (Fig 1B).

In the spleen, IgG levels were significantly (307%) higher in *Aldh1A1* +/- than WT mice as analyzed by ELISA (Fig 2A, C). There was however no differences in total IgA levels (shown by ELISA and western blot) or in kappa light chains between WT and *Aldh1A1* +/- mice (Fig 2B).
Aldh1A1 deficiency decreased body weight but increased spleen weights.

Aldh1A1-/- mice on regular chow had significantly lower body weight (P<0.003 in females and P< 0.012 in males) than WT mice (Fig 3A). However, spleens from Aldh1A1-/- mice were enlarged (140%) compared to WT. (Fig 3B). Hematoxylin and eosin (H&E) staining shows similar spleen morphology in Aldh1A1-/- and WT females (Fig. 3C).

Aldh1A1 increases expression of CD19, CD8 and CD 68 in spleen in mice on regular chow

TaqMan was used to quantify mRNA levels for common inflammatory cell types in WT and Aldh1A1-/- spleens. We used the following markers: CD19 for B cells, CD 68 for macrophages, Itgax for dendritic cells, and CD 8a and CD4 for T cells. Individual mRNA levels for these markers are shown in Fig 4A. Despite of larger spleen mass, Aldh1A1-/- mice express significantly lower levels of B cells, macrophages, and CD8 cells markers with the following P values respectively; P<0.04 (73%), P< 0.003 (60%) and P< 0.026 (70%).

Aldh1A1 suppresses IgG, but not IgA production in fecal in mice on regular chow

Fecal IgG and IgA was measured using ELISA in same mice as in Figure 4. Fecal IgA levels were same between the genotypes (Fig 5A). In contrast, fecal IgG levels were significantly elevated (4000%) higher in Aldh1A1-/- mice on regular chow compared to WT (Fig 5B).
High Fat Diet influences immunoglobulin production in Aldh1A1-/- mice

A high fat diet fed for 180 days (45% saturated fat) augmented the effects of Aldh1A1 on immunoglobulin production. Plasma IgA and IgG levels were significantly increased. IgA levels were 575% higher (Fig 6A) while IgG levels were 741 % (Fig 6B) higher in Aldh1A1-/- as compared to the WT mice. Both immunoglobulin A and G were measured using ELISA. Western blot revealed that these differences were due to the increased expression of kappa light chain in plasma of Aldh1A1-/- as compared to WT mice (Fig 6C & D). There were significantly higher IgG levels in spleen (297%) in Aldh1A1-/- as compared to WT mice (Fig. 7A). Western blot shows increased expression of IgA levels of kappa light chains in both sex groups in Aldh1A1-/- as compared to WT (Fig 7 B & C).
Discussion

Our studies demonstrate that Aldh1A1 participates in the regulation of light and heavy immunoglobulin chains and influences IgA and IgG production. The immunoglobulin concentrations increased or remained unaffected in different tissues of Aldh1A1-/- compared to WT mice with the increase in the immunoglobulin concentration being amplified on a high-fat diet. Aldh1A1 enzyme plays a key role in vitamin A metabolism by generation of RA from retinal, and, thereby, determines concentration of these retinoids. Taken together, our data shows that endogenous metabolism of vitamin A is an important contributor of immunity depending on heavy and light immunoglobulin concentrations in the body. Whereas heavy immunoglobulin chains increase resistance to pathogens, high levels of light immunoglobulins are associated with pathogenic processes including severe inflammation, oxidative stress, and amyloidosis [21-23, 27-28].

Our studies are a first step at understanding physiologic or pathologic processes related to immunoglobulin production and additional studies will need to be performed to more fully understand the role of Aldh1A1 in immunoglobulin production.
Previous studies have investigated how diets enriched in vitamin A or its metabolite RA influence IgA production in animals \[^{10-19}\]. Co-stimulation of RA with elevated IL-5, and IL-6 are associated with IgA secretion in rats \[^{14-16}\]. RA has also been reported to induce gut-homing receptors on B cells and IgA-antibody secreting cells \[^{15-19}\]. However, RA therapies could not be safely used to increase immunoglobulin production. RA is a transcriptionally active metabolite and its administration in humans leads to a common complication known as ‘retinoic acid syndrome’ \[^{24-26}\]. In the body, RA synthesis is mediated by several enzymes, including Aldh1A1 and is under spatiotemporal endocrine control \[^{4-5}\]. These studies were undertaken to investigate whether other vitamin A metabolites in addition to RA influenced immunoglobulin production similar to the effects induced by RA.

Genetic studies done with *Aldh1A1*\(/\)- mice have demonstrated that Aldh1A1 enzyme metabolizes large quantities of retinaldehyde to RA in adult mice \[^{2-6}\]. Aldh1A1 reduces toxic levels of retinol, which is, first, oxidized by ADH1 to retinal \[^{2-4}\]. Studies in Aldh1A1 showed that Aldh1A1 is a major enzyme involved in RA production \[^{4, 6}\]. Based on previous observations on the role of RA in IgA production, we expected a reduction in IgA production in *Aldh1A1*\(/\)- mice. Surprisingly, IgA levels were increased in the plasma (fig. 1 A) and IgG levels were elevated in spleen (fig. 2 C) and feces (fig. 5 B) of *Aldh1A1*\(/\)- mice on a chow diet. Moreover, both IgA (fig. 6 A) and IgG (fig. 6B, 7A) in *Aldh1A1*\(/\)- mice were significantly increased on a high fat diet. The reason for the discrepancies in
the immunoglobulin production in previous studies with RA [13-18], and our investigation in Aldh1A1-/- mice is not known but could be due to various metabolites depending on the influence of Aldh1A1 enzyme deficiency on levels of RA, retinol, retinal, and lipid aldehydes.

Duester and colleagues [3-7], measured the amount of RA in plasma and liver of Aldh1A1-/- mice; two major tissues representative for differences in vitamin A metabolism. They report significantly lower levels of RA in both plasma and livers of Aldh1A1-/- mice as compared to WT. In Aldh1A1-/- the ratio of retinoic acid versus retinal is decreased.

Mice deficient of the Aldh1A1 enzyme showed impaired Rald oxidation and altered fat accumulation when they were fed vitamin A and or a high fat diet [5-6]. This deficiency in RA could indirectly impair B cells differentiation causing pathological immunoglobulin production. We observed that Aldh1A1-/- mice had spleens that were larger in size and weighed significantly higher than the spleens of WT mice (Fig.3 B). Expression of B cell, macrophage and T cells markers were significantly decreased in the spleens of the Aldh1A1-/- as compared to the WT mice suggesting that these cells did not contribute to the increased spleen size (fig. 4). However, we did not measure the B-cells expressing the marker of malignant cells producing high light chains immunoglobulin, such as CD 138, CD79a, and CD56 or other markers of B-cells to account for the increased spleen mass in Aldh1A1-/- mice. Pathological increases in B cell populations could be one reason behind increased levels of IgA and IgG production in the plasma, spleen and feces of Aldh1A1-/- mice fed on a regular chow or a high fat diet. More studies are
needed to address whether Aldh1A1-mediated RA deficiency alters immunoglobulin production or is implicated in the differentiations and maturation of B-cells.

*Aldh1A1* deficiency changes concentration of other vitamin A metabolites that might be responsible for the increased amounts of IgA and IgG in the plasma, spleen and feces of the Aldh1A1-/- mice. For instance, there could be increased levels of retinol in *Aldh1A1*-/ mice as compared the WT due to the absence of a major enzyme that converts retinal to RA. The excess retinal could be reduced to retinol by enzymes such as short chain dehydrogenases/reductases enzymes (SDR). Increased retinol levels can mediate increases in IgA production found in plasma and spleen in *Aldh1A1*-/ similar to the observations in other studies where dietary vitamin A increases IgA production in both rats and mice \[10–13\]. Similar accumulation of retinol, but deficiency in retinal and RA production, was also found in *Adh1A1*-/ \[2, 4–6\]. To gain insight into possible role of retinol, IgA levels were measured in the spleen and plasma of *Adh1*-/ mice fed on a high fat diet (data are not included in this thesis). Data showed no differences in IgA production in the spleen and plasma of *Adh1*-/ and WT mice fed on a high fat diet. The data from these animal models argue against central role of retinol in the increased levels of immunoglobulin found in *Aldh1A1*-/ mice.

It has been reported that retinal is increased in plasma and fat of *Aldh1A1*-/ compared to WT mice \[2-7\]. It is, therefore, plausible that the changes in immunoglobulin levels depend on retinal. Retinal is a transcriptionally-active metabolite \[5–7\]. Retinal could have direct or indirect effects in immunoglobulin production, nonetheless, these effects have
not been investigated yet. Data demonstrating increased immunoglobulin levels in Aldh1A1 deficient mice appear (Figures; 1A, 2C, 5B, 6 A-D, 7A-C) supports possible retinal effects, warranting further investigation. A limitation to this study in relation to vitamin A and its metabolites is that the amounts of vitamin A metabolites retinol, retinal, and retinoic acid were not measured in the plasma, spleen, and bone marrow of the animals in our study. Thus, further studies are recommended to address retinoids concentrations and their effects on immunoglobulins.

In our study, marked changes in immunoglobulin concentrations and increased levels of light chains immunoglobulins were induced by a high-fat diet. It is widely established that high fat feeding increases the production of reactive lipid aldehydes, which modify proteins and induce immune response \[^{[26]}\]. Increased inflammation in mice is associated with increased plasma concentration of light chain immunoglobulins \[^{[21-23,27-28]}\]. We also observed increased light chain immunoglobulins in plasma (fig. 6 C&D) and spleen (fig. 7 C&D) of Aldh1A1-/- as compared to WT mice on a high fat diet. Aldh1A1 is a versatile enzyme, which can utilize many different substrates in addition to retinal \[^{[3]}\]. In vitro studies showed that Aldh1A1 can oxidize lipid aldehydes \[^{[4,5]}\]. Although the relevance Aldh1A1 in the in vivo detoxification of lipid aldehydes is not clear, it is possible that in the absence of Aldh1A1 lipid aldehyde decomposition could be increased and, thereby, augment inflammation. Analysis of lipid aldehyde conjugates and inflammatory
cytokines in plasma and tissue could test this hypothesis and elucidate the Aldh1A1 role in lipid aldehyde clearance in vivo.

One additional explanation for the high light chain immunoglobulin production is the development of multiple myeloma in Aldh1A1-/- mice [20, 28-29]. Some of the symptoms of multiple myeloma include abnormal immunoglobulin A, G and M production in the body. The abnormality occurs in form of increased amount of production in light chain immunoglobulins or an overall increase or decrease in immunoglobulin classes A, G and M production [8, 20]. Our data shows increased concentrations of kappa light immunoglobulins in the spleen and plasma of Aldh1A1-/- mice, especially on a high-fat diet. Whereas increased IgG levels in the plasma of the Aldh1A1-/- mice on a regular chow could be beneficial for the immune response, the abnormal light chain production could be associated with a pathogenesis [21-23, 28-29]. Although our data suggests the possibility of Aldh1A1-/- mice suffering from multiple myeloma, more specific tests such as immunoelectrophoresis for both serum and urine or immunofixation and thorough phenotyping of pathological lesions would provide a definitive proof of multiple myeloma in Aldh1A1-/- mice.

Of note, Aldh1A1 deficiency altered immunoglobulin production differently in various tissues. For instance in there was significantly increased IgG levels in the spleen (fig. 2C) and feces (fig. 5B) of Aldh1A1-/- mice on a chow diet as compared to the WT with no changes in the IgA levels (fig. 2A) in the spleen and feces (fig. 5A) of the same animals. This effect could result from a tissue- or organ-specific Aldh1A1 expression and its
effects on RA generation \[^{3-5}\]. Consequently, knocking out this enzyme in mice led to the disruption of the RA production/pathway differently in various body tissues/organs. This could account for the increased IgA levels (fig.1A) in the plasma of Aldh1A1-/- mice as compared to WT and yet no changes in IgA levels in the spleen (fig 2A) or feces (fig 5A) of the same animals.
Conclusion

Novel therapies targeting immunoglobulin production may provide a solution for millions of patients suffering from mucosal and other immune disorders. Environment and diets influence immunoglobulin production; the identification of genes responsible for immunoglobulin production in response to environment and dietary modification could enable the development of potential novel and effective therapies. Data presented in this thesis support the hypothesis that Aldh1A1 affects immunoglobulin production. It also shows that Aldh1A1 gene could potentially influence light chain and heavy chain immunoglobulin production in mice. While it is evident that Aldh1A1 influences immunoglobulin production in plasma, spleen and feces in mice, further studies are recommended using other immune tissues such as mucosal sites (i.e. Peyer’s patches, mesenteric lymph nodes and lamina propria of the small intestines) or bone marrow. More studies are needed to understand therapeutic or pathogenic potential of Aldh1A1 in the context of different diets and other environmental factors.
Reference:


immunodeficiency virus-infected children in Uganda: a controlled clinical trial. 


Appendix

Figure 1. Increased IgA, but not IgG Production in Plasma in Aldh1A1-/- Compared to WT Mice on Regular Chow. A & B ELISA was used to determine IgA (A) and IgG (B) levels in plasma of Aldh1A1-/- (7 females and 5 males) and WT (5 females and 3 males). Asterisk indicates P< 0.016 (T test). IgG levels were similar in both groups (ns- not significant).
Figure 2. Increased IgG production and Similar IgA levels in Spleen in Aldh1A1−/− Compared to WT Mice on Regular Chow. IgA was analyzed by ELISA (A) and western blot (B) in the same group of mice as in Fig1. Antibodies detects both heavy and light Kappa chains in IgA. (C) IgG levels in spleen measured by ELISA. Asterisk indicates higher levels of IgG in the Aldh1A1−/− than in WT
Figure 3. Body Weights and Increased Spleen Weights in *Aldh1A1*−/− Mice Compared to WT on regular chow. Asterisk indicates significantly higher body weights in WT than *Aldh1A1*−/− mice; *P* < 0.003 and 0.012. A) Body weights of *Aldh1A1*−/− and WT mice. B) Spleen weights of 19 *Aldh1A1*−/− young mice and 15 WT and 10 *Aldh1A1*−/− and 9 WT old mice. Spleen weight was significantly higher in *Aldh1A1*−/− than WT in both groups. C) Spleen from female *Aldh1A1*−/− mice and WT mice were embedded in paraffin followed by H & E staining.
Figure 4. Decreased CD 19, CD 68 and CD8a mRNA Expression in the Spleen of Aldh1A1−/− Compared to WT mice on Regular Chow. Comparative real-time PCR was performed in triplicates to determine mRNA expression of CD19, CD4, CD3e, CD5, CD8a, Itgax, and CD68 in the same group of mice as in Fig 1. P values were determined using T-test. DC 19, CD 8a and CD 68 were significantly decreased in Aldh1A1−/− than WT P<0.04, P< 0.026 and P< 0.003 respectively.( T-test).
Figure 5. Increased IgG production and similar IgA levels in Fecal in Aldh1A1-/- compared to WT Mice on Regular Chow. A & B ELISA was used to determine IgA (A) and IgG (B) levels in feces of Aldh1A1-/- (7 females and 5 males) and WT (5 females and 3 males). IgA levels were similar in both groups (ns- not significant). IgG levels were significantly higher in Aldh1A1-/- than in WT.
Figure 6. Increased IgA, IgG and kappa Light Chains Concentration in Plasma in Aldh1A1-/- Compared to WT Mice on a High Fat Diet. Fig 6 A; ELISA was used to measure IgA levels in plasma in Aldh1A1-/- (4 females and 3 males) and (3 females and 5 males) WT mice. Fig 6 B; Plasma IgG levels in were measured in Aldh1A1-/- mice (4 females and 5 males) and WT (5 females and 7 Males) mice. Fig 6 C; Western blot was used to measure IgA heavy chain and kappa light chains expression in plasma in 4 Aldh1A1-/- female and 2 WT mice. Fig 6 D; Western blot was used to measure IgA heavy chain and kappa light chains expression in plasma in 3 Aldh1A1-/- male mice and 3 WT mice. All mice were fed on 45% saturated high fat diet for 180 days. Asterisk indicates higher levels of IgG in the Aldh1A1-/- than in WT mice(P< 0.008 T-test ).
Figure 7. Increased IgA, IgG and Kappa Light Chain Concentration in Spleen in *Aldh1A1*-/‐ Compared to WT Mice on a High Fat Diet. Animals were same as in group 6. A; ELISA was used to measure IgG levels. Asterisk indicates higher levels of IgG in the *Aldh1A1*-/‐ than in WT mice (P< 0.0001 T test).B; Western blot shows IgA heavy chain and kappa light chains levels in spleen in similar mice as described in group 6C. There a was higher expression of IgA heavy chain and kappa light chains in *Aldh1A1*-/‐ than in WT. C; Western blot was used to measure IgA heavy chain and kappa light chains expression in plasma in the same group of male mice as those in fig 6 D. There was higher expression of IgA heavy chain and kappa light chains in *Aldh1A1*-/‐ than in WT male mice.