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Potential Role for Oral Tolerance in Gene Therapy

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Abstract

Oral immunotherapies are being developed for various autoimmune diseases and allergies to suppress immune responses in an antigen-specific manner. Previous studies have shown that anti-drug antibody (inhibitor) formation in protein replacement therapy for the inherited bleeding disorder hemophilia can be prevented by repeated oral delivery of coagulation factor antigens bioencapsulated in transplastomic lettuce cells. Here, we find that this approach substantially reduces antibody development against factor VIII in hemophilia A mice treated with adeno-associated viral gene transfer. We propose that the concept of oral tolerance can be applied to prevent immune responses against therapeutic transgene products expressed in gene therapy.

Introduction:

Oral delivery of antigens to the immune system of the small intestine often leads to an immune regulatory response that promotes antigen-specific unresponsiveness and is crucial for maintaining tolerance to food antigens [1]. Interestingly, even when this balance is lost and food allergy develops, unresponsiveness can be restored by careful oral dosing of the

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Author statement:

JSSB performed experiments and analyzed data; XL, AS, and KCK contributed to experiments; HD and RWH designed experiments and supervised the study. JSSB, HD, and RWH wrote the manuscript.

Declaration of competing interest:

Henry Daniell and Roland Herzog are inventors in several patents on expression of blood clotting factors in chloroplasts and oral tolerance induction. Their hemophilia research was funded in the past by several major pharmaceutical companies including Bayer, Novo Nordisk, and Shire/Takeda. All other authors declare no conflict to report.

Ethics Statement:

Mice were housed in the laboratory animal resource center facility at Indiana University–Purdue University, Indianapolis (IUPUI). All animal procedures were performed as per the guidelines of Institutional Animal Care and Use Committee (IACUC) under approved protocols.

antigen [2]. Such oral immunotherapies (OIT) have now been successfully developed in the clinic for a variety of food allergies, leading for example to regulatory approval for treatment of peanut allergy [3, 4]. Key attributes of an efficacious method for oral tolerance induction include protection of the antigen from degradation in the stomach and efficient delivery to the immune system across the epithelium of the small intestine. Previously, we showed that bioencapsulated antigens expressed at high levels in chloroplasts of edible plant cells are effective in suppressing the formation of anti-drug antibodies (ADAs) in protein replacement therapy for genetic diseases such as the inherited bleeding disorder hemophilia or lysosomal storage disease [3, 5]. Induction of antigen-specific regulatory T cells (Treg) in the small intestine's immune system is a critical component of the underlying tolerance mechanism [6–9]. To achieve optimal delivery in this system, the antigens are expressed as fusions to the transmucosal carrier CTB (cholera toxin subunit B) [3]. This technology enabled us to extend the concept of oral tolerance beyond treatment of allergies and autoimmune diseases to suppression of ADAs [10, 11].

Plant-based oral antigen administration suppresses gene therapy-induced antibody formation against factor VIII

Building on our previous studies on suppression of neutralizing antibodies (inhibitors) that form upon repeated intravenous delivery of coagulation factors during replacement therapy for hemophilia, we hypothesized that oral administration of bioencapsulated antigen could also suppress inhibitor formation in the context of gene therapy. Inhibitor formation occurs in 20-30% of hemophilia A patients receiving coagulation factor FVIII (FVIII) replacement therapy, representing a serious complication of treatment. Clinical immune tolerance protocols (ITI) are available for reversal of inhibitors [12]. However, ITI protocols are based on daily high-dose intravenous FVIII infusion that may take months to > 1 year and are effective in only ~70% of cases, and no prophylactic protocol for prevention of inhibitors is available. Most current clinical gene therapy approaches for hemophilia employ adeno-associated viral (AAV) gene transfer to hepatocytes [13]. Hepatic gene transfer by itself may induce immune tolerance to coagulation factors or other transgene products, representing another natural route of tolerogenic antigen administration that is characterized by induction of Treg and immune suppressive cytokines [12]. However, coagulation FVIII, the protein that is deficient in hemophilia A (the more common form of the disease), is a highly immunogenic protein [12, 14]. For instance, we found in our preclinical studies that hemophilia A mice on BALB/c background (BALB/c F8e16$^{-/-}$ mice) form high-titer inhibitors against human FVIII upon hepatic AAV gene transfer [15]. Using our published protocol, we fed a mixture of lyophilized lettuce cells expressing the light chain (LC) or heavy chain (HC) of B-domain deleted (BDD) human FVIII (BDD-hFVIII), fused to CTB, by oral gavage twice per week for 8 weeks (1.5 μg per antigen per dose) [10]. Alternatively, a mixture of CTB-HC and CTB-C2 (C2 domain of human FVIII) was gavaged (using the same antigen dose). After 4 weeks, all animals were intravenously injected with 1x10^{11} vector genomes [vg] of hepatotropic AAV8 vector expressing BDD-hFVIII from a liver-specific promoter as published (Fig. 1A) [15]. Control animals that received vector without oral tolerance developed very high-titer inhibitor titers that peaked at week 12 (on average nearly 90 BU/ml, Fig. 1B). In contrast, average inhibitor titers were up to 10-fold lower in animals that were treated with the oral tolerance regimen. CTB-HC/LC

treatment was slightly more effective (although not significantly different) than CTB-HC/C2 treatment (Fig. 1B). Binding antibody titers were also measured for the last time point of the experiment, which showed a similar difference as the Bethesda titers between orally treated and control mice (compare week 16 in Fig. 1B and Fig. 1C).

Other evidence for effectiveness of oral tolerance in gene therapy

Our results support the concept of combining antigen-specific oral tolerance with gene therapy to prevent unwanted immune responses against therapeutic transgene products. Findings by Hardet *et al.* add further support [16]. Inclusion of chicken ovalbumin in drinking water of mice prevented antibody and $CD8^+$ T cell responses against an ovalbumin transgene product expressed from an AAV1 vector upon intramuscular administration. The authors found evidence for upregulation of PD-1 checkpoint molecule and for enhanced deletion/apoptosis of $CD8^+$ T cells but not for dependence of tolerance on $CD4^+CD25^+FoxP3^+$ Treg. However, out studies showed that oral antigen administration induces multiple subsets of Treg that have somewhat redundant immune suppressive functions, with $CD4^+CD25^-FoxP3^-LAP^+$ Treg often being induced at higher frequencies [3, 6, 8]. Others propose that induction of Treg by commensal gut bacteria may aid in maintenance of tolerance to gene therapy approaches that utilize bacterial nucleases for gene editing applications [17]. Further studies are needed to determine if our plant cell-based approach can suppress not only antibody but also $CD8^+$ T cell responses. The plant cell-based delivery platform offers multiple advantages, including efficacy at low antigen doses (owing to bioencapsulation and use of transmucosal carrier), low production costs, stability of the antigen upon long-term storage at ambient temperature (due to freeze drying during the manufacturing process), and lack of toxicity [3, 18]. Indeed, success of oral delivery of Ara h proteins bioencapsulated in peanut cells is the first FDA approved treatment to treat peanut allergy [3], and opened the door for treating protein drug related allergies [3]. However, this drug does not use a transmucosal carrier for enhanced delivery to the immune system, limiting the success of treatment to duration of oral delivery of the antigen. CTB fusion of antigen may facilitate longer duration of tolerance duration (we found up to 7 months or 40% of the average lifespan the mouse strain used in experiments on oral tolerance to factor IX) [19]. In this context, recent FDA approval of CTB fusion protein bioencapsulated in lettuce cells for human clinical trials [IND 154807, NCT05433181], augurs well for advancing plant cell based tolerance induction therapy to the clinic [3, 20].

Outlook

Current approvals for hemophilia gene therapies and enrollment in ongoing clinical trials is limited to adult patients lacking a history of inhibitor formation. In these cases, tolerance was maintained after initiation of gene therapy [13]. The risk of inhibitor formation for gene therapy in pediatric patients with no or limited exposure to FVIII or FIX is unknown. Looking into the future, the risk of inhibitor formation against FVIII in gene therapy may be unknown even in many adult patients due to the use of emicizumab, the bispecific antibody that mimics FVIII function and that is getting more popular for use in pediatric patients [12]. These subjects will therefore have very limited exposure to FVIII antigen by the time they may consider gene therapy. Clinical experience with OIT for food allergies indicates that success and durability of oral tolerance is much enhanced when the protocol is initiated

early in life [2, 3]. While we were not able to completely abolish inhibitor formation in the adult mouse model, starting oral tolerance early in life is likely to further improve the outcome and is feasible given the non-invasiveness of the approach. Combination with hepatic gene transfer, another route that is effective in Treg induction, may be ideal to achieve sustained correction of the disease with much reduced risk of antibody formation against the therapeutic protein.

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References:

[1]. Herzog RW, Weiner HL, Biswas M, Innovation and clinical progress in oral tolerance, Cell Immunol, 386 (2023) 104695. [PubMed: 36898277]

[2]. Kazmi W, Berin MC, Oral tolerance and oral immunotherapy for food allergy: Evidence for common mechanisms?, Cell Immunol, 383 (2023) 104650. [PubMed: 36543052]

[3]. Daniell H, Kulis M, Herzog RW, Plant cell-made protein antigens for induction of oral tolerance, Biotechnol Adv, 37 (2019) 107413. [PubMed: 31251968]

[4]. Macdougall JD, Kim EH, A clinical focus on oral tolerance in the development, prevention, and management of food allergy, Cell Immunol, in press (2023).

[5]. Rana J, Munoz MM, Biswas M, Oral tolerance to prevent anti-drug antibody formation in protein replacement therapies, Cell Immunol, 382 (2022) 104641. [PubMed: 36402002]

[6]. Bertolini TB, Biswas M, Terhorst C, Daniell H, Herzog RW, Pineros AR, Role of orally induced regulatory T cells in immunotherapy and tolerance, Cell Immunol, 359 (2021) 104251. [PubMed: 33248367]

[7]. Gonzalez-Visiedo M, Kulis MD, Markusic DM, Manipulating the microbiome to enhance oral tolerance in food allergy, Cell Immunol, 382 (2022) 104633. [PubMed: 36347161]

[8]. Kumar SRP, Wang X, Avuthu N, Bertolini TB, Terhorst C, Guda C, Daniell H, Herzog RW, Role of Small Intestine and Gut Microbiome in Plant-Based Oral Tolerance for Hemophilia, Front Immunol, 11 (2020) 844. [PubMed: 32508814]

[9]. Bertolini TB, Herzog RW, Kumar SRP, Sherman A, Rana J, Kaczmarek R, Yamada K, Arisa S, Lillicrap D, Terhorst C, Daniell H, Biswas M, Suppression of anti-drug antibody formation against coagulation factor VIII by oral delivery of anti-CD3 monoclonal antibody in hemophilia A mice, Cell Immunol, 385 (2023) 104675. [PubMed: 36746071]

[10]. Kwon KC, Sherman A, Chang WJ, Kamesh A, Biswas M, Herzog RW, Daniell H, Expression and assembly of largest foreign protein in chloroplasts: oral delivery of human FVIII made in lettuce chloroplasts robustly suppresses inhibitor formation in haemophilia A mice, Plant Biotechnol J, 16 (2018) 1148–1160. [PubMed: 29106782]

[11]. Su J, Sherman A, Doerfler PA, Byrne BJ, Herzog RW, Daniell H, Oral delivery of Acid Alpha Glucosidase epitopes expressed in plant chloroplasts suppresses antibody formation in treatment of Pompe mice, Plant Biotechnol J, 13 (2015) 1023–1032. [PubMed: 26053072]

[12]. Arruda VR, Lillicrap D, Herzog RW, Immune complications and their management in inherited and acquired bleeding disorders, Blood, 140 (2022) 1075–1085. [PubMed: 35793465]

[13]. Samelson-Jones BJ, George LA, Adeno-Associated Virus Gene Therapy for Hemophilia, Annu Rev Med, 74 (2023) 231–247. [PubMed: 36103998]

[14]. Kaczmarek R, Piñeros AR, Patterson PE, Bertolini TB, Perrin GQ, Sherman A, Born J, Arisa S, Arvin MC, Kamocka MM, Martinez MM, Dunn KW, Quinn SQ, Morris JJ, Wilhelm AR, Kaisho T, Munoz-Melero M, Biswas M, Kaplan MH, Linnemann AK, George LA, Camire RM, Herzog RW, Factor VIII trafficking to CD4+ T cells shapes its immunogenicity and requires several types of antigen presenting cells, Blood, in press (2023).

[15]. Butterfield JSS, Yamada K, Bertolini TB, Syed F, Kumar SRP, Li X, Arisa S, Pineros AR, Tapia A, Rogers CA, Li N, Rana J, Biswas M, Terhorst C, Kaufman RJ, de Jong YP, Herzog RW, IL-15 blockade and rapamycin rescue multifactorial loss of factor VIII from AAV-transduced hepatocytes in hemophilia A mice, Mol Ther, 30 (2022) 3552–3569. [PubMed: 35821634]

[16]. Hardet R, Chevalier B, Dupaty L, Naimi Y, Riou G, Drouot L, Jean L, Salvetti A, Boyer O, Adriouch S, Oral-tolerization Prevents Immune Responses and Improves Transgene Persistence Following Gene Transfer Mediated by Adeno-associated Viral Vector, Mol Ther, 24 (2016) 87–95. [PubMed: 26265250]

[17]. Wagner DL, Peter L, Schmueck-Henneresse M, Cas9-directed immune tolerance in humans-a model to evaluate regulatory T cells in gene therapy?, Gene Ther, 28 (2021) 549–559. [PubMed: 33574580]

[18]. Srinivasan A, Herzog RW, Khan I, Sherman A, Bertolini T, Wynn T, Daniell H, Preclinical development of plant-based oral immune modulatory therapy for haemophilia B, Plant Biotechnol J, 19 (2021) 1952–1966. [PubMed: 33949086]

[19]. Verma D, Moghimi B, LoDuca PA, Singh HD, Hoffman BE, Herzog RW, Daniell H, Oral delivery of bioencapsulated coagulation factor IX prevents inhibitor formation and fatal anaphylaxis in hemophilia B mice, Proc Natl Acad Sci U S A, 107 (2010) 7101–7106. [PubMed: 20351275]

[20]. Daniell H, Nair SK, Esmaeili N, Wakade G, Shahid N, Ganesan PK, Islam MR, Shepley-McTaggart A, Feng S, Gary EN, Ali AR, Nuth M, Cruz SN, Graham-Wooten J, Streatfield SJ, Montoya-Lopez R, Kaznica P, Mawson M, Green BJ, Ricciardi R, Milone M, Harty RN, Wang P, Weiner DB, Margulies KB, Collman RG, Debulking SARS-CoV-2 in saliva using angiotensin converting enzyme 2 in chewing gum to decrease oral virus transmission and infection, Mol Ther, 30 (2022) 1966–1978. [PubMed: 34774754]

Highlights:

1. Oral immunotherapy actively suppresses antibody formation against the transgene product in gene therapy for hemophilia
2. Oral immunotherapy may be more broadly beneficial in combination with gene therapy to promote antigen-specific tolerance

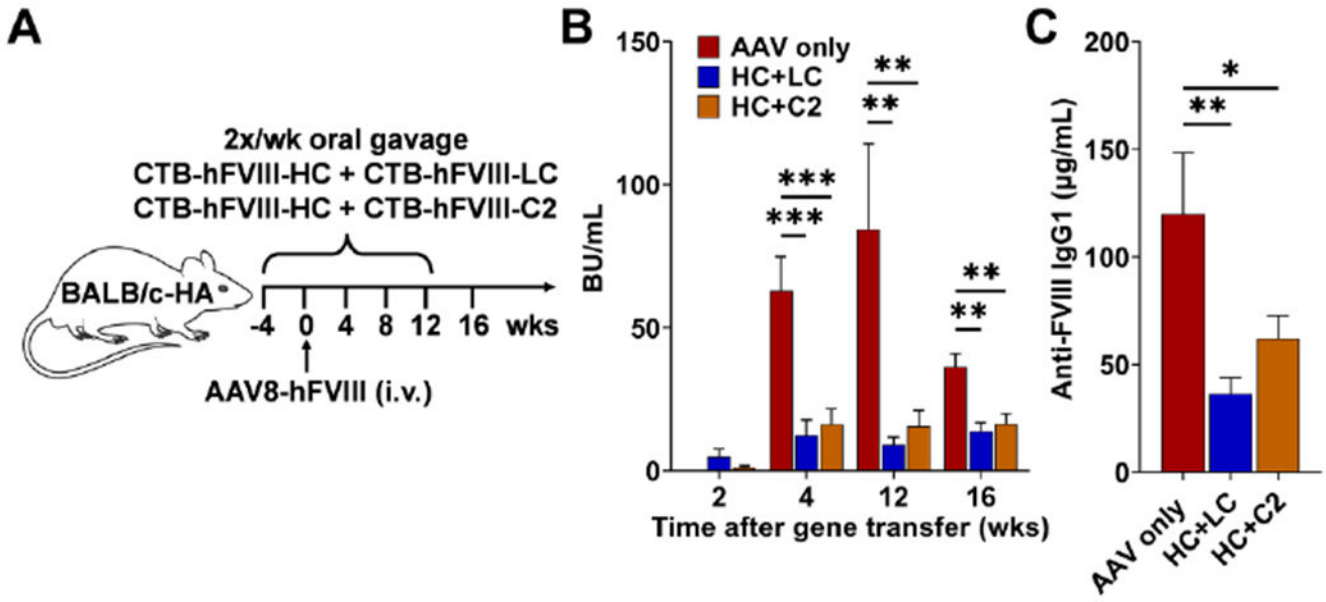

Fig. 1.

Oral tolerance induction in hemophilia A mice (male BALB/c F8e16$^{-/-}$, 6 weeks of age). Lyophilized lettuce leaf cells were administered by oral gavage twice per week after suspension in sterile PBS. The chloroplast transgenic cells expressed cholera toxin B subunit (CTB) fused to heavy chain, light chain, or C2 domain of B-domain deleted hFVIII. HC/LC or HC/C2 antigen combinations were given at doses of 1.5 µg/antigen. **A.** Experimental outline: Oral delivery was performed for 8 weeks, and AAV8-hFVIII vector was administered after 4 weeks at a dose of 1x10^{11} vg/mouse. **B.** Inhibitor titers against FVIII as a function of time after gene transfer. **C.** IgG1 anti-hFVIII titers 16 weeks after gene transfer. Group sizes: AAV only (control group, no oral gavage): n=5, HC+LC: n=6, HC+LC: n=8. Statistical comparisons were performed using separate one-way ANOVA at each time point corrected with Tukey post hoc test. * P<0.05. ** P<0.01. *** P<0.001. **** P<0.0001.