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Increased sensitivity of Apolipoprotein E knockout mice to swainsonine dependent immunomodulation

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ABSTRACT

The mechanisms that mediate accelerated atherosclerosis in autoimmune diseases remain unclear. One common mechanism that has been documented in autoimmune diseases and atherosclerosis is formation of hypoglycosyalted N-glycans on the cell surface. In this study we tested the effects of swainsonine, a class II α-mannosidase inhibitor which results in formation of hypoglycosylated N-glycans, on atherogenesis and immune cell dynamics in the atheroprone and hypercholesterolemic ApoE −/− mouse. Wild type or ApoE−/− mice (8 weeks of age) were fed a normal chow diet and administered swainsonine via the drinking water for 8 weeks at which time, atherosclerosis, and systemic markers of markers of inflammation were evaluated. Interestingly, no change in the rate of atherosclerosis development was observed in ApoE −/− mice treated with swainsonine. However, swainsonine significantly increased the number of peripheral blood leukocytes in ApoE −/− mice, with trends toward similar increases in swainsonine treated wild type mice noted. Assessment of leukocyte subsets using specific markers of all major blood lineages indicated that the increase in circulating leukocytes was due to the elevated number of progenitor cells. Consistent with swainsonine having a greater effect in ApoE −/− vs. wild type mice, increases in circulating inflammatory markers (IgA, IgG and chemokines) were observed in the former. Collectively, these data demonstrate that predisposition of ApoE −/− mice to vascular disease is associated with sensitization to the immunomodulatory effects of swainsonine and indicate that changes in N-glycans may provide a mechanism linking autoimmunity to atherogenesis.

Introduction

Autoimmune diseases represent one of the fastest growing categories of disease afflicting over 9% of the population (Cooper et al., 2009). A common sequelae of these diseases is accelerated cardiovascular disease including atherosclerosis (Skaggs et al., 2012; Gerli et al., 2007; Karmon et al., 2012). While the genetic and environmental risk factors underlying development and onset of autoimmune diseases vary, most are characterized by changes in protein N-glycosylation with enrichment in mannose rich structures (van Kooyk and Rabinovich, 2008; Green et al., 2007; Chui et al., 2001), and recently it has been demonstrated that these same alterations on endothelial cells are associated with atherosclerosis development (Scott et al., 2012, 2013).

N-glycosylation is an enzyme driven post-translational modification of proteins whereby carbohydrates are added onto the amide residue of asparagines in an N-X-S/T sequon. N-glycans mature from a high-mannose to hybrid to complex state as they transit through the endoplasmic reticulum and Golgi complex. Critical to this maturation process is removal of mannose residues by a family of alpha-mannosidase enzymes that allows for addition of N-acetylgluosamine branches which support further monosaccharide additions. Under normal physiologic conditions the overwhelming majority of N-glycans are thought to be complex. During autoimmune disease however, these N-glycans fail to develop into the fully complex state with their maturation halted at the high mannose and hybrid state (hereafter referred to as hypoglycosylated N-glycans) with these epitopes now being recognized by the hosts innate immune system as “non-self” danger signals which promote

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systemic immune activation (Rabinovich et al., 2012; Rachmilewitz and Glycosylation, 2010).

In support, transgenic mice that fail to generate complex N-glycans develop autoimmune associated inflammation (Green et al., 2007; Chui et al., 2001; Lee et al., 2007; Malhotra et al., 1995) and display elevated T-cell activation (Demetriou et al., 2001; Bowlin et al., 1989) and proliferation (Mkhikian et al., 2011). Pharmacological inhibition of N-glycan maturation *in vivo* is possible through inhibition of alpha-mannosidase with compounds such as swainsonine, a class II alpha-mannosidases inhibitor which restricts N-glycan maturation at the hybrid state. Prolonged administration of swainsonine is known to induce autoimmune-like phenotypes including lupus like renal disease (Huxtable and Dorling, 1983) and exposure of mice or cells to swainsonine leads to elevated secretion of certain glycoproteins and inflammatory cytokines including interferon-γ (Bowlin et al., 1989; Morgan et al., 2004; Yeo et al., 1985). Additionally, swainsonine is a known immunomodulator that induces progenitor cell proliferation and release into the circulation in rodents and has been considered to boost immune cell function in cancer patients (Oredipe et al., 2003; White et al., 1991). Mice deficient in the MAN2A gene, one of the protein targets of swainsonine, are susceptible to development of autoimmunity characterized by increased T-cell activation, increased levels of circulating immunoglobulins and immune complex mediated glomerular nephritis (Chui et al., 2001). Indeed, several studies have now shown that loss of N-glycan branching in T-cells is associated with hyper-activation and increased proliferation (Lee et al., 2007; Mkhikian et al., 2011).

Because of this apparent association of hypoglycosylation with both atherosclerosis and autoimmune diseases, and due to the correlation of the diseases states with each other, in the current work we sought to examine if atherosclerosis prone ApoE −/− mice, which have also been used in models of autoimmunity and atherosclerosis (Aprahamian et al., 2004; Richez et al., 2013), would be more susceptible to the immunomodulatory effects of swainsonine than wild type mice. Herein we show that swainsonine induces increased immunomodulatory effects on ApoE −/− mice compared to wild type as measured by increased levels of circulating leukocytes, increases in serum IgG and IgA, and increased levels of circulating cytokines.

Results

Phenotypic effects of swainsonine ingestion

ApoE −/− mice are intrinsically hypercholesterolemic and therefore eventually develop atherosclerosis even when fed a standard chow diet. In contrast, many studies utilize atherogenic diets (high fat/high cholesterol diets or diets supplemented with cholate) in order to accelerate the disease. In the current study we opted for a standard chow diet regimen as opposed to a atherogenic diet to test the effects of swainsonine in the presence of a milder hypercholeterolemic/inflammatory background. Fig. 1 shows that increased cholesterol (Fig. 1A) and triglyceride (Fig. 1B) levels in ApoE −/− mice compared to WT mice was not affected by swainsonine treatment. As mentioned above, previous reports indicate that swainsonine induces progenitor cell expansion in normocholesterolemic mice and increases total circulating leukocyte counts so we next determined if a similar effect would be observed in ApoE −/− mice. As seen in Fig. 1C, a trend toward increased levels of circulating leukocytes in wild type mice treated with swainsonine was observed (not significant by one-way ANOVA, but $p = 0.01$ by *t*-test between wt and wt + SW). This effect was however amplified and significant in ApoE −/− where swainsonine increased the levels of leukocytes significantly above all other groups including the treated wild type animals. These data show that swainsonine administration does not alter the hypercholesterolemic phenotype of ApoE −/− mice, but that these mice display an increased sensitivity to swainsoinine dependent expansion of leukocyte populations.

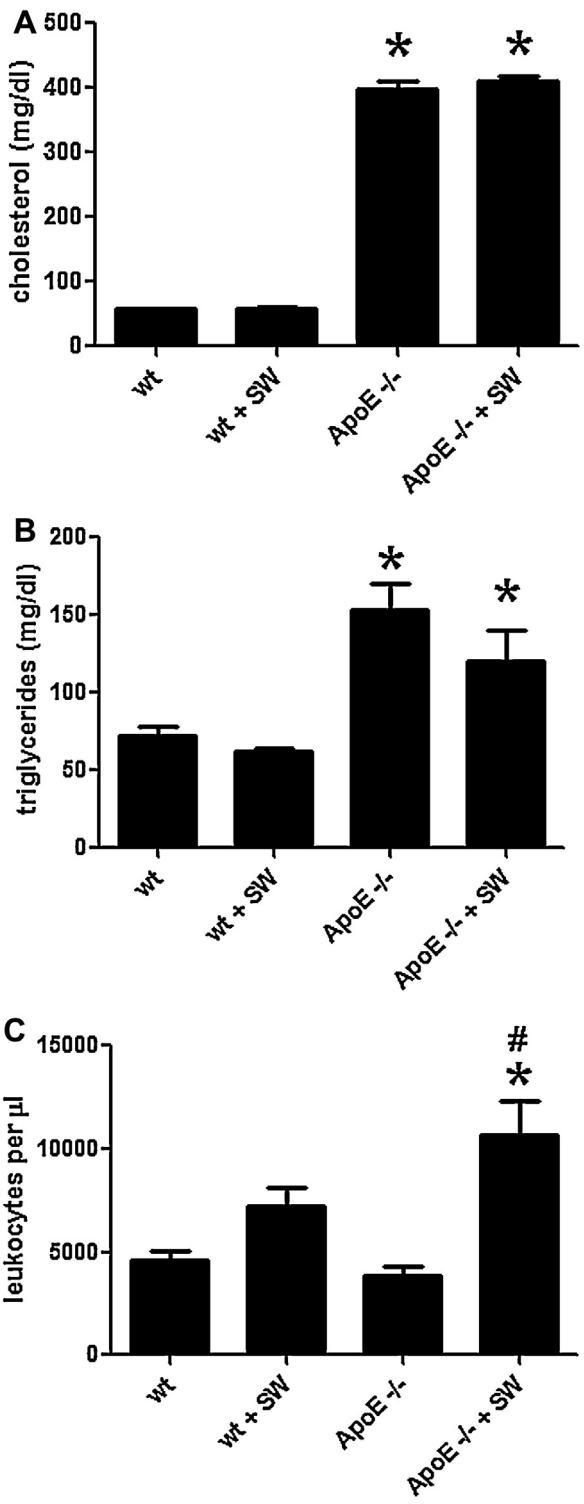

Fig. 1. Swainsonine does not alter the hypercholesterolemia phenotype of ApoE −/− mice. Mice were treated as described in Section "Methods" and serum was collected by retro orbital bleed and analyzed for total cholesterol (A), triglycerides (B), and total leukocyte counts (C). $^{*}p < 0.05$ by one-way ANOVA versus wt and wt + SW in (A, B, and C) and $^{\#}p < 0.05$ versus ApoE −/− in (C). There were four mice analyzed for each treatment condition.