



Goat Milk–Derived Lipids Restrain NK T Cell–Dependent Eosinophilic Inflammation in a Murine Model of Atopic Dermatitis

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TO THE EDITOR

Atopic dermatitis (AD) is a debilitating disease that disproportionately affects infants and young children and is considered the first step of the atopic march—the age-related progression of allergic disease. Consequently, there is an urgent unmet need to elucidate the root causes of AD and evaluate potential prevention mechanisms.

Anecdotal evidence suggests that cow milk avoidance and substitution with other mammalian milk sources, such as goat milk (GM), has the potential to ameliorate AD symptoms. However, it remains unclear to what extent underlying cow milk allergy may have confounded these observations. In this study, we assessed the therapeutic benefit of GM in AD in the absence of cow milk allergy and present a set of data suggesting that the noticeable therapeutic activity of GM against AD is mediated through the sensing of GM-derived lipids by NK T (NKT) cells and the effects of it on NKT cell–dependent recruitment of inflammatory eosinophils.

To determine whether the consumption of GM infant formula (GMF) can decrease AD disease severity, we used a previously described MC903-driven model of AD-like disease (Li et al., 2009, 2006; Naidoo et al., 2021, 2018). All strains of mice were housed under specific pathogen-free condition at the Malaghan Institute of Medical Research (Wellington, New Zealand). All experimental procedures were approved by the Victoria University of Wellington Animal Ethics Committee and performed according to institutional guidelines. Because milk-derived lipids have been previously shown to

bind CD1d and modulate NKT-cell activity (Brennan et al., 2017), we hypothesized that the potential therapeutic activity of GMF may be at least partly mediated by CD1d/NKT cells. Thus, both wild-type C57BL/6 and NKT cell–deficient *Cd1(d)*^{−/−} mice were supplemented daily with GMF for a week before and during the 19 day–long MC903 AD model (Figure 1a). Mucosal-associated invariant T cell–deficient *Mr1*^{−/−} mice served as positive controls for therapeutic activity, as recently described (Naidoo et al., 2021), as well as negative controls for the abrogation of any lipid-mediated disease reduction.

Interestingly, in the absence of GMF supplementation, *Cd1*^{−/−} and *Mr1*^{−/−} mice showed comparably reduced ear thickening and skin barrier dysfunction (as assessed by transepidermal water loss), indicating that both mucosal-associated invariant T and NKT cells promote disease progression in this model of AD (Figure 1b and c). Daily administration of GMF did also exhibit therapeutic activity, leading to significant reductions of ear thickness in wild-type and *Mr1*^{−/−} mice, but as hypothesized, provided no further benefit to *Cd1*^{−/−} mice. Next, we compared GMF with two other GM products with contrasting lipid contents, that is, reconstituted whole-GM powder (29.5% milk fat in dry matter) and skim GM powder (1% milk fat in dry matter). Consistent with a lipid-mediated mode of action, GMF (26% total fat, 13% milk fat in dry matter) and whole-GM powder had a similar effect on disease progression, whereas skim GM powder had no detectable activity (Figure 1d). Because the lipid- and CD1-dependent

reduction in disease severity may have been mediated by either type 1 (invariant) or type 2 NKT cells, we compared *Cd1*^{−/−} mice, which are deficient in both types of NKT cells, with mice lacking the invariant NKT (iNKT) cell receptor α -chain gene segment TRAJ18 (*Ja18*^{−/−}), which express CD1 but lack iNKT cells. As shown in Figure 1e, *Ja18*^{−/−} mice closely phenocopied *Cd1*^{−/−} mice, in support of an iNKT cell–dependent mechanism. Completing these observations, GM-derived lipids displayed iNKT cell agonistic activity when presented by plate-bound CD1d in vitro, as evidenced by side-by-side comparison with the prototypical iNKT cell agonist α -galactosylceramide (Figure 1f). GM-derived lipids may thus have both iNKT cell stimulatory and regulatory properties, similar to those of recently described endobiotic glycosphingolipids (Oh et al., 2021).

Because the role of NKT cells in the context of AD remains to be elucidated, we sought to provide a tentative mechanism whereby NKT cells contribute to disease progression—a mechanism that could ultimately be modulated through dietary lipids. We previously reported the eosinophil dependence of MC903-driven AD (Naidoo et al., 2018) and, using the same model, have more recently described the function of mucosal-associated invariant T cells as cellular checkpoints for eosinophil activation in situ (Naidoo et al., 2021). Because we comparatively quantified eosinophilia in wild-type, *Mr1*^{−/−}, and *Cd1*^{−/−} ears, it became apparent that the significant reductions in ear thickness (Figure 1b) and transepidermal water loss (Figure 1c) observed in *Mr1*^{−/−} and *Cd1*^{−/−} mice reflected distinct mechanistic pathways. Indeed, although mucosal-associated invariant T cells exclusively control eosinophil activation (Figure 1g and h) (Naidoo et al.,

Abbreviations: AD, atopic dermatitis; GM, goat milk; GMF, goat milk infant formula; iNKT, invariant NK T; NKT, NK T

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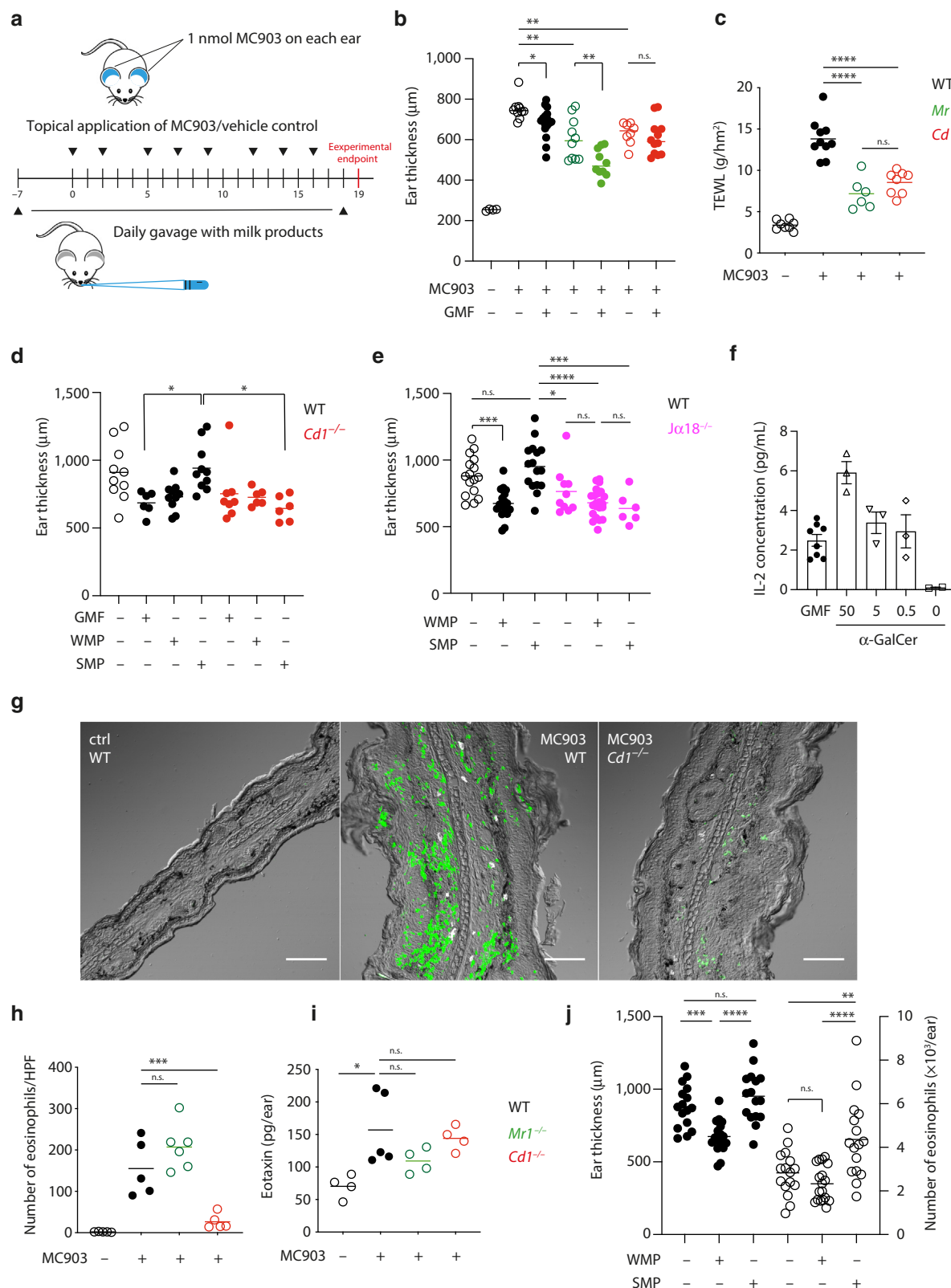


Figure 1. Goat milk-derived lipids modulate NKT-eosinophil axis in atopic dermatitis. (a) Treatment scheme. Male and female mice were used between ages 6 and 14 weeks. Milk products (GMF, WMP, SMP) were reconstituted in water at the concentration used for human consumption (14.6 g/100 ml) and fed either by gavage or provided ad libitum, which yielded similar results. At the experimental endpoint, ear thickness, TEWL, and eosinophil quantification by confocal microscopy and flow cytometry were performed as previously described (Naidoo et al., 2021). (b) Effect of GMF on ear thickness on day 19 of MC903 treatment. (c) Comparative skin barrier dysfunction, as measured by TEWL, of WT, *Mr1^{-/-}*, and *Cd1^{-/-}* mice treated with MC903. (d, e) Ear thickness on day 19 of MC903 treatment, after daily administration of GMF, WMP, or SMP. (f) NKT-cell antigenic activity of GMF (146 mg/ml) and the NKT-cell agonist α -GalCer (in ng per well), as assessed by IL-2 quantification in the supernatants of 10⁵ mouse NKT hybridoma cells (DN32.D3) after 24 h incubation. (g) Confocal imaging of Siglec-

2021), the recruitment of eosinophils seems in turn to largely rely on NKT cells, in an eotaxin-independent manner (Figure 1i). Eosinophil recruitment was also responsive to dietary GM intervention, in a milk fat-dependent manner (Figure 1j). However, the ability of whole-GM powder to thwart eosinophil recruitment did not fully match its effect on ear thickness (Figure 1j).

Together, these results suggest that consumption of full-fat GM products can have a beneficial effect on AD disease severity, possibly by restraining iNKT cell-dependent eosinophilia. Because topical administration of MC903 drives AD-like skin inflammation in a thymic stromal lymphopoietin-dependent and -obligate manner, it is tempting to speculate that MC903 exerts its activity, at least partly, through thymic stromal lymphopoietin-mediated NKT-cell activation (Nagata et al., 2007; Ziegler and Artis, 2010) and subsequent NKT cell-dependent eosinophil recruitment, as observed in this study. However, because the MC903 AD-like model is conducted in the absence of allergen, the therapeutic effect of dietary NKT-cell modulation in disease settings comprising allergen-specific T helper 2 cells and/or additional alarmins (e.g., IL-33) remains to be determined. Finally, further research is warranted to appropriately compare GM with cow and human milk and determine the interspecies variations in milk-derived NKT cell modulators.

Data availability statement

All data underlying the results are available as part of the article.

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AUTHOR CONTRIBUTIONS

Conceptualization: EC, OG; Data Curation: KW, AC, KG, KN, CB; Writing - Original Draft Preparation: OG; Writing - Review and Editing: KW, AC, KG, KN, CB, EC

CONFLICT OF INTEREST

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F⁺ eosinophils (green). Bar = 50 μ m. (h) Associated eosinophil counts per HPF. (i) In situ eotaxin concentrations for WT, $Mr1^{-/-}$, and $Cd1^{-/-}$ mice. (j) Comparative ear thickness (solid circles, left y-axis) and eosinophilia (open circles, right y-axis) in WMP- and SMP-supplemented WT mice. Plots show individual data points and their means. Data are representative of up to three independent experiments. Statistics were calculated using one-way ANOVA with Tukey posthoc test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and **** $P < 0.0001$. α -GalCer, α -galactosylceramide; ctrl, control; GMF, goat milk infant formula; h, hour; HPF, high-powered field; NKT, NK T; n.s., nonsignificant; SMP, skim goat milk powder; TEWL, transepidermal water loss; WMP, whole goat milk powder; WT, wild type.