Role of keratinocytes GPR109A and COX-2 in nicotinic acid and monomethyl fumarate induced flushing

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Introduction

The anti-dyslipidemic drug nicotinic acid and the anti-psoriatic drug monomethyl fumarate induce cutaneous flushing through the activation of the G-protein-coupled receptor GPR109A. Flushing is a troublesome side effect of nicotinic acid, but may be a direct reflection of the wanted effects of monomethyl fumarate. Here we analysed the mechanisms underlying GPR109A-mediated flushing and show that both Langerhans cells and keratinocytes express GPR109A. Using cell ablation approaches and transgenic cell type-specific expression of GPR109A in Gpr109a$^{-/-}$ mice, we provide evidence that the early phase of flushing depends on GPR109A expressed on Langerhans cells, whereas the late phase is mediated by GPR109A expressed on keratinocytes. Interestingly, the first phase of flushing is blocked by a selective cyclooxygenase-1 (COX-1) inhibitor, and the late phase is sensitive to a selective COX-2 inhibitor. Both, monomethyl fumarate and nicotinic acid, induce PGE_2 formation in isolated keratinocytes through activation of GPR109A and COX-2.

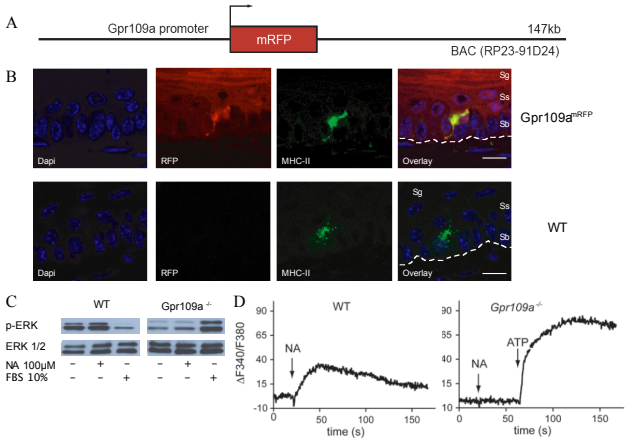

Fig. 1. Keratinocytes express GPR109A. A, scheme of the Gpr109a reporter transgene. B, Gpr109a expression in epidermis. Shown are sections through the epidermis of wild-type and of Gpr109a^{mRFP} mice. Cell nuclei were stained with DAPI, and keratinocytes and Langerhans cells were visualized by immunofluorescence labeling with antibodies directed against cytokeratin and MHC-II. The mRFP-fluorescence was detected in parallel to visualize GPR109A expression. Dotted line, basal membrane; Sb, Stratum basale; Ss, Stratum spinosum; Sg, Stratum granulosum. C, effect of 100 µM nicotinic acid (NA) and fetal bovine serum (FBS) on ERK1/2 phosphorylation in keratinocytes prepared from wild-type (WT) or GPR109A-deficient mice (Gpr109a$^{-/-}$). D, effect of 100 µM nicotinic acid (NA) on the intracellular free $[Ca^{2+}]_i$ in Fura-2-loaded keratinocytes from wild-type (WT) or GPR109A-deficient mice (Gpr109a$^{-/-}$).

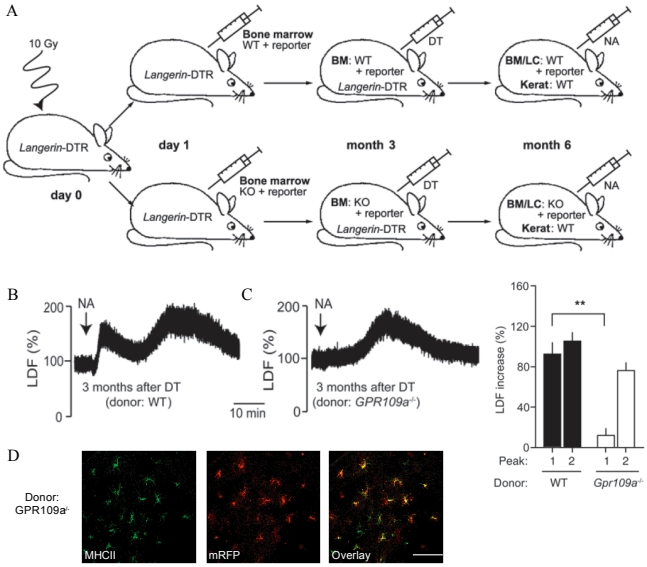

Fig. 2. Gpr109a expressed by Langerhans cells mediated only the early phase of nicotinic acid–induced flushing.
A, experimental scheme. One day after irradiation, Langerin-DTR mice were transplanted with bone marrow from wild-type (Gpr109a$^{+/+}$) or Gpr109a-deficient mice (Gpr109a$^{-/-}$) carrying in both cases the Gpr109a expression reporter transgene (Gpr109a^{mRFP}). Three months after the transplantation, both groups were treated with diphtheria toxin, and three months later, nicotinic acid-induced flushing was evaluated. B-C, nicotinic acid (NA) induced flushing three months after diphtheria toxin treatment. D, fluorescence image of epidermal sheet from transplanted animals three months after diphtheria toxin treatment stained with an anti-MHC-II antibody and analyzed for mRFP-expression.

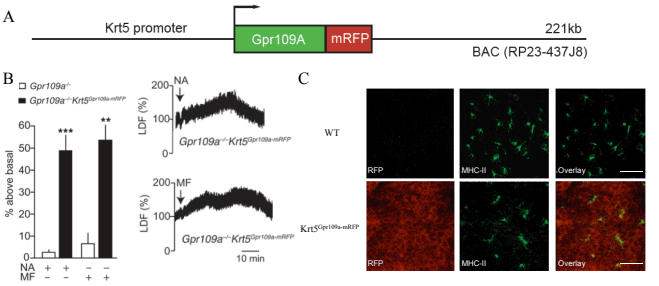

Fig. 3. Expression of GPR109A in keratinocytes is sufficient to mediate the late phase of nicotinic acid-induced flushing.
A, scheme of the Krt5$^{Gpr109a-mRFP}$ transgene. B, effect of nicotinic acid (NA) and monomethyl fumarate (MF) on flushing in Gpr109a-deficient mice and on Gpr109a-deficient mice carrying the Krt5$^{Gpr109a-mRFP}$ transgene. Data are presented as mean ± S.E.M. (n>6). C, fluorescence image of epidermal sheets prepared from wild-type and Krt5$^{Gpr109a-mRFP}$ mice. Shown are en face views of epidermal sheets stained with anti-MHCII antibodies and analyzed for expression of mRFP.

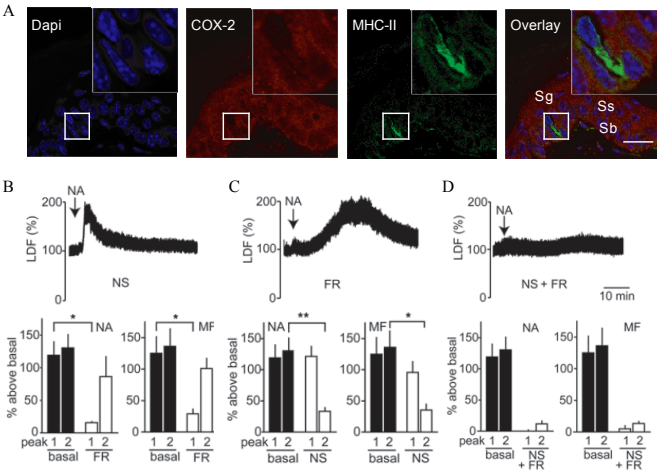

Fig. 4. Roles of COX-1 and COX-2 in GPR109A-mediated flushing. A, analysis of COX-2 expression in the epidermis. Shown are transversal sections of the tail epidermis stained with DAPI and with antibodies specific to COX-2 and MHC-II. Dotted line, basal membrane; Sb, Stratum basale; Ss, Stratum spinosum; Sg, Stratum granulosum. B-D, flushing responses induced by nicotinic acid (NA) and monomethyl fumarate (MF) in wild-type mice treated in the presence of 5 mg/kg of the COX-1 inhibitor FR122047 (B), 10 mg/kg of the COX-2 inhibitor NS398 (C), or pre-treated with both inhibitors (D). Shown are representative traces as well as the quantification of at least four experiments. Data are presented as means ± S.E.M. (n ≥ 4).

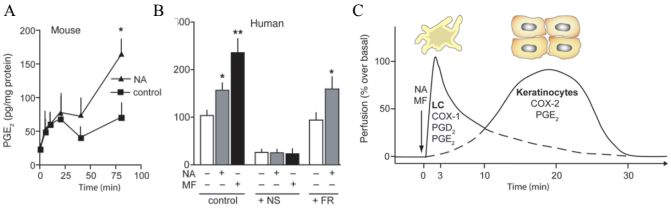

Fig. 5. GPR109A-mediated stimulation of prostanoid release from keratinocytes.
A, time course effect of nicotinic acid (NA) on the release of PGE_2 from mouse keratinocytes. B, effect of nicotinic acid (NA) and monomethyl fumarate (MF) on the release of PGE_2 from human keratinocytes. Keratinocytes were left untreated or were pretreated with the COX-2 inhibitor NS398 (10 µM) or COX-1 inhibitor FR122047 (5µM). Data shown are mean values +/- S.E.M (n ≥ 4). C, proposed model for the local mechanism underlying GPR109A-mediated flushing. Application of GPR109A agonists results in biphasic increase in dermal blood flow, which results from activation of GPR109A on Langerhans cells, which is responsible for the first phase and from the activation of GPR109A on keratinocytes, which is responsible for the late phase of the response.

Conclusions

Here we show that nicotinic acid and monomethyl fumarate induced flushing results from two GPR109A-mediated mechanisms which involve Langerhans cells and keratinocytes as well as different prostanoid forming enzymes. These data will help to further improve flush-reducing strategies in patients taking GPR109A agonists like nicotinic acid by combined inhibition of COX-1 and COX-2 or PGD_2 and PGE_2 activities. In addition, the presented data shed new light on the mechanisms of action of the anti-psoriatic drug monomethyl fumarate and suggest that GPR109A expressed on epidermal cells but also on other cells mediates anti-inflammatory effects.