

Supplementary Information

A *Trichuris* whipworm parasite releases its p43 immunomodulatory protein loaded with selected eicosanoid signalling lipids

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1. Expanded liquid chromatography/mass spectrometry (LC-MS) and analytical methods.

Global Metabolomic Analysis

Solvent extraction was performed by adding 1 ml of chloroform:methanol:water (1:3:1, v/v/v) to 25 µL purified p43 protein before centrifuging (13,000g for 3 min at 4°C). The supernatant was analysed by liquid chromatography-mass spectrometry (LC-MS) using a Thermo Orbitrap Q-Exactive mass spectrometer coupled to a Dionex UltiMate 3000 RSLC platform (Thermo Fisher Scientific, Hemel Hempstead, UK). All samples were analysed in both positive and negative ion mode over the mass to charge (m/z) range 70-1050 at a resolution of 70,000. The samples (10 µL) were injected onto a ZIC-pHILIC column (150 mm × 4.6 mm, 5 µm column, Merck Sequant) maintained at 25°C. Mobile phase A consisted of water containing 20 mM ammonium carbonate and mobile phase B consisted of acetonitrile. The initial conditions for analysis were 80% A/20% B. The percentage of mobile phase B was increased to 80% over 15 min using a linear gradient, followed by an increase to 95% B, which was held for 2 min before re-equilibration with the starting conditions for 4 min. The flow rate was 300 µl/min. All solvents were LC-MS grade (Rathburn Chemicals, Walkerburn, UK).

The raw LC-MS data were processed with IDEOM (with the default parameters) (1), which uses the XCMS (2) and mzMatch (3) software in the R environment. Putative metabolite identification was performed by matching mass to charge ratios (m/z) of detected peaks with entries in-house databases and a mass error set to 5 ppm.

Targeted Lipid Analysis

Methanol was added to purified P43 protein after which samples were centrifuged (13,000g for 10 min at 4°C), adjusted to <10% methanol by the addition of water and acidified to pH 3.5 with 1 M HCl. The sample was applied to a solid-phase extraction (SPE) cartridges (Isolute C18 500 mg/6 ml, Biotage, Uppsala, Sweden), which had been conditioned with 2 × 6 ml methanol, followed by 2 × 6 mL water. Samples were washed with 6 ml water and 2 × 6 mL hexane before elution of lipid mediators with 2 × 3 ml ethyl acetate. The eluate was evaporated to dryness under nitrogen and reconstituted in 200 µl methanol:water (1:1, v/v).

Analysis of the lipid mediators was performed on a Thermo Altis triple quadrupole mass spectrometer interfaced with a Vanquish Flex UHPLC System (Thermo Scientific, Hemel Hempstead, UK). Samples (10 µl) were injected onto Thermo Hypersil Gold C18 column (2.1 mm×100 mm; 1.9 µm) maintained at 35 °C. Mobile phase A consisted of 90:10 (v/v) water: methanol with 0.1% acetic acid and mobile phase B contained methanol with 0.1% acetic acid. The gradient increased from 45-100% mobile phase B over 18 min and the flow rate was 400 µl/min. All solvents used were LC-MS grade (Rathburn Chemicals, Walkerburn, UK). The mass spectrometer was operated in negative ion mode and data were acquired using the selected reaction monitoring (SRM). Lipids were identified on the basis of characteristic ion pair transitions (EPA, 301/257; DHA, 327/283; PGE2, 351/189; 5.6-EET, 319/191; 15-HETE, 319/ 175) and matching retention time with authentic standards purchased from Cayman Chemical (Ann Arbor, MI, USA). The LC-MS/MS data were processed with Thermo Xcalibur v2.1 (Thermo Scientific).

2. Picture credits for Figure 1.

Credits - eden.gov.uk; kalusugan.ph; stock.adobe.com; qiagen.com; dentalzone.com; birchbiotech.com; thermofisher.com; chemical structures all common property

3. References

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