Maresin conjugates in tissue regeneration biosynthesis enzymes in human macrophages

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Macrophages are central in coordinating immune responses, tissue repair, and regeneration, with different subtypes being associated with inflammation-initiating and proresolving actions. We recently identified a family of macrophage-derived proresolving and tissue regenerative molecules coined maresin conjugates in tissue regeneration (MCTR). Herein, using lipid mediator profiling we identified MCTR in human serum, lymph nodes, and plasma and investigated MCTR biosynthetic pathways in human macrophages. With human recombinant enzymes, primary cells, and epi-anatomically pure compounds we found that the synthetic maresin epoxide intermediate 13S,14S-eMaR (13S,14S-epoxy-4Z,7Z,9E,11E,16Z,19Z-docosahexaenoic acid) was converted to MCTR1 (13R-glutathionyl, 14S-hydroxy-4Z,7Z,9E,11E,13R,14S,16Z,19Z-docosahexaenoic acid) by LTC4s and blocked LTC4 and increased resolvins and lipoxins. The conversion of MCTR1 to MCTR2 (13R-cysteinylglycinyl, 14S-hydroxy-4Z,7Z,9E,11E,13R,14S,16Z,19Z-docosahexaenoic acid) by LTC4s inhibitors blocked LTC4 and increased resolvins and lipoxins. The conversion of MCTR1 to MCTR2 (13R-cysteinylglycinyl, 14S-hydroxy-4Z,7Z,9E,11E,13R,14S,16Z,19Z-docosahexaenoic acid) was catalyzed by γ-glutamyl transferase (GGT) in human macrophages. Biosynthesis of MCTR3 was mediated by dipeptidases that cleaved the cysteinylglycinyl bond of MCTR2 to give 13R-cysteine, 14S-hydroxy-4Z,7Z,9E,11E,13R,14S,16Z,19Z-docosahexaenoic acid. Of note, both GSTM4 and GGT enzymes displayed higher affinity to 13S,14S-eMaR and MCTR1 compared with their classic substrates in the cysteinylglycine metabolome. Together these results establish the MCTR biosynthetic pathway and provide mechanisms in tissue repair and regeneration.

Resolution of acute inflammation is an orchestrated host response to injury and/or infection that leads to the clearance of bacteria and tissue debris as well as tissue repair and regeneration (1–3). Central to the regulation of resolution responses is a novel genus of endogenous mediators termed specialized proresolving mediators (SPM) (2). They actively counterregulate production of inflammation-initiating signals including cytokines, chemokines, and lipid mediators and regulate leukocyte trafficking and phenotype as well as promote tissue repair and regeneration (1, 2, 4–6). At the site of inflammation leukocytes are key in the production of both inflammation-initiating (7, 8) and proresolving mediators (2, 4) because they carry the necessary enzymatic machinery for the stereoselective conversion of precursor essential fatty acids to the bioactive mediators.

Macrophages are central players in the acute inflammatory response governing both initiation and resolution phases (3, 4, 9–12). Distinct macrophage subtypes are involved in the regulation of these different phases of acute inflammatory responses, with macrophages from the resolution phase expressing higher levels of SPM biosynthetic enzymes (12). Recent evidence also demonstrates that lipid mediator profiles change with macrophage phenotype. Classic macrophages express higher levels of inflammation-initiating eicosanoids, whereas alternatively activated cells display higher levels of proresolving mediators (6, 13). Recently, we reported that macrophages produce a family of bioactive peptide-conjugated mediators coined maresin conjugates in tissue regeneration (MCTR) (4) and the complete stereochemistries of MCTR1 (13R-glutathionyl, 14S-hydroxy-4Z,7Z,9E,11E,13R,14S,16Z,19Z-docosahexaenoic acid) (International Union of Pure and Applied Chemistry nomenclature: 13R,7Z,9E,11E,13R,14S,16Z,19Z-docosahexaenoic acid) (4). MCTR2 (13R-cysteinylglycinyl, 14S-hydroxy-4Z,7Z,9E,11E,13R,14S,16Z,19Z-docosahexaenoic acid) and MCTR3 (13R-cysteine, 14S-hydroxy-4Z,7Z,9E,11E,13R,14S,16Z,19Z-docosahexaenoic acid) were established (14). Each displays potent bioactions in stimulating human phagocyte functions, promotes the resolution of bacterial infections, counterregulates the production of proinflammatory mediators, and promotes tissue repair and regeneration (14).

In the proposed MCTR biosynthetic pathway, (4), human macrophage 12-lipoxygenase is the initiating enzyme, converting docosahexaenoic acid to 14S-hydro(peroxy)-4Z,7Z,10Z,12E,16Z,19Z-docosahexaenoic acid and then to 13S,14S-epoxy-4Z,7Z,9E,11E,13R,14S,16Z,19Z-docosahexaenoic acid (13S,14S-eMaR). The epoxide intermediate is then enzymatically converted to MCTRs.

Significance

We recently uncovered a family of macrophage-derived molecules, coined maresin conjugates in tissue regeneration, that regulate the system’s ability to clear bacteria as well as repair and regenerate damaged tissues. In the present study, we identified enzymes involved in the formation of these potent molecules in human macrophages. These enzymes were shared with the classic cysteinyl leukotrienes, underscoring the presence of conserved biosynthetic motifs in these two functionally distinct lipid mediator families. Inhibition of these pathways upregulated the formation of several specialized proresolving mediator (SPM) families including D- and E-series resolvins. Thus, these illustrate the dynamic nature of the SPM biosynthetic pathways and provide new targets in the resolution of inflammation and regulation of tissue repair and regeneration.

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The authors declare no conflict of interest.

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Results

Enzymes That Catalyze These Reactions in Human Macrophages.

Results establish the MCTR biosynthetic pathway and identify the enzymes that catalyze these reactions in human macrophages. For example, in planaria, a GST catalyzes the conversion of the epoxide to MCTR1, which in turn is the proposed precursor to MCTR2 and MCTR3 (4). Therefore, we investigated the expression of both GSTM4 and LTC4S in human macrophages. Using flow cytometry and fluorescently conjugated antibodies, we found that human macrophages expressed both LTC4S and GSTM4 (Fig. 2A). We next tested whether these enzymes were involved in MCTR biosynthesis. To this end, human macrophages were transfected with shRNA targeting LTC4S or GSTM4 or control sequences. In cells transfected with the shRNA to LTC4S or GSTM4, we found >50% reduction in the expression of these enzymes compared with control scrambled (CS) shRNA (n = 4 independent experiments). We next investigated MCTR production in these cells, and using LC-MS-MS–based lipid mediator profiling found that transfection of cells with shRNA to GSTM4 led to a reduction in MCTR1 (∼60%), MCTR2 (∼60%), and MCTR3 (∼55%; Fig. 2D) compared with CS-shRNA transfection. Of note, in these incubations we also observed a significant increase in both maresin (MaR) 1 and MaR2 (Fig. 2E). Similar results were obtained when macrophages were transfected with shRNA for LTC4S (Fig. 2 D and E).

To further test the role of LTC4S in MCTR biosynthesis and dynamic modulation of lipid mediator pathways in human macrophages we investigated the regulation of endogenous lipid mediator–SPM pathways by LTC4S and LT biosynthesis inhibitors. Incubation of human macrophages with MK886 significantly reduced cysteinyll leukotrienes, with LTC4 levels reduced by ∼41%, LTD4 by ∼36%, and LTE4 by ∼29%, in line with published findings (15). MCTR levels were also reduced, with MCTR1 levels reduced from 3.0 ± 0.1 pg/4 × 10^6 cells to 1.3 ± 0.4 pg/4 × 10^6 cells, MCTR2 from 1.5 ± 0.5 pg/4 × 10^6 cells.

Table 1. MCTR in Human Tissue: Relation to cysteLT and PCTR

<table>
<thead>
<tr>
<th>Mediator</th>
<th>Q1</th>
<th>Q3</th>
<th>Lymph node, pg/150 mg</th>
<th>Serum, pg/mL</th>
<th>Plasma, pg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>LTC4</td>
<td>626</td>
<td>189</td>
<td>6.7 ± 3.1</td>
<td>5.9 ± 3.4</td>
<td>2.3 ± 1.0</td>
</tr>
<tr>
<td>LTD4</td>
<td>497</td>
<td>189</td>
<td>0.2 ± 0.1</td>
<td>0.4 ± 0.1</td>
<td>0.2 ± 0.0</td>
</tr>
<tr>
<td>LTE4</td>
<td>440</td>
<td>189</td>
<td>0.5 ± 0.2</td>
<td>1.7 ± 0.4</td>
<td>0.3 ± 0.2</td>
</tr>
<tr>
<td>MCTR1</td>
<td>650</td>
<td>211</td>
<td>1.1 ± 0.3</td>
<td>1.9 ± 0.5</td>
<td>0.8 ± 0.1</td>
</tr>
<tr>
<td>MCTR2</td>
<td>521</td>
<td>191</td>
<td>0.3 ± 0.2</td>
<td>0.3 ± 0.1</td>
<td>0.3 ± 0.0</td>
</tr>
<tr>
<td>MCTR3</td>
<td>464</td>
<td>191</td>
<td>1.4 ± 0.3</td>
<td>1.1 ± 0.5</td>
<td>0.3 ± 0.0</td>
</tr>
<tr>
<td>PCTR1</td>
<td>650</td>
<td>231</td>
<td>2.9 ± 0.6</td>
<td>1.6 ± 0.5</td>
<td>0.6 ± 0.2</td>
</tr>
<tr>
<td>PCTR2</td>
<td>521</td>
<td>231</td>
<td>1.6 ± 0.9</td>
<td>0.8 ± 0.3</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>PCTR3</td>
<td>464</td>
<td>231</td>
<td>1.6 ± 0.8</td>
<td>1.7 ± 0.8</td>
<td>0.7 ± 0.1</td>
</tr>
</tbody>
</table>

LMs were extracted using C18 SPE columns and products profiled using LC-MS–MS–based LM metabololipidomics. Products were identified from MS spectra and quantified using MRM with calibration curves specific to each compound. Results are mean ± SEM, n = 7 for axillary human lymph nodes and serum and 10 for plasma (see Fig. S1).

*Below the limit of detection (~0.1 pg).
proresolving mediators including resolving (Rv) D2, lipoxin (LX), and docosahexaenoic acid bioactive metabolomes, and cells to 0.8 ± 0.2 pg/4 × 10⁶ cells, and MCTR3 from 2.4 ± 0.4 pg/4 × 10⁵ cells to 0.9 ± 0.1 pg/4 × 10⁵ cells (Fig. 3 and Table S1). In these incubations we also identified and quantified representative of 1.5 mmol/min and a Kₘ of 42.5 ± 3.8 μM (Fig. 4B, Left) and a k_{cat}/Kₘ of 1.4 ± 0.1 M⁻¹ s⁻¹, whereas incubation of LTA₄ with GSTM4 gave V_{max} of 1.6 ± 0.6 mmol/min and Kₘ of 98.0 ± 6.6 μM (Fig. 4B, Right) and a k_{cat}/Kₘ of 2.1 ± 0.2 M⁻¹ s⁻¹. Together these results demonstrate that LTC₄S and GSTM4 each convert 13S, 14S-eMaR to MCTR1 in human macrophages. In addition, GSTM4 gave higher affinity to 13S, 14S-eMaR, whereas LTC₄S has a higher affinity to LTC₄S.

Allylic epoxides such as 13S, 14S-eMaR and LTC₄S can directly interact with biosynthetic enzymes, regulating their activity, as in the case of LTC₄S hydrolyase that is inactivated by its substrate LTC₄S inhibiting the production of LTB₄ (7, 16). Thus, we next questioned whether these epoxides regulated the activity of either LTC₄S or GSTM4. Incubation of LTC₄S with 13S, 14S-eMaR did inhibit the conversion of 13S, 14S-eMaR to MCTR1, as evidenced by a doubling in MCTR1 levels in incubations with a second addition of the epoxide compared with incubations where only vehicle was added (Fig. 5A and B). Addition of LTC₄S to hrLTC₄S also did not interfere with the conversion of LTC₄S to LTC₄S. Similar results were also obtained with hrGSTM4 (Fig. 5C).
Having found that hrGGT converts MCTR1 to MCTR2, we next tested whether MCTR1 was a precursor to MCTR2 and the role of GGT in catalyzing this step in human macrophages. Incubation of human macrophages with MCTR1 and either acivicin or serine borate, two GGT enzyme inhibitors, significantly reduced the MCTR2 and MCTR3 and significantly increased MCTR1. These results implicate GGT in macrophage production of MCTR2 (Fig. 4A).

To further test this, we incubated hrGGT with MCTR1 and assessed the kinetics of conversion to MCTR2. MCTR1 was rapidly converted to MCTR2 with 50% maximal kinetics similar to those observed for the conversion of LTC₄ to LTĐ₂ (Fig. 6B). Having found that hrGGT converts MCTR1 to MCTR2, we next assessed the catalytic efficiencies of hrGGT. MCTR1 gave a Vₘₐₓ of 8.1 ± 0.4 mmol/min and a Kₘₐₚ of 4.6 ± 1.0 μM, and a kₖₑₐₚ/Kₙ₉ of 6.0 ± 0.6 M⁻¹s⁻¹ for hrGGT. For direct comparison, LTC₄ gave a Vₘ₂ₓ of 8.9 ± 0.8 mmol/min, a Kₘ₂ₓ of 18.7 ± 5.0 μM, and a kₖₑ₂ₓ/Kₙ₉ of 1.6 ± 0.4 M⁻¹s⁻¹ (Fig. 6C), suggesting that GGT has a higher affinity for MCTR1 than LTC₄. Together these results indicate that MCTR1 is a precursor to MCTR2 via enzymatic conversion by GGT as demonstrated in human macrophages and using recombinant human enzyme.

**Discussion**

In the present paper we establish the MCTR production in human tissues and biosynthetic pathway with human macrophages together with recombinant enzymes. Using material prepared by total organic synthesis, we found that 13S, 14S-eMaR is converted to MCTR1, a step that in human macrophages is catalyzed by both LTC₄S and GSTM4. Cleavage of the γ-glutamyl moiety of MCTR1 by GGT yields MCTR2. This mediator is then a precursor in the biosynthesis of MCTR3, where in human macrophages the cysteinyl-glycyl bond is cleaved by a dipeptidase enzyme. Using LM metabololipidomics, we profiled human tissues identifying MCTR in human plasma, serum, and lymph nodes at concentrations (0.5–4.5 pM) commensurate with their known bioactive ranges.

**Tissue repair and regeneration are essential in the reestablishment of barrier function and return to homeostasis (1–3, 10, 17).** Macrophages are central in orchestrating these responses, with cells of the alternative activated lineage being primarily linked with tissue repair and regeneration. In this context identification of MCTRs as macrophage-derived mediators with potent tissue protective and regenerative actions (4, 14) provides...
LTC4S catalyze the formation of MCTR1 from 13S,14S-eMAR during both health and disease. In the present study, we demonstrated that the dipeptidase enzyme(s) that catalyzes the conversion of MCTR1 to MCTR2 (Fig. 6). This enzyme, and the third enzyme identified in the present study, the dipeptidase enzyme(s) that catalyzes the conversion of MCTR2 to MCTR3, are also shared with the cysteinyl leukotriene pathway (Figs. 6 and 7). Of note, substrate affinity for the GGT enzyme to MCTR1 was higher than to LTC4, a finding that further underscores the role of these enzymes in determining the macrophage lipid mediator phenotype.

leads into pathways and mechanisms that control reestablishment of functions to damaged tissues. We also recently found that alternatively activated human macrophages produce higher levels of MCTRs than classically activated macrophages (6), underscoring the potential role of this pathway in tissue and organ repair in human tissue.

Bioactive mediators are produced via the stereoselective conversion of essential fatty acids that give rise to molecules with defined stereochemistries (2, 7). Hence, identifying the enzymes responsible for the formation of lipid mediators is of fundamental importance. This is because establishing the identity of these enzymes allows for a better appreciation of their biological roles during both health and disease. In the present study, we demonstrated that two enzymes from the GST family, GSTM4 and LTC4S, catalyze the formation of MCTR1 from 13S,14S-eMAR (Figs. 2–5). Both of these enzymes also catalyze the conversion of LTA4 to LTC4, a lipid mediator that displays potent vasoactive and smooth muscle constricting actions (7). Of note, the two enzymes displayed different affinities to these substrates and whereas LTC4S displayed a higher affinity to LTA4, GSTM4 displayed a higher affinity toward 13S,14S-eMAR (Fig. 4). These findings suggest that in addition to substrate availability, the relative expression of the two enzymes in one cell type may determine the balance between the inflammation-, contraction-, and stress-initiating LTC4 (7) vs. the tissue-regenerative pathway of MCTRs. The second enzyme in the MCTR biosynthetic pathway that was identified in this report is GGT, which catalyzes the conversion of MCTR1 to MCTR2 (Fig. 6). This enzyme, and the third enzyme identified in the present study, the dipeptidase enzyme(s) that catalyzes the conversion of MCTR2 to MCTR3, are also shared with the cysteinyl leukotriene pathway (Figs. 6 and 7). Of note, substrate affinity for the GGT enzyme to MCTR1 was higher than to LTC4 (Fig. 6), a finding that further underscores the role of these enzymes in determining the macrophage lipid mediator phenotype.

Fig. 6. Human macrophage GGT converts MCTR1 to MCTR2. KG1a cells (1 × 10^6 cells/mL) were incubated with acivicin (2.5 mM), serine borate (45 mM), or vehicle (PBS, pH 7.45, 15 min) then MCTR1 (0.33 µM) and serum-treated zymosan (0.1 mg, 37 °C, PBS, pH 7.45, 180 min). Incubations were stopped with ice-cold methanol and products profiled using lipid mediator metabololipidomics. (A, Left) Representative MS-MS spectrum of MCTR2 and (Right) MCTRs amounts in macrophage incubations. Results are mean ± SEM, n = 4 macrophage preparations. *P < 0.05 vs. macrophages + MCTR1. (B) Time course: 4.4 mM of MCTR1 (Left) or LTC4 (Right) were each incubated with human recombinant GGT (147 ng/20 µL, 185 mM Tris-HCl, pH 8.2, room temperature) for the indicated intervals. Results are mean ± SEM; n = 4 macrophage preparations. (C) Human recombinant GGT (147 ng/20 µL) was incubated with the indicated concentrations of (Left) MCTR1 or (Right) LTC4 (185 mM Tris-HCl, pH 8.2, room temperature). All incubations were stopped with ice-cold methanol and extracted and products were profiled using LM metabololipidomics. Results are mean ± SEM; n = 3 independent incubations.

Fig. 7. MCTR3 is formed by human macrophage dipeptidase from MCTR2. (A–C) KG1a cells (1 × 10^6 cells/mL) were incubated with clastatin sodium (2.3 mM), or vehicle (PBS, pH 7.45, 15 min) then MCTR2 (66.9 nM) or MCTR1 (83.4 nM) and serum-treated zymosan (0.1 mg, 37 °C, PBS, pH 7.45, 360 min). Incubations were stopped and extracted and products were profiled using LM metabololipidomics. (A) Representative MS-MS spectrum of MCTR3. (B and C) MCTR in macrophage incubations. Results are mean ± SEM; n = 4 independent experiments. (B) *P < 0.05 vs. KG1a cells + MCTR2. (C) *P < 0.05, **P < 0.01 vs. KG1a cells + MCTR1.

Fig. 8. MCTR biosynthetic pathway. Structures are illustrated in most likely conformations based on biosynthetic evidence (4, 14). Stereochemistry of MCTR1, MCTR2, MCTR3, MaR1, and the maresin-epoxide intermediate are established (14, 16). The lipoxigenase responsible for 14-lipoxygenation and epoxidation reactions in human macrophages is human 12-LOX (16, 21). DPEP, dipeptidase; EH, epoxide hydrolase; LOX, lipoxigenase.
In summation, in the present experiments using primary human macrophages, stereochemically defined materials prepared using total organic synthesis and human recombinant enzymes, we establish the MCTR biosynthetic pathway and precursor-product relationship(s) for MCTR1, MCTR2, and MCTR3. The identification of these potent proresolving and tissue-regenerative immunomodulators in other human organs and tissues, including lymph nodes and serum, suggests that these pathways and mediators may be of interest in other human tissues. Given the differential affinity of enzymes identified herein to the cysteinyi leukotriene and MCTR pathways, their relative expression at sites of injury and/or inflammation may also assist in understanding disease processes. In addition, they also provide leads for targeted therapeutic strategies that may preferentially inhibit formation of inflammation-initiating cysteinyl leukotriene and up-regulate SPM formation.

Materials and Methods

Human Tissues and Cells. This study was conducted in accordance with Partners Human Research Committee Protocols 1999P001297 and 1999P001279 and a protocol approved by Barts and the London Research Ethics Committee [London (QMREC 2014.61)]. Informed consent was obtained from all participants.

LM Metabololipidomics. Human lymph nodes (∼150 mg) were defrosted on ice and carefully weighed, then 1 mL ice-cold methanol was added to each (see Table S2 for patient demographics and tissue source). Fresh serum and plasma (1 mL) were obtained from healthy donors and 4 mL of ice-cold methanol was added to each sample. Five hundred micromolars of internal standards d13-LTAc, d13-LTE4, d12-LTB4, d14-PGE2, d15-RvD2, and d15-LXA4 were added to each sample to facilitate quantification and samples. Studies were then kept at −20 °C for 1 h to allow for protein precipitation and products isolated as detailed in SI Materials and Methods.

Incubation Conditions: Enzymes. hLTCCS (40 ng/20 μL; Origene) and hgGSTM4 (61 ng/20 μL; Creative Biomart) was suspended in 25 mM Tris-HCl containing 5 mM reduced glutathione and 0.05% Triton X-100 (pH 7.8) and incubated with 135, 145-eMaR or LTa (0.3, 1, 3, 10, 30, and 100 μM) at room temperature for 2 min. The incubations were quenched using 2 volumes of MeOH and were profiled using lipid mediator metabololipidomics. Synthetic epoxide eMaRa was prepared as in ref. 16, and MCTR1, 2, and 3 were prepared as in ref. 19. hLTCCS (40 ng/20 μL; Origene) was suspended in 25 mM Tris-HCl containing 5 mM reduced glutathione and 0.05% Triton X-100 (pH 7.8). This was incubated with 135, 145-eMaR (5 μM, 2 min, 37 °C) then with 135, 145-eMaR (5 μM, 2 min, 37 °C) or vehicle. Incubations were then quenched using 2 volumes of ice-cold methanol and products profiled using lipid mediator metabololipidomics. In separate experiments hLTDCS (0.12 μM; 25 mM Tris-HCl, 5 mM reduced glutathione, and 0.05% Triton X-100, pH 7.8), was incubated with LTa from Cayman Chemical (5 μM, 2 min, 37 °C) then with LTa (5 μM, 2 min, 37 °C) or vehicle. Incubations were then quenched using 2 volumes of ice-cold methanol and products profiled using LM metabololipidomics.

HgGSTM4 (61 ng/20 μL; Creative Biomart) was suspended in 25 mM Tris-HCl containing 5 mM reduced glutathione and 0.05% Triton X-100 (pH 7.8). This was incubated with synthetic 135,145-eMa-Ra (5 μM, 2 min, 37 °C) then with 135,145-eMa-Ra (5 μM, 2 min, 37 °C) or vehicle. Incubations were then quenched and products profiled as above. Also, 61 ng/20 μL GSTM4 (25 mM Tris-HCl, 5 mM reduced glutathione, and 0.05% Triton X-100, pH 7.8) was incubated with LTa (5 μM, 2 min, 37 °C) then with LTa (5 μM, 2 min, 37 °C) or vehicle. Incubations were then quenched and products profiled as above.

MCTR1 (4.4 mM) and LTc4 (4.4 mM) were suspended separately in Tris-HCl (185 mM, pH 8.2) and were incubated with GGT (147 ng/20 μL; Lee Bio-solutions) for a total of 10 min. Aliquots were taken at predetermined intervals, placed in two volumes of ice-cold methanol, and mediator levels determined. GGT (147 ng/20 μL) was suspended in 185 mM Tris-HCl (pH 8.2) and incubated with 135, MCTR1 or LTc4 (0.3, 1, 3, 10, 30, and 100 μM) at room temperature for 2 min. All incubations were stopped using 2 volumes of MeOH, extracted, and profiled using LM metabololipidomics. Human lymph nodes (deidentified) were purchased from Science Care and Ohio State University (OSU) Tissue Procurement Services.

Statistics. All results are expressed as means ± SEM. Differences between groups were compared using Student t test (two groups). The criterion for statistical significance was P < 0.05. Sample sizes for each experiment were determined on the variability observed in preliminary experiments and prior experience with the experimental systems. The criterion for statistical significance was P < 0.05. Partial least squares discriminant analysis (PLS-DA) was conducted as described in ref. 20 with mediators and macrophage lineage markers giving variable importance in projection scores greater than 1 taken as displaying significant correlation.

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