Supporting Information

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SI Materials and Methods

Materials. The following materials were purchased from the designated suppliers: FCS, streptomycin, penicillin, L-glutamine, RPMI-1640, PBS free of Ca^{2+} and Mg^{2+} (Gibco); Lymphoprep (Axis-Shield); TriReagent, endotoxin-free dimethylsulphoxide (DMSO), phenylmethylsulfonyl fluoride (PMSF), polymyxin B sulfate (Sigma Chemical), GA (Sanofi-Aventis); PI3K pan-inhibitor Ly294002, MEK1/2 inhibitor, U0126 and MEK1 inhibitor, PD98059 (LC Laboratories); PI3Kα, β and γ selective inhibitors, compound 15e, TGX-221 and AS-604850, respectively (Alexi Corp.); and GSK3α/β inhibitor, SB216763 (Sigma Chemical); Kinase specific Stealth RNAi siRNA and Stealth RNAi negative control duplex (Invitrogen). IC87114 was kindly provided by Calistoga Pharmaceuticals. Other reagents were of analytical grade or better.

Monocytes. Peripheral blood monocytes were isolated from buffy coats of blood of healthy volunteers provided by the Geneva Hospital Blood Transfusion Center as previously described (1). In accordance with the ethical committee of the Geneva Hospital, the blood bank obtained informed consent from the donors, who are thus informed that part of their blood will be used for research purposes. To avoid activation by endotoxin, polymyxin B sulfate (5 μg/mL) was added in all solutions during isolation procedure and in all experiments. Monocyte purity routinely consisted of >90% CD14+ cells, <1% CD3+ cells, and <1% CD19+ cells as assessed by flow cytometry.

sIL-1Ra Production. Monocytes (5×10^4 cells/200 μL/well) were preincubated for 45 min in RPMI medium 1640 supplemented with 10% heat-inactivated FCS, 50 μg/mL streptomycin, 50 U/mL penicillin, 2 mM L-glutamine, and 5 μg/mL polymyxin B sulfate (medium) in 96-well plates at the indicated concentration of kinase inhibitors or DMSO, and then activated 48 h with 25 μg/mL GA. The latter concentration was previously determined as optimal (2). All conditions were carried out in triplicate. The production of sIL-1Ra was measured in culture supernatants by commercially available enzyme immunoassay (Quantikine, R&D). All experiments were carried out with monocytes isolated from at least three different blood donors. DMSO used as a control for inhibitors did not display any effects.

mRNA Quantification. Monocytes (2×10^6 cells/3 mL/well) were cultured in six-well plates in medium containing 5 μg/mL polymyxin B sulfate for 45 min with the indicated inhibitor or DMSO and then activated by 25 μg/mL GA for 18 h. Total mRNA was prepared by TriReagent (Sigma) according to the provider protocol. Quantitative real-time duplex PCR analysis was conducted on a TaqMan 7300 quantitative real time PCR system (Applied Biosystems) after reverse transcription by SuperScript II (Invitrogen). The levels of mRNA expression were normalized with the expression of a housekeeping gene (18S) analyzed simultaneously. sIL-1Ra and 18S probes were purchase form Applied Biosystems. All measurements were conducted in triplicates. DMSO used as a control for inhibitors did not display any effects.

Western Blot Analysis. Human monocytes were resuspended at 6×10^6 cells/mL in medium supplemented with 5 μg/mL polymyxin B sulfate and 500 μL was placed in 2-mL polypropylene tubes (Eppendorf) at 37 °C for 1 h before addition of kinase inhibitors or DMSO for 45 min. Cells were then activated with 25 μg/mL GA. At the indicated time, the activation was stopped by the addition of 800 μL ice-cold PBS and underwent centrifugation. Total cell lysates were prepared and subjected to Western blot analysis as described previously (3). Nitrocellulose membranes were probed with anti–phospho-p44/42 MAP Kinase (Thr202/Tyr204), anti-p44/42 (ERK 1/2), anti–phospho-Akt (Ser_{473}), anti-Akt, anti–phospho-GSK3α/β (Ser21/9), anti-MEK 1, anti GSK3α and anti-GSK3β (Cell Signaling Technology), anti-PI3Kδ (Calbiochem), anti-PI3Kα (BD), anti–β-tubulin (Sigma), and anti-MEK2, anti-PI3Kβ, and anti-PI3Kγ (Santa Cruz). Secondary IR700/800 conjugated goat antirabbit or goat antimouse antibodies (Rockland) were used, and antibody-bound proteins were detected and quantified with an Odyssey system (Li-Cor). DMSO used as a control for inhibitors did not display any effects.

mRNA Silencing. PI3Ks, MEK1, MEK2, ERK1/2, Akt, and GSK3α/β were silenced with 4 μM stealth siRNA or Stealth RNAi negative control duplex (mock) designed by the provider (Invitrogen). Monocytes were transfected using Nucleofector device and Nucleofector human Monocyte kit according to the provider protocol (Amaxa). Transfected monocytes (6×10^6 cells/1.6 mL/well) were seeded into 24-well ultra-low attachment plates (Corning). After 48 h, transfected cells were harvested. PI3Ks, MEK1/MEK2, ERK1/2, Akt, and GSK3α/β silencing was ascertained by Western blot using anti-PI3Kα, anti-PI3Kβ, anti-PI3Kγ, anti-PI3Kδ, anti-MEK1, anti-MEK2, anti-p44/42 ERK1/2, anti-Akt, and anti-GSK3α/β–specific antibodies. The ability of silenced monocytes to produce sIL-1Ra upon GA-treatment was measured as described above.

Translocation of PI3Kδ at Monocyte Membranes. Monocytes (10×10^6 cells/mL; 1 mL) were activated by GA (25 μg/mL) for 2 h. The activation was stopped by the addition of ice-cold PBS and centrifugation. Monocytes were then resuspended in 50 mM Hepes (pH 7.3) containing 150 mM NaCl, 5 mM EDTA, 1 mM $NaVO_4$ 50 mM NaF, 1 μM leupeptin, 1 μM pepstatin ,and 20 μM PMSF and lyzed by 10 freeze–thaw cycles. Membranes were isolated by ultracentrifugation at 100,000 × *g* for 45 min. The pellets were resuspended in sample buffer and subjected to Western blot analysis with anti-PI3Kδ as described above.

Statistical Analysis. When required, significance of differences between groups was evaluated using the Student paired *t* test.

1. Hyka N, et al. (2001) Apolipoprotein A-I inhibits the production of interleukin-1beta and tumor necrosis factor-alpha by blocking contact-mediated activation of monocytes by T lymphocytes. *Blood* 97:2381–2389.
2. Burger D, et al. (2009) Glatiramer acetate increases IL-1 receptor antagonist but decreases T cell-induced IL-1beta in human monocytes and multiple sclerosis. *Proc Natl Acad Sci USA* 106:4355–4359.
3. Molnarfi N, Gruaz L, Dayer JM, Burger D (2007) Opposite regulation of IL-1beta and secreted IL-1 receptor antagonist production by phosphatidylinositide-3 kinases in human monocytes activated by lipopolysaccharides or contact with T cells. *J Immunol* 178:446–454.

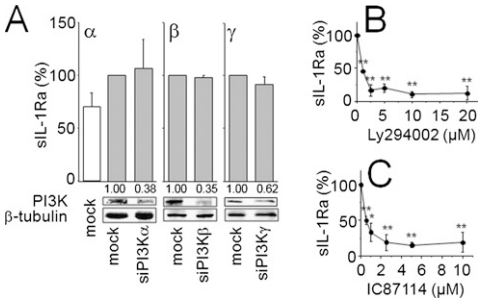

Fig. S1. PI3Kδ controls sIL-1Ra induction by GA in human monocytes. (*A*) Monocytes were nucleofected with stealth siRNA for the indicated PI3K isoform or negative control (mock). PI3K silencing was assessed by Western blot (*Lower*). PI3K knocked-down or mock-transfected monocytes were activated (gray columns; 100% = 1806 ± 634 pg/mL sIL-1Ra) or not activated (white column; sIL-1Ra concentration = 1173 ± 696 pg/mL) with GA and sIL-1Ra measured in culture supernatants. (*B* and *C*) Monocytes were preincubated with increasing concentration of Ly294002 (*B*) or IC87114 (*C*) before the addition of GA. sIL-1Ra production was measured in harvested supernatants and presented as percentage of sIL-1Ra production induced by GA in the absence of inhibitor (100% = 2347 ± 956 pg/mL sIL-1Ra). Results are presented as mean ± SD of three independent experiments carried out with monocytes isolated from three different individuals. $^{**}P < 0.01$ and $^{*}P < 0.05$ as determined by Student *t* test.

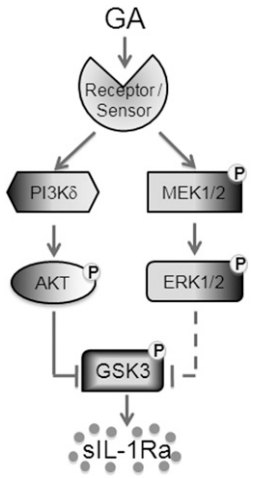

Fig. S2. Model of how GA activates PI3Kδ/Akt and MEK/ERK pathways to induce sIL-1Ra production. GA is recognized by a receptor (cell surface) or a sensor (inside the cell) that transduces signal via activation of both the PI3Kδ/Akt and MEK/ERK pathways. The two pathways then converge to phosphorylate/inactivate GSK3, resulting in the induction of sIL-1Ra production in monocytes.