Figure S1. IL-10 inhibits transcription elongation at TNF. (A) Cells were stimulated for the indicated times with LPS, or with LPS with or without IL-10 for 30 or 120 min, after which total RNA was isolated and subjected to quantitative RT-PCR using primers specific for mature or primary TNF transcript. (B) Comparison of the effect of IL-10 on the recruitment of Pol II to the TSS+1 or the downstream region of TNF using n = 4 blood donors. Black bars denote the level of LPS+IL-10 costimulation, normalized as a percentage of the LPS alone induction (white bars). Pooled data from the four donors are shown and are expressed as mean ± SEM. Statistical significance was assessed using the paired Student’s t test. *, P < 0.05; **, P < 0.01; ***, P < 0.001. (C) Cells were left unstimulated or preincubated with 500 nM flavopiridol, followed by LPS stimulation for 30 min, and then subjected to ChIP using primers specific for the NFKBIA TSS+1. Data in A and C are expressed as mean ± SD of triplicate measurements for a single donor representative of three independent experiments using different donors.
Figure S2. IL-10 requires elements 3′ and downstream of the TNF 3′ UTR for inhibition. (A) Adenoviral TNF reporters were infected into macrophages and stimulated with LPS with or without IL-10 for 4 h, after which luciferase assays were performed. Data are expressed as mean ± SE of eight separate donors and are represented as the percent inhibition in comparison to its LPS-induced value. Statistical significance was assessed by an unpaired Student’s t test. ***, P ≤ 0.001; ns, not significant. (B) Cells were either transfected with siControl or siRelA oligonucleotides, followed by stimulation with LPS for the indicated times. RNA was isolated and used in quantitative RT-PCR with probes specific for the indicated mRNA. (C) Cells were either transfected with siControl or siRelA oligonucleotides followed by stimulation with LPS for the indicated times. Protein secretion into the supernatants was determined by ELISA. Data are expressed as mean ± SD of triplicate measurements for a single donor representative of three independent experiments using different donors.
Figure S3. **IL-10 does not inhibit NF-κB activation.** Western blot analysis of the nuclear translocation of RelA in response to LPS with or without IL-10. Cytoplasmic fractions are shown on the top and nuclear fractions are shown on the bottom. Antibodies used are indicated on the left. Tubulin and lamin A/C were used as cytoplasmic and nuclear protein loading controls, respectively. This blot is a representative of three independent experiments.
Figure S4. IL-10 inhibits the recruitment of RelA to proinflammatory genes. (A) Cells were stimulated with LPS for the indicated times, after which ChIP was performed using antibodies to RelA and primers specific to the κB sites of the human TNF gene. (B) Cells were left unstimulated or stimulated with LPS with or without IL-10 before ChIP assay using anti-RelA or a rabbit isotype control and primers to target κB sites at TNFAIP3, CCL20, and NFKBIA. (C) Kinetics of RelA recruitment to the κB site in the IL6 promoter and the effect of IL-10 costimulation on its recruitment at 120 min after LPS stimulation. Data are expressed as mean ± SD of triplicate measurements for a single donor representative of three independent experiments using different donors.