The role of IL-1 and MyD88-dependent signaling in rhinoviral infection

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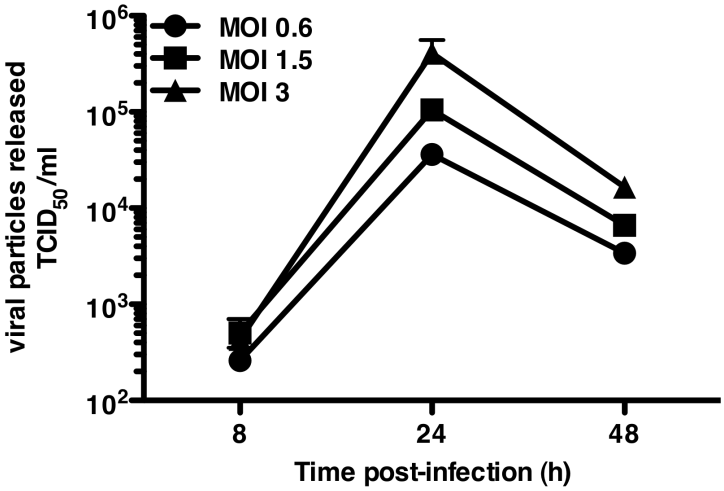
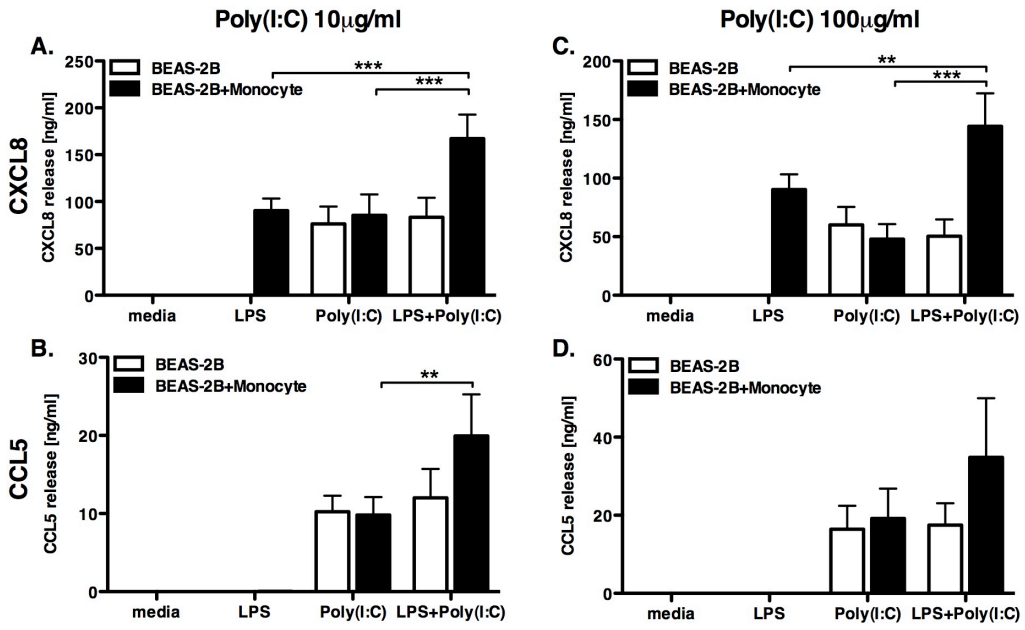
Supplemental figure 1



Supplemental figure 1. RV-1B infection of BEAS-2B cells triggers release of active viral particles into the supernatant

BEAS-2B cells were grown to 95% confluency in 12 well plates. Cells were infected for 1h with RV-1B at MOI of 0.6, 1.5 and 3 and then washed twice to remove nonadherent virus. Fresh media was added and cell free supernatants were collected at 8, 24 and 48h post-infection. Viral particle release into the supernatant was quantified by titration in subconfluent Ohio HeLa cell in 96 well plates. Serial dilutions of supernatants were carried out and the development of cytopathic effect (CPE) was visualized after 4 days. Titres are presented as $TCID_{50}$/ml. Data shown are mean ± SEM of n=6 with each replicate performed on separate passages of cells.

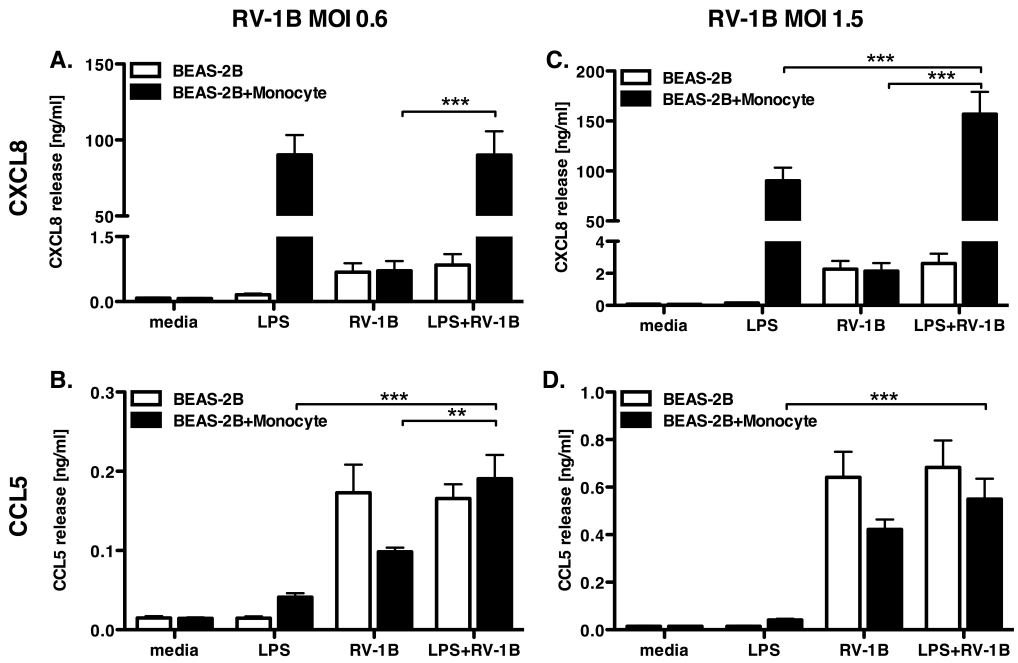
Supplemental figure 2



Supplemental figure 2. Poly(I:C) and LPS co-stimulation potentiates epithelial cell pro-inflammatory responses

BEAS-2B cells were grown to 95% confluency in 12 well plates. Cells were stimulated with media, LPS (0.1ng/ml), Poly(I:C) (10μg/ml or 100μg/ml), or LPS/Poly(I:C) (10μg/ml or 100μg/ml) in combination (A-D). Cocultures were created with the addition of 9500 highly purified $CD14^+$ monocytes. Monoculture controls were also created. After 24h cell free supernatants were generated and release of CXCL8 (A, C) or CCL5 (B, D) measured by ELISA. Data shown are mean ± SEM of n=4, with each replicate performed on separate passages of BEAS-2B cells with freshly prepared monocytes from independent donors. Data were analysed by two-way ANOVA with Bonferroni's posttest. Significant differences are indicated by **P<0.01 and ***P<0.001.

Supplemental figure 3



Supplemental figure 3. Co-activation of viral and bacterial signalling pathways potentiates epithelial cell pro-inflammatory responses at low viral titres

BEAS-2B cells were grown to 95% confluency in 12 well plates. Cells were infected for 1h with RV-1B (MOI 0.6 or MOI 1.5), followed by stimulation with media or LPS (A-D). Cocultures were created with the addition of 9500 highly purified $CD14^+$ monocytes. Monoculture controls were also created. After 24h cell free supernatants were generated and release of CXCL8 (A, C) or CCL5 (B, D) measured by ELISA. Data shown are mean ± SEM of n=4, with each replicate performed on separate passages of BEAS-2B cells with freshly prepared monocytes from independent donors. Data were analysed by two-way ANOVA with Bonferroni's posttest. Significant differences are indicated by **P<0.01 and ***P<0.001.