Supportive Information

Interleukin-1 beta Reduces Tonic Contraction of Mesenteric Lymphatic Muscle Cells: Involvement of Cyclooxygenase-2 / Prostaglandin E2

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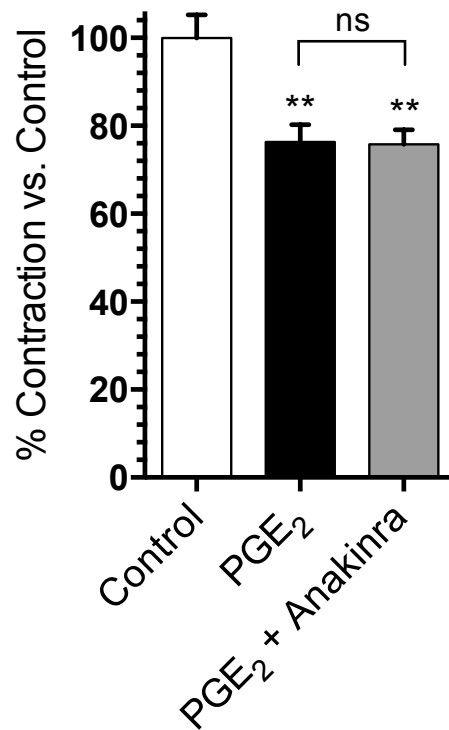Louisiana State University Health Sciences Center-Shreveport
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Supportive Figure 1. PGE$_2$ decreased rat mesenteric lymphatic muscles cell (RMLMC) contractility independent from Anakinra treatment. RMLMC treated with PGE$_2$ (10^{-5} M) for 4 days showed a significantly lower contractility (expressed in % of contraction vs. control) compared with control treated RMLMC, while Anakinra (5 μg/ml) treatment showed no significant effect on the PGE$_2$ induced relaxation. n=4, ** $p < 0.01$ vs. control, One-way ANOVA, with Bonferroni's post-hoc test; data are mean ± standard error of the mean (SEM).
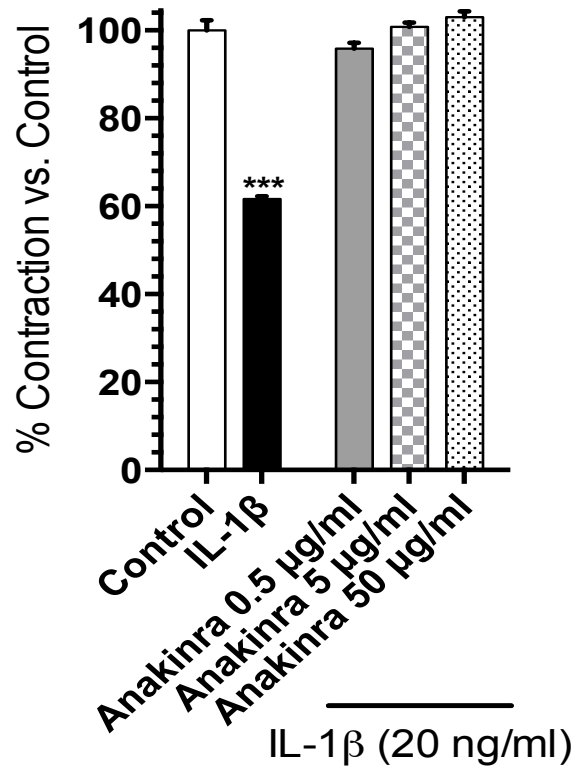
Supportive Figure 2. Different doses of Anakinra restored IL-1ß-induced decreased rat mesenteric lymphatic muscles cells (RMLMC) contractility. RMLMC treated with IL-1ß (20ng/ml) for 4 days showed a significantly lower contractility (expressed in % of contraction vs. control) compared with control treated RMLMC while co-treatment with Anakinra (0.5, 5, 50 µg/ml) restored RMLMC relaxation. n=5, *** $p < 0.001$ vs. control, One-way ANOVA, with Bonferroni's post-hoc test; data are mean ± standard error of the mean (SEM).
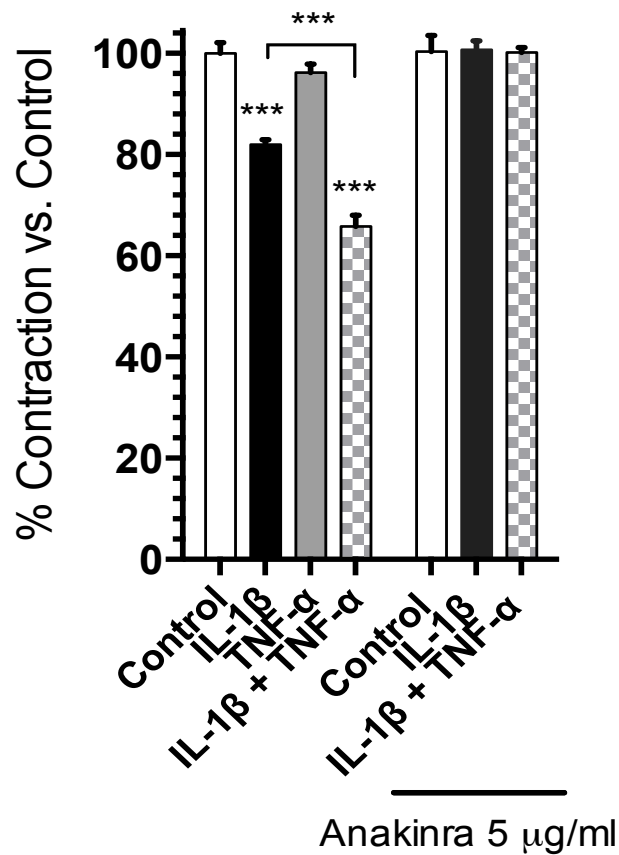
Supportive Figure 3. IL-1β + TNF-α exhibit synergistic effects in decreasing RMLMC contractility. IL-1β (20 ng/ml), but not TNF-α (20 ng/ml), decreased RMLMC contractility (expressed in % of contraction vs. control) while IL-1β (20 ng/ml) + TNF-α (20 ng/ml) decrease RMLMC contractility to a greater extend then IL-1β (20 ng/ml) alone. IL-1 receptor antagonist (Anakinra 5 μg/ml) maintained RMLMC contractility in the presence of IL-1β or IL-1β+TNF-α at day 4. n= 4, *** $p < 0.001$ vs. control, *** $p < 0.01$ IL-1β 20 ng/ml vs. IL-1β (20 ng/ml)+TNF-α (20 ng/ml). One-way ANOVA, with Bonferroni's post-hoc test; data are mean ±SEM.

$$\left(\frac{\left(1 - \left(\frac{t4}{t0} \right)^{\text{exp.}} \right)}{\left(1 - \left(\frac{t4}{t0} \right)^{\text{con.}} \right)} \right) \times 100$$

Supportive Figure 4. Equation for gel contraction. *exp.* represents the values of the treated gels, whereas *con.* represents the values for the untreated internal control gels. t0 = day 0, t4 = day 4. Since we observed slightly different contractile response within our control groups, an own control for each single experimental treatment was set. These individual controls were set as 100% and the results of parallel conducted treatment studies were normalized to this internal control. When for example the control group produced a 70% contraction and a treatment a 35% contraction, then the data are presented as 100% contraction for control and 50% contraction for the treatment group.