Supplementary Data

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Elevated expression of PDGF-C in Coxsackievirus B3 (CVB3)-induced chronic myocarditis

Virus titre
A half mouse heart was weighed, rinsed for 1 to 2 hours in sterile 0.9 % NaCl solution, homogenized by pounding material in a sterile mortar with sand and bloated in sterile minimum essential medium (MEM/E) supplemented with 100 µg/ml penicillin (Sigma, Deisenhofen), 0.1 mg/ml streptomycin (Sigma, Deisenhofen), 2 % neonatal calf serum (NCS) (Sigma, Deisenhofen), and re-suspended to give a 2 % (w/v) suspension. The intracellular virus was released by 3 cycles of freezing and thawing. The solution was serially diluted tenfold and titrated using mycoplasma free HeLa cell monolayers. The detection limit is 2.33 (ln₁₀ of TCID₅₀ per 100 mg organ weight).

Semi quantitative PCR
Primer pairs, annealing temperatures and the lengths of the amplified products were as follows: β-actin, 590 bp (primer sequences see ¹); VP1 (VP1 sense primer 5´-GGC CCA GTG GAA GAC GCG-3´ and VP1 antisense primer 5´-AAA TGC GCC CGT ATT TGT CAT TG-3´), 851 bp; PDGF-A (PDGF-A sense primer 5´-GCC TGT GCC CAT TCG CAG GAA GA-3´ and PDGF-A antisense primer 5´-GGC CAC CTT GAC ACT GTG CAA-3´ and PDGF-A antisense primer 5´-GCC CAC CTT GAC ACT GTG CAA-3´, annealing temperature 56°C), 395 bp; IL-1α, 491 bp; IL-1β, 336 bp; TNF-α, 354 bp (CLONTECH).

In-situ hybridisation
Total RNA was isolated from CVB3-Mü/J-infected HeLa cells and reverse transcribed. The DIG-labelled DNA probe was generated by PCR with the PCR DIG probe synthesis kit (Roche) using the specific VP1 primers. 6 µm thick sections from formalin-fixed and paraffin-embedded heart tissue were de-waxed by treating with
xylene, hydrated by descending ethanol row, and incubated with protease VIII (SIGMA-Aldrich) at RT for 30 min. Hybridisation with the DIG-labelled VP1-DNA probe was performed in hybridisation buffer (50 mM Tris-HCl, 300 mM NaCl, 2 mM EDTA, pH 7.4, 10% dextran sulphate, 0.01% herring sperm DNA, 0.05 % SDS, 0.05 % polyvinylpyrrolidone, 50 % deionised formamide) at 37°C in a humid chamber for 16 h. Unbound VP1 probe was removed by washing with 5x SSC, 0.01 % N-laurylsarcosine, and 0.02 % SDS, and the sections were subsequently incubated with blocking reagent (Roche) for 30 min, and alkaline-phosphatase-conjugated anti-DIG-antibody in blocking buffer (1:500) for 1 h. The sections were washed with PBS, and then incubated with 0.18 mg/ml 5-bromo-4-chloro-3-indoyl phosphate (BCIP), 0.34 mg/ml nitroblue tetrazolium (NBT), and 240 µg/ml levamisole NBT/BCIP at RT for 2 h. The reaction was stopped with PBS. Sections were counterstained with haematoxylin.

For the detection of PDGF-C mRNA paraffin-embedded heart sections were treated as mentioned before, however, sections were either incubated with 200 ng/ml of antisense or sense DIG-labelled cRNA probe in hybridisation buffer at 37°C for 16 h and then subjected to the following washings: 5 min with 2x SSC at 37°C, 3 x 5 min with 60 % formamide in 0.2x SSC at 37°C, and 2 x 5 min with 2x SSC at RT.

References