

# Pretransplant Islet Culture Influences Local TH1 Cytokine mRNA Expression after Allogeneic Islet Transplantation

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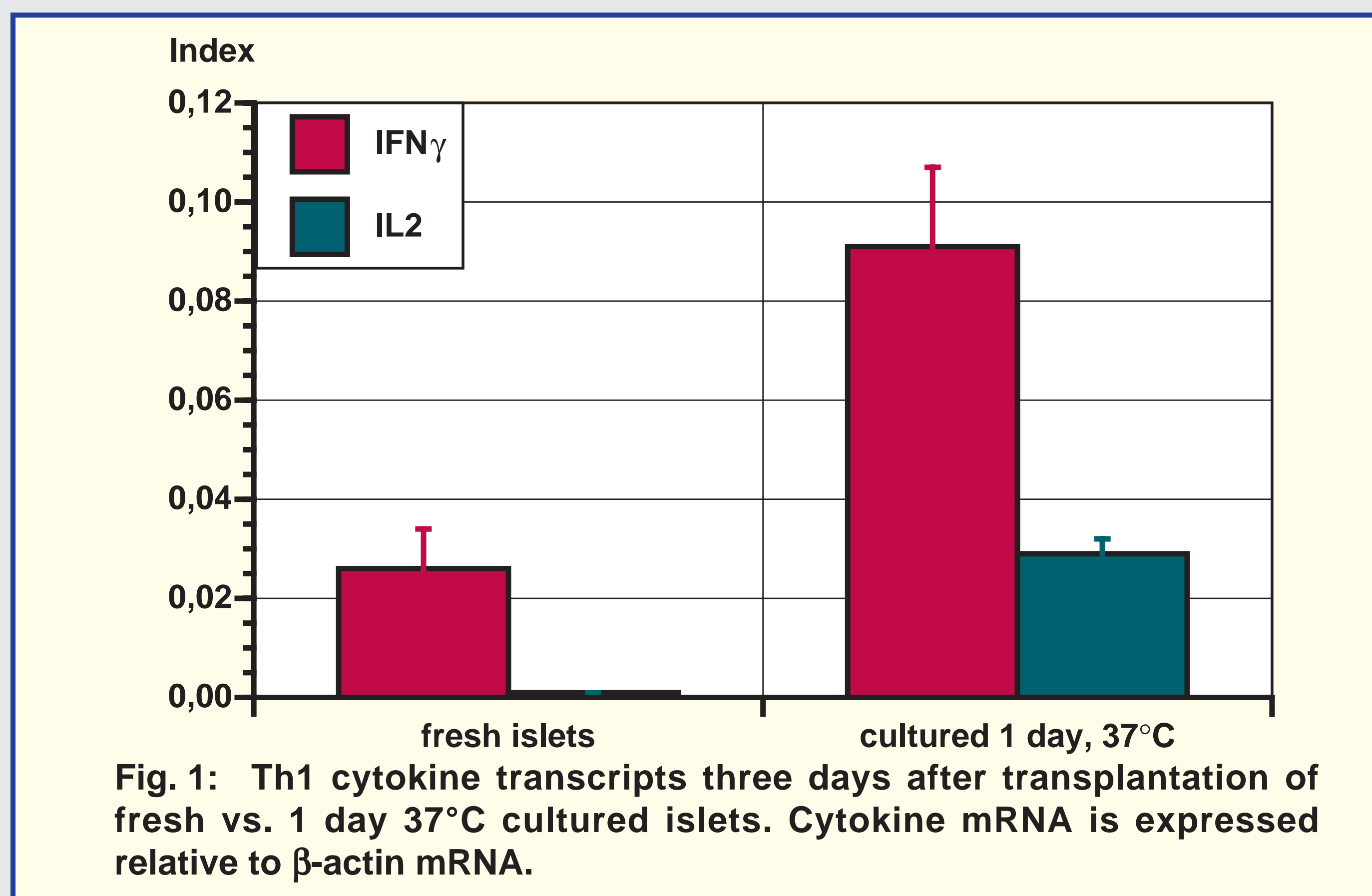


## INTRODUCTION

In islet allograft transplantation (ITx), the relevant quantity of islets required to reverse diabetes is the amount of islets effectively engrafted rather than the number of islets transplanted. In rodent studies only about 25-50% of the transplanted islets engrafted and survived. Pretransplant islet culture may play an important role for the early engraftment of islets.

Proinflammatory cytokines have been implicated to play an important role in early attack on islet cells during allogeneic islet transplantation. Therefore the aim of this study was to investigate the influence of pretransplant islet culture on cytokine mRNA expression, at the graft site, after allogeneic rat islet transplantation.

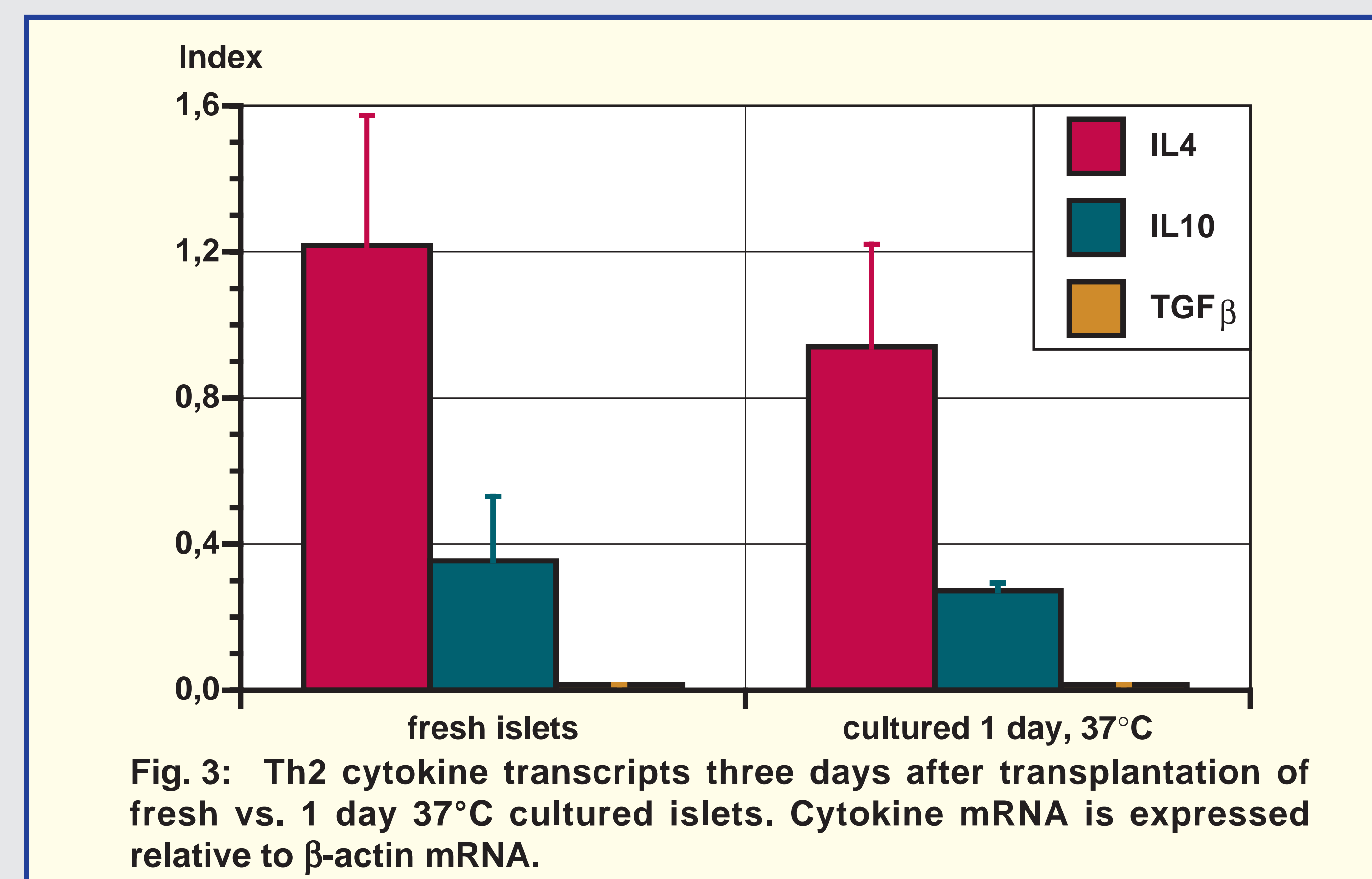
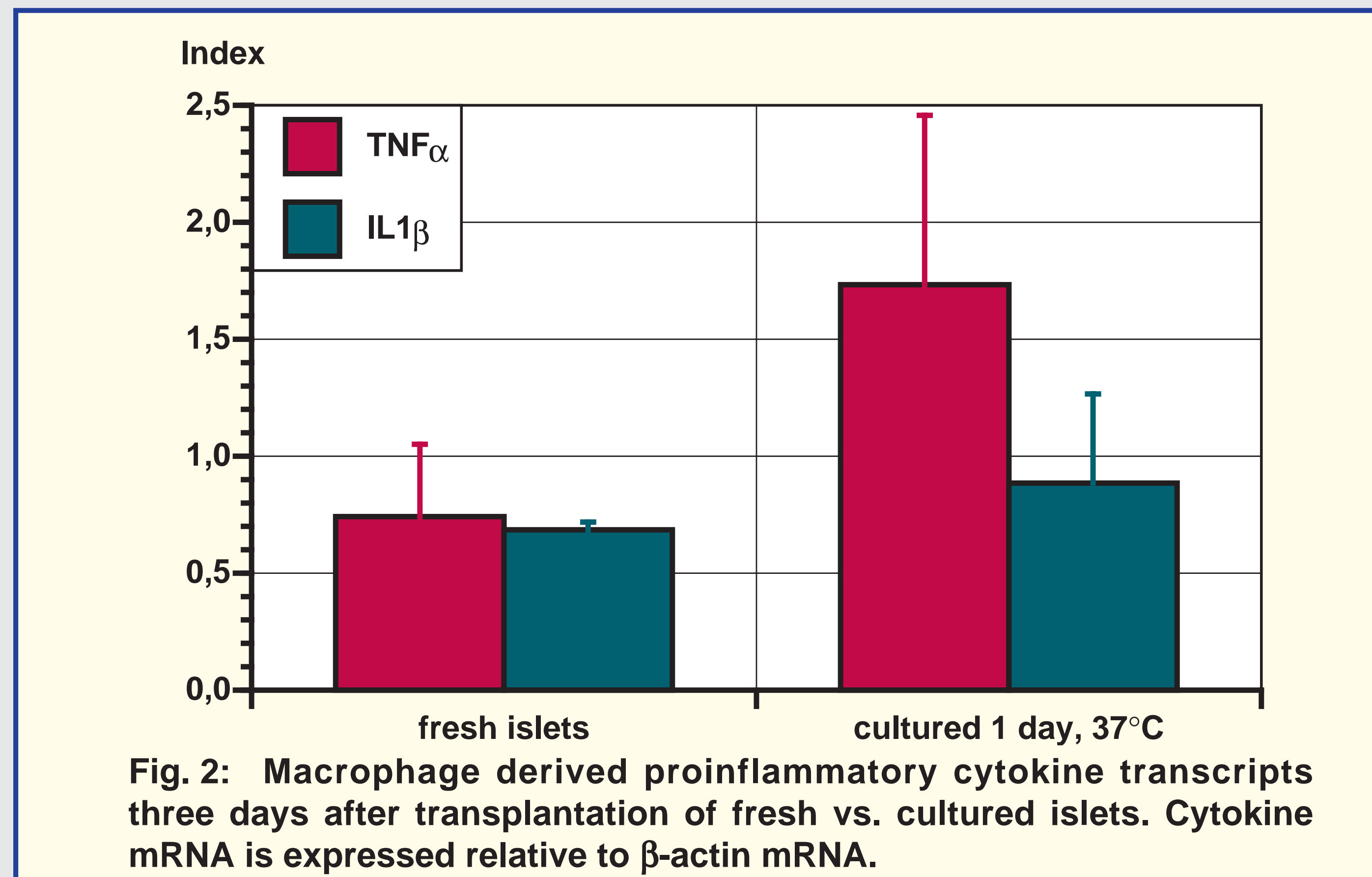
control gene ( $\beta$ -actin) with the cytokine gene of interest. PCR products were separated by gel electrophoresis, documented by a video system and analyzed using the CUE-2 image analyzer (Olympus). The amount of cytokines was expressed relative to the amount of  $\beta$ -actin in the sample.



## RESULTS

The expression of pro-inflammatory/Th1 cytokine transcripts was enhanced in islet grafts from one day freshly prepared vs. cultured islets (IFN $\gamma$ : 0.026 ± 0.008 vs. 0.091 ± 0.016, IL-2: 0.000 ± 0.000 vs. 0.029 ± 0.003, TNF $\alpha$ : 0.742 ± 0.309 vs. 1.733 ± 0.724) (Fig. 1 and 2).

No difference was found for Th2 cytokine and IL-1 $\beta$  mRNAs (IL-4: 1.217 ± 0.356 vs. 0.940 ± 0.281, IL-10: 0.354 ± 0.177 vs. 0.272 ± 0.022, TGF $\beta$ 1: 0.000 ± 0.000 vs. 0.000 ± 0.000, IL1 $\beta$ : 0.685 ± 0.033 vs. 0.885 ± 0.381) for fresh vs. precultured islets, respectively (Fig. 2 and 3).



## MATERIAL AND METHODS

Donor islets were prepared from Lewis rats by collagenase digestion and gradient centrifugation. Subsequently the islets were handpicked and 400 islets each were transplanted under the kidney capsula of Wistar Furth rats either immediately after preparation or following 1 day culture at 37°C in TCM-199 medium.

Three days post transplantation the graft was removed by nephrectomy and total RNA was extracted. Cytokine mRNA expression in these specimen was measured by reverse transcriptase polymerase chain reaction (RT-PCR).

The method of semiquantification involved the co-amplification of an endogenously expressed con-

## CONCLUSION

We conclude that the early engraftment of islets will be determined by both the magnitude of the inflammatory attack (higher after transplantation of 37°C cultivated islets) and the susceptibility of islets to this attack (higher in freshly prepared or 22°C cultivated islets, data not shown). The relative importance of these two parameters may depend on the transplantation model used.