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DETERMINATION OF TH1 / TH2 TYPE CYTOKINES IN THE INFLAMMATORY RESPONSE TO XENOGENIC PORCINE DERMAL COLLAGEN OR SYNTHETHIC POLYPROPYLENE IMPLANTS IN MICE

Hypothesis / aims of study

We have shown in different animal models for abdominal wall hernia repair that xenogenic cell-free, cross linked porcine dermal collagen implants (Pelvicol; CR Bard) induces a lesser inflammatory response, dominated by macrophages particularly after 7 days post implantation. Synthetic implants induce a much more pronounced inflammatory response. It has been suggested that other non-cross linked collagen implants, like small intestinal submucosa derived implants, induce a T-helper 2 (Th2) rather than T-helper1 (Th1) type of inflammatory response[1]. Th2 type cells and their derived cytokines play an important role in modulating the inflammatory response and inducing acceptance of xenogenic organ grafts[2]. In the present study we have investigated the expression cytokines known to play a role in the regulation of the pattern of inflammatory reaction, using a mouse model and different types of implants.

Study design, materials and methods

Twenty four C3H mice were implanted with 1 cm ^{2} of either polypropylene (Prolene, Johnson & Johnson) or Pelvicol subcutaneously. At day 3, 7 and 28 four animals from each group were sacrificed and the implant and surrounding host tissue, further called the explant, were harvested. The explant was immediately divided in several strips, one fixed for routine histopathology, one snap frozen for immunohistochemistry (IHC), and the largest part for homogenisation and further molecular studies. Intracellular cytokines (IL-10 and TGF-β as representitive for Th2 versus IFN-γ and TNF-α for Th1) were determined by IHC. The following rat anti-mouse antibodies (BD Inc.) were used: anti- IFN-γ (clone: XMG1.2), anti-TNF-α (clone: MP6XT3), anti-IL-10(clone: SXC1) and anti-TGF-β (clone: A75-3.1). The cytokine profile was further analysed in tissue homogenate at mRNA expression level by reverse transcription-polymerase chain reaction (RT-PCR). Those primers were used: for IFN-γ: 5'-TGA ACG CTA CAC ACT GCA TCT TGG-3' and 5'-CGA CTC CTT TTC CGC TTC CTG AG-3'; TNF-α:5'-TGC CTA TGT CTC AGC CTC TT-3'and 5'-ATA GCA AAT CGG CTG ACG GT-3'; IL-10: 5'-ATGCAGGACTTTAAG GGTTACTTGGGTT-3' and 5'-ATT TCG GAG AGAGGTACAA ACGAGGTTT-3'; and TGF-β: 5'-ACC TGCAAG ACC ATC GAC AT-3'and 5'ACA TGT TGC TCC ACA CTT GA-3'.

Results and Interpretation of results

Based on histology, Prolene provoked a marked inflammatory response, stronger than with Pelvicol, consisting of mainly polymophonuclear cells at day 3, and later on macrophages. These cells expressed predominantly Th1-type intracellular cytokines such as IFN-γ and TNF-α, mostly abundant at day 7. In contrast, Pelvicol induced generally a milder inflammatory response, with a similar time pattern. However there was upregulation of Th2-type cytokines, such as IL-10 and TGF-β, again at 7 d. In tissue homogenates of Prolene explants, there was higher mRNA expression of Th1 type cytokines: IFN-γ: and TNF-α. Pelvicol implanted mice expressed more Th2 type cytokines: TGF-β and IL-10 mRNA in their tissue homogenates. For both, expression involved cytokines was decreased to nearly undetectable levels by 28 d.

Concluding message

A xenogenic, cross linked porcine dermal collagen implant (Pelvicol) induces a milder inflammatory reaction as compared to polypropylene. We determined the cytokine profile associated to this response. Pelvicol provokes a Th2-type of immune response, which is known to down-regulate the pro-inflammatory response and reportedly contributes to the acceptance and better remodelling of implants.

References

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2. Bach FH, Ferran C, Candinas D, et al. Accommodation of xenografts: expression of "protective genes" in endothelial and smooth muscle cells. Transplant Proc. 1997 Feb-Mar;29(1-2):56-8.

Fig.1 Immunohistochemistry analysis of intracellular cytokines in both Prolene and Pelvicol implanted mice 7 days after implantation (arrows: positive staining).

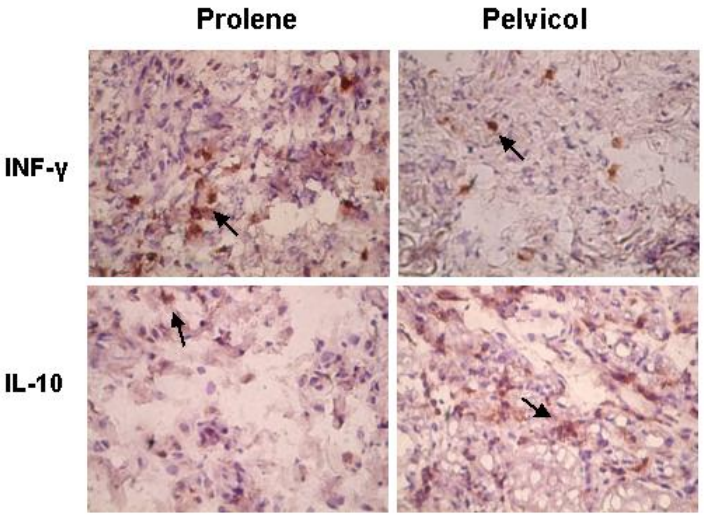

Fig.2 RT-PCR evaluation: mRNA expression of intracellular cytokines in both Prolene and Pelvicol implanted mice 7 days after implantation.