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APOPTOTIC RESPONSE TO XENOGENIC ACELLULAR COLLAGEN IMPLANT AND POLYPROPYLENE IN A MURINE MODEL

Hypothesis / aims of study

Implantation of foreign materials to reinforce fascial defects inevitably results in an inflammatory response by the host. Locally infiltrating cells play an important role and may be responsible for adverse local side effects, or determine the long term fate of the implant. We earlier showed that bio-derived xenogenic Pelvicol (Bard, Olen, Belgium) implants induced a weaker inflammatory response compared to synthetic Prolene (Johnson and Johnson, Dilbeek, Belgium). We hypothesized that apoptosis of the infiltrating cells may play a role in the specific nature of the immune response to Pelvicol resp. Prolene.

Study design, materials and methods

Female C3H mice were implanted subcutaneously with Pelvicol or Prolene (1x1 cm). The 'explants', containing the original implant, underlying abdominal wall, overlying skin and neighboring subcutaneous tissues were harvested at 3 and 7 days postoperative for analysis. Histopathology was assessed using light microscopy. Cytometric estimates of apoptosis were determined by TUNEL, Annexin V/PI double staining as well as DNA content assays.

Results

In Pelvicol explants, a significant number of TUNEL-positive apoptotic cells were detected by day 3, and that increased by day 7 day. In contrast, less apoptotic cells were seen in Prolene during the experiment. This phenomenon was confirmed by Annexin V/PI (Fig. 1), DNA content staining (Fig. 2) and flow cytometry analysis. In Pelvicol, the percentage of apoptotic cells (Annexin V +/PI-), was significantly higher at d 7 as compared to Prolene (9.8 $\pm$ 1.3% vs 4.0 $\pm$ 1.1%; $p< 0.05$); whereas, the percentage of necrotic cells (Annexin V + /PI+ and Annexin V -/PI+) was significant lower (4.7 $\pm$ 1.6% vs. 11.6 $\pm$ 2.0%; $p< 0.05$). Increased numbers belonging to the subG1 cell population (apoptotic cells) were detected at d3 (6.7 $\pm$ 1.3% vs 2.5 $\pm$ 0.7%; $p< 0.05$) and d7 (9.6 $\pm$ 1.5 vs 3.4 $\pm$ 0.8; $p< 0.05$) in Pelvicol explants as compared to Prolene. Flow cytometry profile of DNA content analysis is shown in Fig 2.

Interpretation of results

Apoptosis has been suggested to be a subtle process, circumscribed to the involved cells, which balances inflammatory response of immune cells[3]. In contrast, during necrosis the plasma membrane is the major site of damage, promoting the inflammatory responses of the surrounding tissues. Our finding of increased apoptosis in the peri-implant area is consistent with previous studies[2]. Increased apoptotic events in Pelvicol explant, coincides and might, at least in part, explain the less strong inflammatory process after Pelvicol implantation as compared to Prolene.

Concluding message

Pelvicol implantation is associated with increased apoptosis of infiltrating cells as compared to Prolene. This may contribute to the milder inflammatory responses seen around Pelvicol implants.

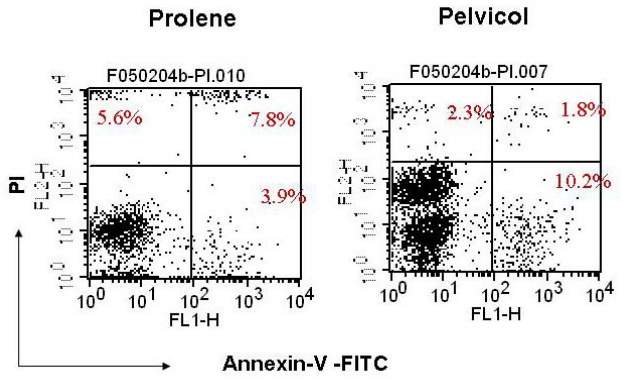

Fig 1. Representative diagram of FITC-Annexin V/PI flow cytometry of infiltrating cells in the explants 7 days after implantation. The lower left quadrants (Annexin V-/PI-) show the viable cells, and the lower right quadrants (Annexin V+/PI-) present the apoptotic cells, and the 2 upper quadrants contain necrotic cells.

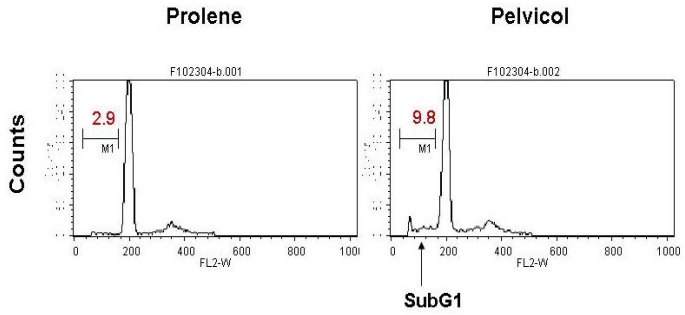

Fig 2. Histogram of DNA-flow cytometry analysis of infiltrating cells in the explants 7 days after implantation. DNA specific fluorochrome PI identified the distinct hypo-diploid cell population, subG1.

References
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