Granulocyte-based immune response against decellularized or glutaraldehyde cross-linked vascular tissue

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Abstract

Supporting structures derived from biological tissue have been used in numerous tissue-engineering applications. This study focuses on the immune response of human leukocytes toward decellularized or glutaraldehyde (GA) cross-linked vascular tissue in vitro.

Porcine and human pulmonary roots were sterilized with antibiotics, decellularized or cross-linked with GA. Proteins of the vascular tissue were extracted and the migratory response of human leukocytes toward protein extracts was examined using an in vitro migration chamber. Transmigrated leukocytes were counted and subsets (lymphocytes, monocytes, granulocytes) analyzed by flow cytometry.

Decellularization significantly reduced the migration of monocytes compared to native porcine tissue. Although the proportion of transmigrating lymphocytes was much lower, decellularization again reduced the migratory response. Surprisingly, after decellularization granulocyte migration was still significantly higher than the negative control. Results comparable to those obtained with porcine material were found when human tissue was used for the experiments. Interestingly, migratory behavior toward extracts of GA-fixed porcine tissue was similar to that of decellularized specimens.

We have shown that decellularization of vascular tissue reduces lymphocyte and monocyte recruitment comparable to cross-linking treatment. However, the migration of granulocytes, which are also known to be strongly involved in early inflammatory reactions, could be abolished neither by decellularization nor by fixation with GA.

Keywords: Tissue engineering; Immune response; ECM; Leukocytes

1. Introduction

Bioprosthetic cardiovascular devices such as heart valves or blood vessels made from xenograft or allograft tissue have long been a focus of research [1]. The core principle behind the use of xenograft tissue is the necessity to mask antigenicity through cross-linking with, e.g. glutaraldehyde (GA). However, it has been demonstrated that even GA-fixed porcine tissue leads to adverse immunological reactions in the host [2]. Fresh or cryopreserved allograft prostheses are also well known to induce an immune response similar to the rejection process, which develops in solid organ transplant recipients [3]. As the cellular components within the bioprosthetic material have been associated with undesired effects, such as calcification and immunological recognition, several methods have been explored to produce acellular tissue scaffolds. Decellularized matrices have been suggested to be ideal natural biomaterials for tissue repair and could be used as supporting structures in tissue engineering applications [4].

An already clinically applied approach is to decellularize allogeneic or xenogeneic heart valves or vascular structures [5–8] since this treatment is considered to eliminate immunogenicity and thereby increase implant durability. In preclinical animal models as well as in human settings it has been shown that the humoral immune response against decellularized heart valves is strongly reduced after implantation, compared to cryopreserved...
tissue [9,10]. Monocytes and granulocytes are well described to infiltrate cryopreserved allograft or cross-linked xenograft prostheses [11,12] but to date only few studies have dealt with the cellular or non-specific immune response against decellularized biomaterials. Findings on organ transplant recognition and rejection cannot be easily transferred to decellularized tissue where viable cells are missing.

Introducing an in vitro migration assay we have recently demonstrated that a decellularization procedure strongly reduces the migratory response of the monoblastoid cell-line U937 [13]. However, in vivo monocytes are not separated from other immunocompetent cells such as lymphocytes or granulocytes. This study was designed to determine the migratory behavior of freshly isolated human leukocyte subsets, not isolated from each other, toward extractable proteins of decellularized vascular tissue and compare it to conventional cross-linked tissue in an in vitro model. Thereby we thought to gain further important knowledge on the response of immunocompetent cells toward decellularized tissue scaffolds. We could demonstrate for the first time that decellularization of vascular tissue reduces monocyte and lymphocyte recruitment similar to GA cross-linking. However, neither decellularization nor cross-linking with GA abolished granulocyte recruitment, known to be strongly involved in the early cellular immune response.

2. Materials and methods

2.1. Porcine pulmonary roots

Porcine pulmonary trunks (n = 9) were obtained from a local slaughterhouse. For cold sterilization the conduits were incubated in sterile water (A.d.) containing multiple antibiotics (12 mg/L vancomycin, 12 mg/L metronidazol, 12 mg/L amikacin and 3 mg/L ciprofloxacin) for 48 h at 4 °C. Decellularization (n = 3) was carried out as previously described [13], based on a triple detergent combination (0.05% tert-octylphenyl-polyoxyethylen (Triton X-100®), Biorad, Hercules, CA, USA), 0.05% sodium-deoxycholate (Merck, Darmstadt, Germany) and 0.05% octylphenyl-polyethylene glycol (IGEPAL-CA630®) with 0.02% EDTA for 48 h at 4 °C. Proteins of specimens were thoroughly washed with cold PBS/C0 for at least 3 days. Tissue specimens (100 mg) were digested for 24 h at 37 °C. To remove residual detergents and cell debris, the conduits were subsequently washed for 12 days in phosphate-buffered saline (without calcium or magnesium, PBS/C0) followed by ribonuclease (RNase, 100 μg/mL, Roche Diagnostics GmbH, Mannheim, Germany) and deoxyribonuclease (DNase, 150 IU/mL Sigma, St. Louis, MO, USA) digestion for 24 h at 4 °C. For a more sensitive evaluation of DNA removal, DNA was extracted from two specimens of each native and decellularized porcine pulmonary wall (native n = 6, decellularized n = 6). Tissue specimens (100 mg) were homogenized and consecutively digested with 30 μg/mg tissue proteinase K in a buffer containing 150 mmol/L NaCl, 1 mmol/L EDTA, 0.5% sodium dodecyl sulfate (pH 10.5) for 65 °C for 2 h. The DNA was extracted by adding butanol/chloroform (1:5) and pelleted at 12,000 g for 2 min. The extracted DNA was precipitated with ice-cold ethanol and incubated on ice for 5 min. After centrifugation at 12,000 g for 5 min the DNA was solubilized in destilled water and visualized by electrophoresis in agarose–ethidium-bromide gels.

For removal of contaminating proteins or lipids, potentially biasing the photometric DNA quantification, the DNA was repurified with a Flexi Gene DNA Kit (Quagen, Hilden, Germany) according to the instructions given by the manufacturer. The amount of extractable DNA was calculated per mg wet weight of tissue. The purity of the DNA was determined by calculating the A260/A280 ratio.

2.2. Human pulmonary roots

Human pulmonary conduits (n = 4) were obtained in the course of heart transplantations. Conduits that could not be used for implantation as homograft substitutes for several reasons were either cryopreserved in RPMI 1640 medium containing dimethylsulfoxid (DMSO) after antibiotic sterilization (n = 2) or decellularized (n = 2), using the procedure described above.

Tissue specimens strictly from above the commisure level, of the native, decellularized, or GA-fixed porcine as well as native or decellularized human pulmonary wall tissue were snap frozen in liquid nitrogen and stored at −80 °C until use for protein extraction.

2.3. Histological examination

For evaluation of cell removal histological examination was performed. Two longitudinal slices from different areas (4 mm width each) were excised of each pulmonary root and were embedded in paraffin. For general morphology, three longitudinal sections (10 μm) of each slice were stained with hematoxylin–eosin (HE) and examined by two independent assistants using high power field light microscopy. Immunohistostaining was carried out using immunofluorescence techniques. The DNA-specific dye TO-PRO®-3 (Molecular Probes, Leiden, The Netherlands) was used to detect remaining cell-nuclei in the heart valve scaffold. The specimens were further stained with polyclonal anti-porcine collagen type I & III (polyclonal rabbit IgG, 1:20, Monosan®, Uden, The Netherlands) cross-reactive with human type I & III collagen. Three sections of each slice (porcine n = 36, human n = 24) were analyzed by confocal laser scanning microscopy (ZEISS LSM 510 laser scanning microscope, Jena, Germany) using a three-dimensional (3D) analysis mode.

2.4. DNA-isolation and quantification

For a more sensitive evaluation of DNA removal, DNA was extracted from two specimens of each native and decellularized porcine pulmonary wall (native n = 6, decellularized n = 6). Tissue specimens (100 mg) were homogenized and consecutively digested with 30 μg/mg tissue proteinase K in a buffer containing 150 mmol/L NaCl, 1 mmol/L EDTA, 0.5% sodium dodecyl sulfate (pH 10.5) for 65 °C for 2 h. The DNA was extracted by adding butanol/chloroform (1:5) and pelleted at 12,000 g for 2 min. The extracted DNA was precipitated with ice-cold ethanol and incubated on ice for 5 min. After centrifugation at 12,000 g for 5 min the DNA was solubilized in destilled water and visualized by electrophoresis in agarose–ethidium-bromide gels.

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2.5. Preparation of tissue extracts

The frozen tissue samples of porcine and human arterial conduits were thoroughly washed with cold PBS/C0. Proteins of specimens were extracted by mechanical homogenization in 2 mL migration medium (RPMI 1640 medium w/o l-glutamine and phenol red, Bio Whittaker) in the presence of protease inhibitors, on ice according to a protocol already described [13]. The debris of each homogenate was sedimented by a subsequent centrifugation at 10,000 g and 18 °C for 30 min. The supernatant was carefully withdrawn and the protein content was determined by a modified Bradford assay (Biorad, Heidelberg, Germany). All protein extracts were diluted with migration medium to an equal final protein concentration of 0.25 μg/mL prior use in the migration assay and exactly 1.5 mL was added into the lower chamber of the migration chamber. Furthermore, extracts of highly purified porcine collagen I/III (Biogide®, Geistlich Biomaterials, Wollhusen, Switzerland) were prepared as described and cell migration toward extracted proteins was examined.

RPMI 1640 medium without any supplements was used to assess random migration and autologous serum (adjusted to a protein concentration of 0.25 μg/mL) was used as negative control. Sterile technique was used throughout.
To examine the influence of different protein concentrations on the migratory response, serial dilutions (1, 0.5, 0.25, and 0.125 μg/μL) of protein extracts and autologous serum were prepared.

### 2.6. Human leukocytes

Blood samples were drawn from healthy volunteers (female n = 5, male n = 7; mean age 39.1 ± 14.0) after giving informed consent (proved by the ethical committee, Medical University of Vienna). Human leukocytes were isolated from EDTA-anticoagulated venous blood by lysing 1 mL blood with 14 mL lysing solution (0.1 mM Na–EDTA, 1.0 mM KHCO₃, 0.17 mM NH₄Cl in A.d., pH 7.3) and subsequent centrifugation at 300g and 25 °C for 7 min. The supernatant was discarded and the cell pellet was washed with PBS⁻/⁻ and subsequently resuspended in migration medium to a final concentration of 3.33 × 10⁶ cells/mL. Purity, as assessed by flow cytometric (FACS) analysis using antibodies against CD45/CD14 (Serotec, Düsseldorf, Germany), was typically greater than 95%. The isolated leukocytes comprised 29.4 ± 0.6% lymphocytes, 60.2 ± 0.5% polymorphonuclear cells (PMNC, granulocytes) and 4.2 ± 0.1% CD14⁺ cells (monocytes).

### 2.7. Migration assay

The in vitro cell migration assays were carried out as described previously [13]. Briefly, a polyethylene-terephtalate (PET) membrane (FalconSM cell culture inserts, pore size 3 μm, Beckton Dickinson Labware, France) was placed in each well of a six-well culture plate, dividing each well into a lower chamber (LCH) and an upper chamber (UCH). The bottom of the wells was covered with 1.5 mL of the different solution (PBS⁻/⁻ supplemented with 0.1% (w/v) sodium azide, 5 mmol/L glucose and 5% fetal calf serum).

The number of transmigrated leukocytes was determined by two independent assistants, using a hemocytometer. Viability, as assessed by propidium iodide (PI) staining and FACS analysis, was typically higher than 90%. Leukocyte subsets were examined by FACS-analysis using FSC and SSC measurements as well as monoclonal antibodies against human CD45 and CD14. PI negative cells were gated and 10,000 events were acquired from each sample on an EPICS profile 2 cytometer (Coulter, Krefeld, Germany).

The proportions of the three CD 45⁺ leukocyte subpopulations (lymphocytes, CD14⁺ monocytes, and granulocytes) in the upper chamber as well as their distribution in the lower chamber were examined by FACS analysis.

### 2.8. Statistical analysis

All values are shown as mean ± standard error of mean (SEM). A Wilcoxon signed rank test with Bonferroni correction for multiple comparisons was used to assess differences between groups. Correlation analysis was performed by computing Pearson’s linear correlation coefficient (R). Values of P < 0.05 were considered significant.

### 3. Results

#### 3.1. Decellularization procedure

Neither by HE- nor by DNA-specific TO-PRO 3® staining residual cells, cell nuclei, or microscopically identifiable debris could be detected within the examined decellularized porcine or human tissue sections, indicating a histologically verified acellular tissue (Fig. 1). Gel-electrophoresis proved that no residual DNA was detectable within decellularized tissue, indicating effective removal of xenogenic DNA (Fig. 2). Photometric DNA quantification revealed 303.8 ± 31.2 ng DNA per mg wet weight within native porcine pulmonary arteries, whereas the DNA-content of the decellularized arterial tissue was below the detection limit (0.01 μg/μL).

#### 3.2. Migration of leukocytes

When RPMI 1640 medium without any supplements was used 55.1 ± 4.4 × 10³ leukocytes migrated across the PET-membrane (random migration, n = 12).

The extracted proteins (0.25 μg/μL) of the porcine native pulmonary wall or the human cryopreserved conduit wall induced a strongly increased migration of leukocytes (porcine: 899.6 ± 68.9 × 10³ cells in LCH; n = 12; human: 829.3 ± 48.02 × 10³; n = 12). After the decellularization procedure the cell migration toward the extracts of the vascular wall (porcine: 587.3 ± 74.3 × 10³, n = 12; human: 726.3 ± 64.6 × 10³; n = 12) was still significantly higher compared to autologous serum used as negative control (200.2 ± 23.7 × 10³, n = 12, P < 0.05). A migration of 420 ± 45 × 10³ leukocytes (n = 10) was elicited by protein extracts of GA-fixed tissue.

Flow-cytometric analysis of the migratory response toward porcine native versus porcine decellularized vascular tissue is shown in Fig. 3.

#### 3.3. Migration of monocytes

Random migration revealed 0.23 ± 0.09% monocytes in the LCH. Proteins extracted from the porcine pulmonary wall led to a strong migration of CD14⁺ cells (11.95 ± 1.53%) compared to autologous serum used as negative control (0.42 ± 0.12%). The non-decellularized allograft tissue also induced a strong migration (11.49 ± 1.30%). Interestingly, the decellularization procedure significantly reduced monocytic migration (P < 0.05) toward extracts of the vascular tissue (porcine: 0.77 ± 0.22%; human: 0.55 ± 0.13%). The extracts of porcine GA cross-linked arteries elicited a transmigration of 0.70 ± 0.06% monocytes.

#### 3.4. Migration of lymphocytes

RPMI 1640 medium without any supplements induced a random migration of 0.14 ± 0.05% lymphocytes. Extracted proteins initiated a minor increase in lymphocyte transmigration. Only 0.62 ± 0.08% of the lymphocytes in the UCH were attracted by the proteins of the native porcine pulmonary root. The proteins of the human pulmonary artery induced the migration of 0.42 ± 0.05% lymphocytes. However, after decellularization the vascular tissue attracted less lymphocytes (porcine: 0.23 ± 0.05%; human:
0.25 ± 0.07%), which was comparable to the migration elicited by autologous serum (0.24 ± 0.05%). Protein extracts of GA-fixed tissue recruited 0.28 ± 0.02% lymphocytes.

3.5. Migration of granulocytes

Random migration revealed 2.60 ± 0.61% transmigrated granulocytes. Compared to the non-decellularized samples (porcine: 26.8 ± 2.03%; human: 24.75 ± 1.65%) the decellularization procedure did not reduce the migratory response of granulocytes (porcine: 17.42 ± 2.13%; human: 22.06 ± 2.27%) to the level of the negative control (6.53 ± 0.65%, P < 0.05), as it was observed for lymphocytes and monocytes. GA-fixation reduced the recruitment of granulocytes to 15.97 ± 1.79%. Comparison is shown in Fig. 4.

Interestingly, the extracts of the BioGide® porcine collagen type I/III scaffold revealed a similar migration of leukocyte subsets as the extracts of decellularized or GA-fixed tissue did (monocytes: 1.05 ± 0.31%; lymphocytes: 0.19 ± 0.04%; and granulocytes: 22.86 ± 4.18%).

Dilution series (n = 5) of the extracts of native porcine tissue revealed that lymphocyte, monocyte as well as granulocyte migration is strongly dependent on the protein concentration. The decellularization procedure seems to remove proteins capable for lymphocyte and monocyte cell recruitment. Higher amounts of protein extracted from decellularized porcine vascular tissue led to increased granulocyte transmigration as it was observed for native tissue. In contrast, the migration of lymphocytes toward decellularized porcine tissue did not increase within the examined range of 0.125–1.000 µg/µL protein. Interestingly, the recruitment of monocytes was not increased until 1.000 µg/µL protein was used within the experiments. This might indicate that proteins capable of monocyte recruitment were effectively, but not completely, removed by the decellularization procedure (Fig. 5).

4. Discussion

The involvement of the immune system in degeneration and calcification of bioprosthetic cardiovascular devices made from either xenogenic or allogeneic tissue is still controversially discussed [2,14,15]. However, gaining
Evidence has been given that humoral as well as cellular immune responses might be—at least in part—responsible for tissue deterioration [16,17]. As it is known that the presence of cells, cell membranes and/or lipids can induce undesirable effects such as calcification as well as a rapid immune reaction [2,18] several cell-extraction procedures have been proposed with some success [4,19,20]. The decellularization process is considered to generate natural scaffolds for use in tissue engineering applications without adverse immune reactions.

Promising results in vitro as well as in preclinical animal models led to the clinical implementation of decellularized heart valves or vascular structures eventually covered with endothelial cells [6–8,21]. Postoperative evaluation of the humoral response initiated by acellular allogeneic heart valves revealed a strongly decreased panel-reactive antibody (PRA) production [10]. These results indicate a high benefit especially in the pediatric patient where increased PRAs might interfere with later heart transplantation [22]. Since it is known that homograft valves become largely acellular even within 1 year post implantation [12] and therefore mimic a decellularized allogeneic heart valve, a superior performance of a priori decellularized homograft valves in the long-term has still to be proven.

Decellularized xenogenic or allogeneic vascular grafts have already been implicated in clinical trials. However, only very few studies have been undertaken to evaluate the cellular immune response against this privileged tissue.

Accumulation of leukocytes is generally attributed to the presence of chemoattractants. The recruitment of immunocompetent cells is an initial step in the immune response towards implanted bioprostheses, whereby the release of extracellular matrix proteins may promote leukocyte adhesion, diapedesis or degranulation.

In a recently established in vitro migration assay, we could clearly demonstrate that the decellularization of porcine or human heart valves significantly reduces their potential to attract cells of a monoblastoid cell line [13]. Since these results had displayed the behavior of just one single cell-type, the current study was designed to examine the migratory response of human leukocyte subsets isolated from different individuals to closer mirror in the in vivo situation where cell–cell interactions take place.

The results of the present study corroborate the finding that decellularization strongly reduces the migration of...
monocytic cells toward extractable proteins of xenogenic as well as of allogeneic vascular tissue.

Focusing on the elimination of the immune response, a strongly decreased monocylic response seems to be crucial. Monocytes and macrophages are important effector cells of host defense and are known to remove cellular debris by digesting foreign material. It is furthermore well described that monocytes are involved in foreign body type reaction as well as in other types of immune reactions. Monocytes and macrophages are known to be predominant in early infiltrates found in cryopreserved homografts [12] and to precede T-cell influx. Furthermore, it is proposed that macrophages might be associated with tissue calcification.
due to their capability to obtain an osteoblast-like calcium depositing phenotype [23]. These clinical data indicate that the elimination or strong reduction of monocyte recruitment could be beneficial for the durability of tissue-engineered constructs.

Postoperative fever of unknown origin exceeding 38.3 °C is well known after the implantation of allograft valves [24]. Interestingly, a significantly lower body temperature was observed after the implantation of decellularized homograft valves [6]. Such non-infectious fever episodes were not described after the implantation of conventional GA cross-linked bioprostheses. In our study, GA-treatment of pulmonary roots did also abolish the migration of monocytes toward the protein extracts of the porcine tissue. On the other hand, in organ transplantation fever is known to occur during allograft rejection [25]. As fever is induced by endogenous pyrogens produced by macrophages, our in vitro results demonstrating a strongly reduced monocytic recruitment toward extracts of decellularized tissue might be a link to the clinical situation. Recently, it was described that a gradual cellular infiltrate consisting predominantly of macrophages was found in a decellularized pulmonary homograft [26]. As it is known that decellularization procedures differ strongly in their efficiency of cell removal [27], it could be hypothesized that residual cells [7,28] led to the observed influx of macrophages. Whether the monocytic infiltrate observed by Sayk et al. resembles an early non-specific inflammatory phase of recellularization preceding the desired repopulation, as mentioned by the author, remains hypothetic as to date it seems that—in contrast to animals [29]—humans do not repopulate implanted decellularized tissue [7]. In vivo, neutrophils are attracted by devitalized tissue and foreign bodies and are thereby involved in foreign body type reactions. Polymorphonuclear infiltrates were found when GA-fixed heart valve tissue was implanted in primates [11] and in humans [7]. In organ transplantation neutrophils are known to amplify allograft and xenograft rejection as well as transplant vasculopathy [30,31], but very less is known about the response of PMNC towards decellularized tissue.

Since neutrophil recruitment can be largely mimicked in vitro by testing the ability of these cells to migrate, this study assessed the granulocyte migratory response towards proteins extracted from pulmonary arteries. Surprisingly, the decellularization procedure did not diminish the strong migratory response of human granulocytes, as it was observed for monocytes and lymphocytes. Additionally, the extractable proteins of the GA cross-linked arteries did also elicit a strong polymorphonuclear migration but nearly abolished monocyte recruitment. Although no differences in viability of migrated leukocytes were observed, it is conceivable that GA residuals within the tissue extracts might have influenced cell migration and GA-detoxification [32] would have changed results within this group. Protein extracts of highly purified porcine collagen I & III (BioGide®) led to a similar migration behavior of human leukocytes. It might be hypothesized that collagen fragments or collagenopeptides themselves are chemoattractive for PMNC. However, early studies demonstrated collagenopeptides derived from human or chicken skin to be chemotactic for monocytes but not for neutrophils [33]. Interestingly, in our study human monocytes did not respond to porcine collagen type I & III.

In this study, we used standardized amounts of proteins in order to exclude any bias in cell migration due to protein concentration dependency. Additionally, we tested serial dilutions of porcine native and decellularized tissue proteins and compared the initiated cell transmigration with the effects of serial dilutions of autologous serum. Hereby, we could clearly show that decellularization effectively reduces proteins capable of lymphocyte and monocyte recruitment but does not diminish granulocyte migration. Interestingly, monocyte migration toward protein extracts of porcine decellularized arteries was not increased until 1.0 µg/µL protein was used where cell migration was similar to that elicited by 0.125 µg/µL protein of porcine native arteries. These data indicate that the decellularization procedure effectively—but not completely—removes proteins capable of recruiting monocytes. We could additionally demonstrate that the decellularization procedure used in this study effectively removes DNA from the tissue.

However, as only a fraction of vascular wall proteins could be obtained by the extraction method any conclusions on the immune response against particular fibrillar elements cannot be drawn.

In our study focusing on the cellular immune response, not even GA-treatment diminished PMNC recruitment. Although much less attention has been paid to the response of granulocytes toward implanted organs or tissue, recent work has shown that PMNC seem to play an important role during xenograft and allograft rejection [30,31].

In the migration assay, only minor responses of non-stimulated lymphocytes within the 4 h incubation period were observed. These in vitro results mirror a typical early inflammatory reaction in vivo. Lymphocytes are known to migrate towards chemoattractants and accumulate in tissue, but monocytes and granulocytes are described to precede T-lymphocyte migration. However, differences were seen comparing native and decellularized tissue. Since graft rejection is known to be highly T-cell dependent, the particular lymphocyte response against decellularized tissue is currently under investigation in our laboratory.

To date it seems not clear to which extent the removal of certain proteins capable of leukocyte recruitment will affect the structural integrity of the tissue. It is known that non-cellular integral components of the extracellular matrix such as glycosaminoglycans (GAG) also play a role in tissue elasticity and specific removal can change the mechanical behavior. Furthermore protein damage, such as damage of elastic structures caused by decellularization procedures, can lead to profound changes in arterial geometry and structure [34]. Whether pre-treatment using
agents such as tannic acid to prevent potential eastin degradation [35] might be an option, has still to be proven. On the other hand, it is known that in vivo blood contact with collagen activates a multitude of events which lead to thrombocyte activation, liberation of chemotactic and proliferation of stimulating factors and consecutively to PMNC and macrophage influx. Both, decellularization and GA-crosslinking abolish monocyte but not granulocyte chemotaxis. One could hypothesize that collagen itself is responsible for the observed granulocytic response and simple GA cross-linking might not be fully successful. However, our data indicate that none of the contemporary approaches used to abolish tissue immunogenicity seems to be sufficient and further developments might be necessary to yield inert tissue scaffolds.

5. Conclusions

We have shown that a decellularization procedure shown to be effective for cell removal effectively reduces the response of monocytes and lymphocytes toward proteins extracted from allogeneic as well as xenogeneic vascular tissue. Interestingly, this could not be achieved for granulocytic cells that are known to be strongly involved in early inflammatory reactions. This granulocyte-based acute response was also observed for GA-fixed tissue. To date it remains unclear whether and how this persistent granulocytic recruitment of decellularized and cross-linked tissue might reflect the in vivo situation and whether a reduced PMNC response would influence the clinical outcome of such bioprosthesis. As the use of decellularized xenogenic instead of allogeneic tissue for tissue-engineering purposes would be of obvious advantage, further investigations on the immunological properties of this privileged tissue have to be undertaken.

References


