Neovascularization and vascular markers in a foreign body reaction to subcutaneously implanted degradable biomaterial in mice

van Amerongen, Machteld J.; Molema, Grietje; Plantinga, Josée; Moorlag, Henk; van Luyn, Marja J.A.

Published in:
Angiogenesis

DOI:
10.1023/A:1023822504224

Publication date:
2002

Angiogenesis **5**: 173–180, 2002.

Neovascularization and vascular markers in a foreign body reaction to subcutaneously implanted degradable biomaterial in mice

Machteld J. van Amerongen[1], Grietje Molema[2], Josée Plantinga[1], Henk Moorlag[2] & Marja J.A. van Luyn[1]

[1]*Laboratory for Tissue Engineering, Department of Pathology and Laboratory Medicine, Medical Biology Section, Groningen University Institute for Drug Exploration (GUIDE), Groningen, The Netherlands;* [2]*Tumor Immunology Laboratory, Groningen University Institute for Drug Exploration (GUIDE), Groningen, The Netherlands*

Received 20 March 2002; accepted in revised form 28 November 2002

Key words: angiogenesis, foreign body reaction, inflammation, vascular markers

Abstract

To study the spatiotemporal processes of angiogenesis during a foreign body reaction (FBR), biodegradable bovine collagen type-1 (COL-I) discs were implanted in mice for a period up to 28 days. The cellular infiltration (consisting mainly of macrophages, giant cells and fibroblasts), and the extent of neovascularization into the discs were determined. Also the expression levels and/or distribution of the endothelial cell markers von Willebrand factor (vWF), platelet endothelial cell adhesion molecule-1 (PECAM-1)/CD31, MECA-32 antigens and endomucin, and of the basal lamina marker collagen type IV (Coll IV) were analysed. In time, a strong neovascularization of the discs was observed, with frequently occurring vascular sprouting, and intussusceptive growth of vessels. In this model, vWF, MECA-32 and endomucin antibodies often failed to stain neovessels in the COL-I discs. In contrast, staining for collagen IV basal lamina component in combination with CD31 covered the complete range of neo-vessels. We conclude that the model described in this study is a useful model to study FBR induced angiogenesis because of the active neovascularization taking place during prolonged periods of time.

Abbreviations: AEC – 3-amino-9-ethyl-carbazole; Ang2 – angiopoetin 2; COL-I – collagen type-1; Coll IV – collagen type IV; FBR – foreign body reaction; GA – glutaraldehyde; HIF – hypoxia-inducible transcription factor; IFN-γ – interferon-γ; PBS – phosphate buffered saline; PDGF – platelet-derived growth factor; PECAM-1 – platelet endothelial cell adhesion molecule-1; RT – room temperature; TNF-α – tumour necrosis factor-α; VEGF – vascular endothelial growth factor; vWF – von Willebrand factor

Introduction

Angiogenesis involves the development of new vessels out of pre-existing ones by sprouting, the insertion of new intercapillary meshes into the lumen of pre-existing vessels, partitioning of the vessel lumen (intussuception), and/or endothelial precursor cell seeding at the site of angiogenesis [1]. Angiogenesis plays a critical role in a variety of pathological events, of which its role in tumour growth, chronic inflammation (e.g. rheumatoid arthritis [2], atherosclerosis [3] and psoriasis) and diabetic retinopathy has been most extensively studied [4].

Correspondence to: Dr Marja J.A. van Luyn, Laboratory for Tissue Engineering, Department of Pathology and Laboratory Medicine, Medical Biology Section, Groningen University Institute for Drug Exploration (GUIDE), Hanzeplein 1, 9713 GZ Groningen, The Netherlands. Tel: +31-50-3632503; Fax: +31-50-3632505; E-mail: m.j.a.van.luyn@med.rug.nl

About the induction and activation of angiogenesis during a foreign body reaction (FBR) in general, and in the herein described collagen type-1 (COL-I) model in specific, much less is known. In general, the FBR resembles a sterile inflammatory reaction, i.e., inflammation in the absence of accumulating granulocytes, lymphocytes and plasma cells, which can eventually become chronic [5]. Besides formation of a capsule and infiltration of cells such as macrophages into the biomaterial and fusion of these cells to giant cells [6, 7], protrusion of neovascular sprouts into the biomaterial and surrounding tissue is a main characteristic of the FBR [8–10]. The goal of this study was to determine how the process of angiogenesis evolves during a FBR. For this purpose we selected a biodegradable bovine COL-I matrix, which was shown to be non-cytotoxic and biocompatible, and elicited a FBR as previously described [11]. After subcutaneous implantation of this

matrix in mice, the FBR was evaluated histologically and immunohistochemically. In the case of immunohistochemical analysis of (neo) vessels, different markers were studied to circumvent a potential underestimation of the extent of neovascularization. In this study the spatiotemporal expression of the well established, frequently used endothelial cell markers von Willebrand factor (vWF), platelet endothelial cell adhesion molecule-1 (PECAM-1)/CD31 and MECA-32 antigen [12], were compared with the more recently identified endothelial cell marker endomucin [13], and with the production of collagen type IV (Coll IV), a component of the basal lamina of vessels.

Materials and methods

Biomaterial

A lyophilized, hexamethylenediisocyanate cross-linked COL-I bovine matrix processed from bovine achilles tendon was obtained from Biomaterials Research B.V. (Vaals, The Netherlands) and is hereafter referred to as COL-I. This material is devoid of immunogenic reactive groups. Discs of 6 mm in diameter were punched and sterilized by ethylene oxide.

Animals

Ten-week-old male C57bl/6 mice (Harlan Nederland, Horst, The Netherlands) were housed individually in an animal room with controlled temperature (20 ± 2 °C) and humidity ($50 \pm 10\%$). Animals received pelleted diet (RMH-B 10 mm, Hope Farms, Woerden, The Netherlands) and water *ad libitum*.

Surgical procedure

The study as presented was approved by the local committee for care and use of laboratory animals and was performed according to strict governmental and international guidelines on experimentation.

Under halothane/N_2O/O_2 anesthesia mice were shaved and disinfected with chloral hexidine, after which subcutaneous pockets were made to the right and left of a midline incision on the back. COL-I discs were implanted at a distance of 1 cm from the incision, one at either side. Discs with surrounding tissue were carefully dissected from the subcutaneous site at 7 ($n = 5$), 14 ($n = 7$), 21 ($n = 6$) and 28 ($n = 7$) days after implantation. Immediately after explantation, one disc was immersion-fixed in 2% (v/v) glutaraldehyde (GA) in 0.1 M phosphate buffer (pH 7.4) for at least 24 h at 4 °C. The other disc was snap-frozen in liquid nitrogen.

Histological evaluation of the foreign body reaction

The GA-fixed COL-I discs were dehydrated in graded series of ethanol and embedded in Technovit 7100 (Heraeus Kulzer, Wehrheim, Germany). Longitudinal semi-thin mid-sections (2 μm) were stained with toluidine blue and mounted in Permount (Fisher, New Jersey, USA). The tissue response in the cross-sectional area of the discs was evaluated by analysis of two sections per disc, and rated independently by at least two people, according to a scoring system ranging from 0 (no vascularization), 1 (low vascularization), 2 (moderate vascularization), 3 (high vascularization) to 4 (extensive vascularization) (Figure 1). Inter-rater agreement was calculated as Cohen's κ with values of 0.91 or higher, indicating a very good agreement [14].

The same score was used for the evaluation of the extent of cellular infiltration.

Immunohistochemical staining

Longitudinal mid-sections (7 μm; two sections per disc) from the snap-frozen discs were cut, fixed with acetone and pre-incubated with phosphate buffered saline (PBS, NPBI, Emmer-Compascuum, The Netherlands) containing 10% serum of the species, which produced the secondary antibody. Slides were incubated with primary antibodies (Table 1) diluted in PBS supplemented with 1% (v/v) bovine serum albumine (Central Laboratory for Blood Transfusions, Amsterdam, The Netherlands) for 60 min at room temperature (RT). After washing, endogenous peroxidase was blocked by incubation with 0.1% H_2O_2 in PBS for 10 min followed by incubation with appropriate secondary antibodies conjugated with peroxidase (Swine anti-Rabbit IgG, Rabbit anti-Rat IgG and Rabbit anti-Goat IgG all obtained from DAKO, Glostrup, Denmark) for 30 min at RT. Conjugates were diluted 1/100 in PBS supplemented with 2% mouse serum.

Colour development was performed with 3-amino-9-ethyl-carbazole (AEC, Sigma, Steinheim, Germany) as substrate dissolved in *N*,*N*-dimethylformamide (Merck, Darmstadt, Germany)/0.5 M acetate buffer, pH 4.9. Slides were counterstained with Mayer's hematoxylin and mounted in Kaiser's glycerin. As positive controls, sections of C57bl/6 livers and kidneys were stained.

Results

Cellular infiltration and neovascularization in time: Histological analysis

The degree of neovascularization as part of the FBR to the biodegradable biomaterial COL-I was established in mice up to 28 days. In time, the number of vessels present in the COL-I matrix increased until day 21 (Figure 2).

At day 7 after implantation, the cellular ingrowth at the outer rim of the implant mainly consisted of macrophages, granulocytes, fibroblasts and some binuclear giant cells. The identification of macrophages and fused macrophages (giant cells) was also

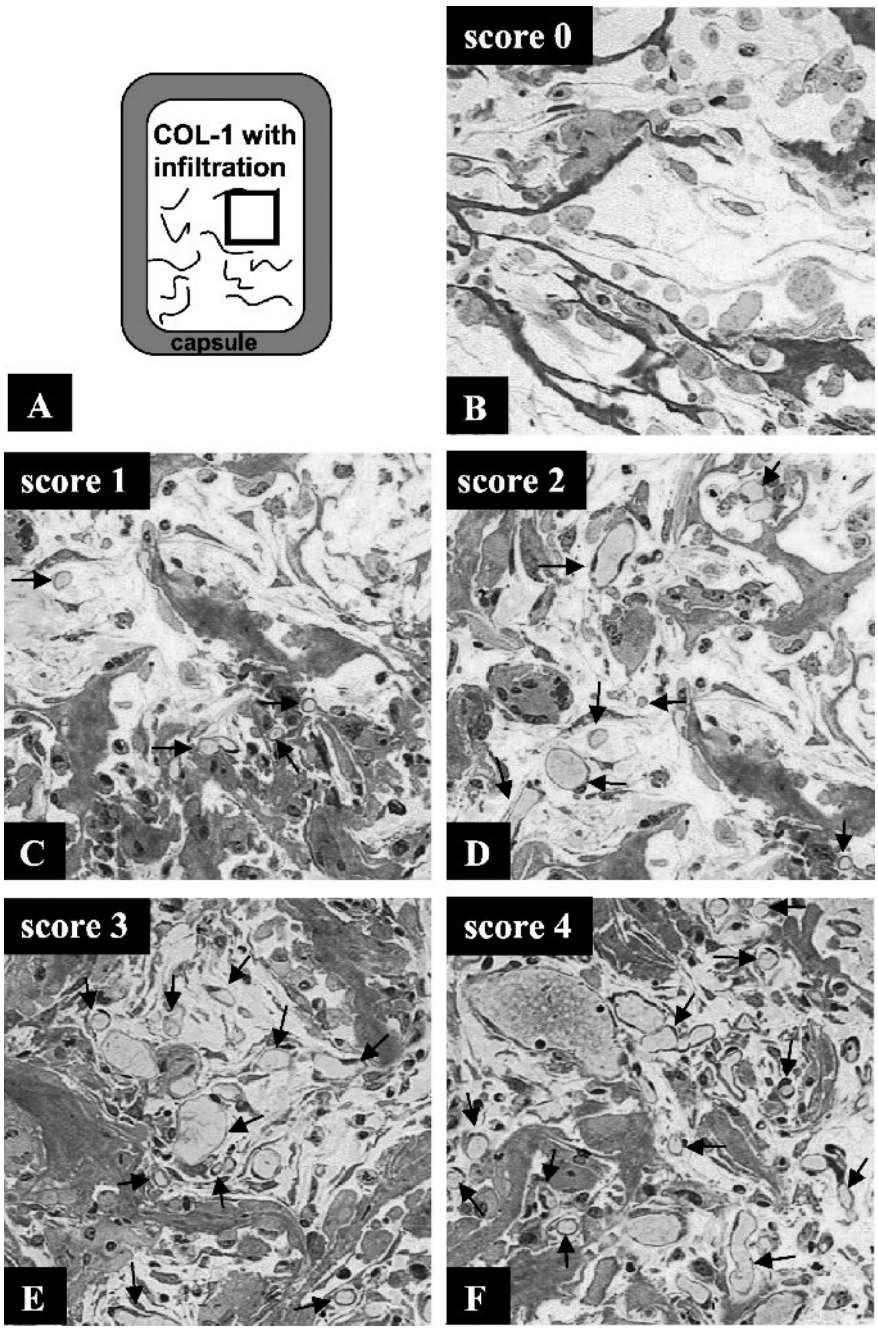

Figure 1. Grading as used in the scoring system for determining neo-vessels. The area of the COL-I discs of which the micrographs were taken is indicated by the square in the schematic overview of the COL-I disc (A). The neovascularization of the total cross-sectional area of the discs was evaluated and rated, according to a scoring system ranging from 0 (no vascularization in between the COL-I bundles (I); B) to 4 (extensive vascularization in between the COL-I bundles (I); F). In all figures arrows indicate a selection of the blood vessels present. Original magnification ×100.

Table 1. Primary antibodies used for immunohistochemistry.

Antibody	Antigen	Dilution	Resource
MEC13.3	Murine PECAM-1/CD31	1:100	PharMingen, Becton Dickinson B.V., Woerden, The Netherlands
Anti-Coll IV	Human collagen IV, cross reacts with mouse collagen IV	1:150	Southern Biotechnology Associates, ITK Diagnostics, Uithoorn, The Netherlands
Anti-vWF	Murine vWF	1:200	Dako, Glostrup, Denmark
V5C7.8	Murine endomucin	Hybridoma supernatant	Dr D. Vestweber, Germany
MECA-32	Murine MECA-32	Hybridoma supernatant	Iowa Hybridoma Bank, USA

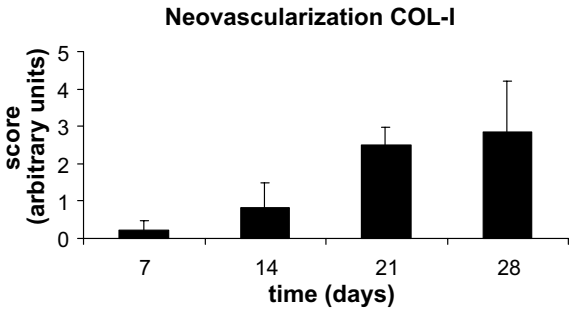

Figure 2. Neovascularization into the COL-I discs in mice as determined by toluidine blue staining of disc cross-sections at days 7, 14, 21 and 28 after implantation. Values are mean ± SD of histologically scored sections as described in materials and methods. Score 0 (no vascularization) to 4 (extensive vascularization).

immunohistologically assessed by staining with the monocyte/macrophage marker MOMA2 (Biosource Netherlands BV; results not shown). Sporadically a lymphocyte could be observed (< 1% of total cell number). Around the COL-I implant, a thin capsule had formed existing of fibroblasts and matrix components. Between the COL-I bundles a fibrin network with little cellular ingrowth was observed. The COL-I discs were hardly vascularized at this time point. Only scarcely small blood vessels were present in the discs, in contrast to the surrounding tissue outside the capsule, which presented as highly vascularized (Figure 3A).

At day 14, the area of the COL-I biomaterial that contained cellular ingrowth had strongly increased, representing on average one quarter of the total disc. This ingrowth mainly consisted of macrophages and giant cells. The total number of giant cells, containing approximately 4–5 nuclei per cell, was clearly increased as compared to day 7. At the same time, the number of granulocytes had strongly decreased. Areas containing fibroblast and matrix components had formed between the COL-I bundles at the edge of the discs. At this stage blood vessels were observed in the biomaterial (Figure 3B). Besides frequently occurring vascular sprouting, also intussusceptive growth of the vessels could be observed (Figure 4).

Twenty-one days after implantation more than half of the disc consisted of cellular ingrowth. The number of macrophages and giant cells had strongly increased. The

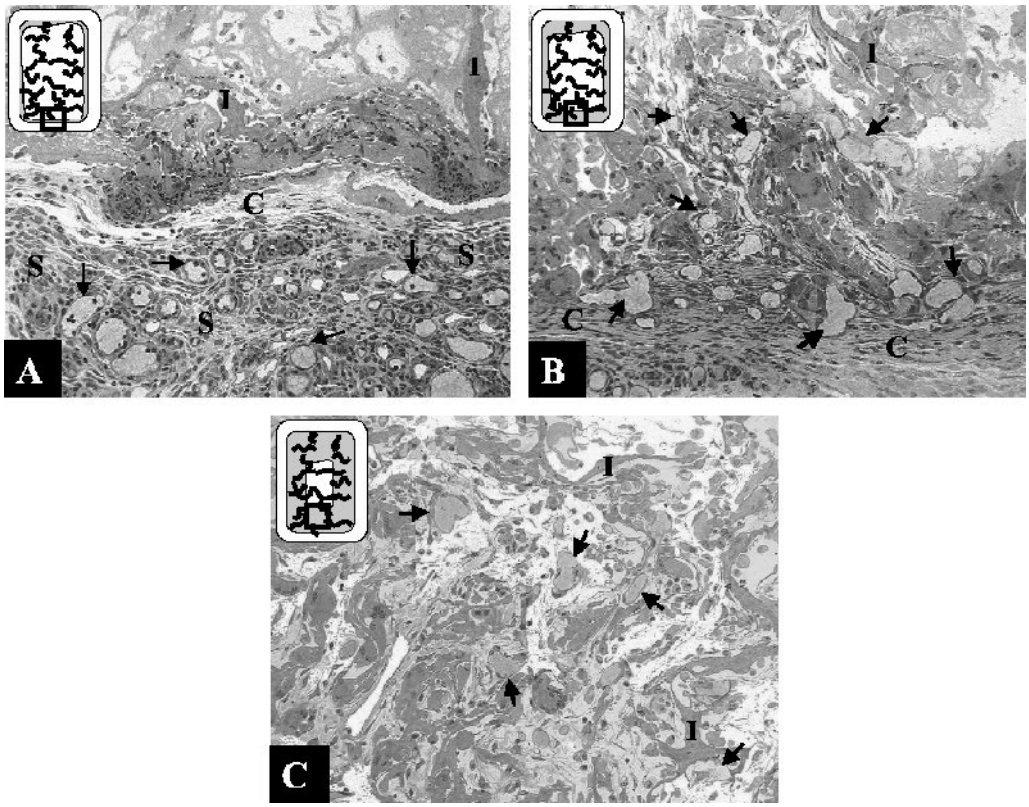

Figure 3. Light micrographs of neovascularization into COL-I discs and surrounding tissue at day 7 (A), 14 (B) and 21 (C). The schematic overview of the COL-I disc in each micrograph illustrates the extent of cellular influx into the COL-I disc (grey area) at the time point under investigation. The square marks the area of the COL-I discs which is shown in the micrograph. At all time points, a (thin) capsule (C) surrounded the discs. In all figures arrows indicate a selection of the blood vessels present. (A) Neovascularization was not observed in between the COL-I bundles (I) (upper part of image), in contrast to the surrounding tissue outside the capsule (C) which was highly vascularized. In the surrounding tissue large stromal areas (S) were formed containing fibroblasts and matrix components. (B) Blood vessels were present in between the COL-I bundles at the periphery of the disc. (C) Vascularization of the COL-I discs had strongly increased. Blood vessels were found in between the COL-I bundles of almost the entire disc. Original magnification ×100.

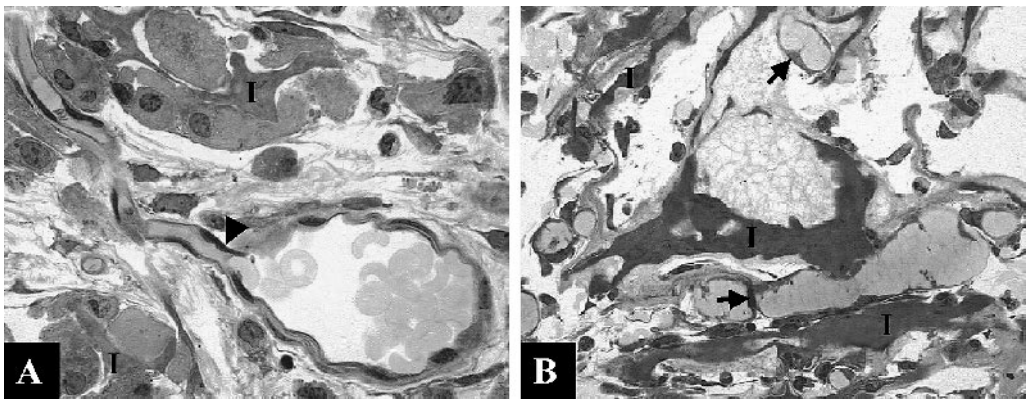

Figure 4. Light micrographs at day 14 of (A) a sprouting (arrowhead) vessel and (B) vessel intussusception (arrows) between the COL-I bundles (I). Original magnification (A) ×400; (B) ×200.

largest giant cells were found at the edge of the discs, containing a lot of cytoplasm and large nuclei. In between the cellular ingrowth, extensive areas with fibroblasts and matrix components were observed. The vascularization of the COL-I implants had also strongly increased, covering almost the entire disc (Figure 3C). Most vessels were found in between infiltrating cells, although occasionally they were also found deeper into the COL-I discs. Whereas large vessels were presented at the periphery, smaller vessels were found in the center of the discs. Sprouting and intussusceptive growth were still present.

Between day 21 and day 28, the cellular ingrowth hardly increased, except for two out of eight COL-I discs that were completely infiltrated with macrophages, giant cells, fibroblasts and matrix components. The number of nuclei of the giant cells in all discs had increased to an average of 10 per cell. Degradation of COL-I could not be observed. High numbers of fibroblast and excessive matrix formation in between the collagen bundles could be observed, which is in accordance with previous observations with biomaterial implants in mice [11]. At day 28, the COL-I discs were entirely vascularized. At the periphery of the discs, the vessels contained a clearly dilated lumen as compared to the vessels in the middle of the discs, although also at the latter site dilated vessels were occasionally observed. Sprouting and intussusception were still present.

Immunohistochemical analysis of neovasculature

Using immunohistochemistry to study neovascularization, the possibility exists that the use of vascular markers would lead to underestimation of the extent of neovascularization, as their expression may be temporarily lost during the angiogenic cascade. We therefore analysed the expression of some established, frequently used, and some more recently identified vascular markers by immunohistochemistry (see Table 1). The results were compared to the data obtained by the histologically quantified neovascularization. A summary of the immunohistochemical staining results of the vascular markers is given in Figure 5. The liver and kidney vasculature, used as controls for the staining procedure, stained markedly with the primary antibodies exploited (data not shown), indicating a proper staining procedure.

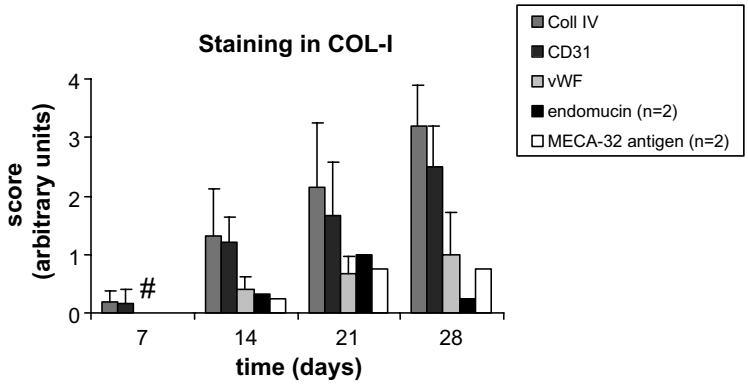

Figure 5. Semi-quantitative analysis of immunohistochemically stained COL-I cross-sections, using antibodies for Coll IV, CD31, vWF, MECA-32 antigens and endomucin, as determined at days 7, 14, 21 and 28. Values for Coll IV, CD31 and vWF are mean ± SD. # At day 7 a high background staining was observed in all COL-I discs using vWF antibody, which made it not possible to evaluate the degree of neovascularization.

As described above, hardly any vessels were observed histologically in the COL-I discs at day 7. This was confirmed by the immunohistochemical analysis of the vascular markers. For all other time points, a summary of immunohistochemically identified markers is given below.

Collagen type IV

The number of vessels and pattern of vascularization as detected by Coll IV staining were at all time points comparable to the number of vessels established in the histologically evaluated COL-I discs. The basal lamina of all large and small vessels in the discs and the surrounding tissue stained markedly (Figures 6A and B). Morphologically, not all Coll IV stained structures could be identified as vessels. However, the fact that Coll IV staining was comparable, qualitatively and semi-quantitatively, to the histological assessment of the vessels, plus the observation that this marker did not have any background staining throughout the COL-I discs, led us to conclude that Coll IV staining allowed proper identification of the neovasculature in this model.

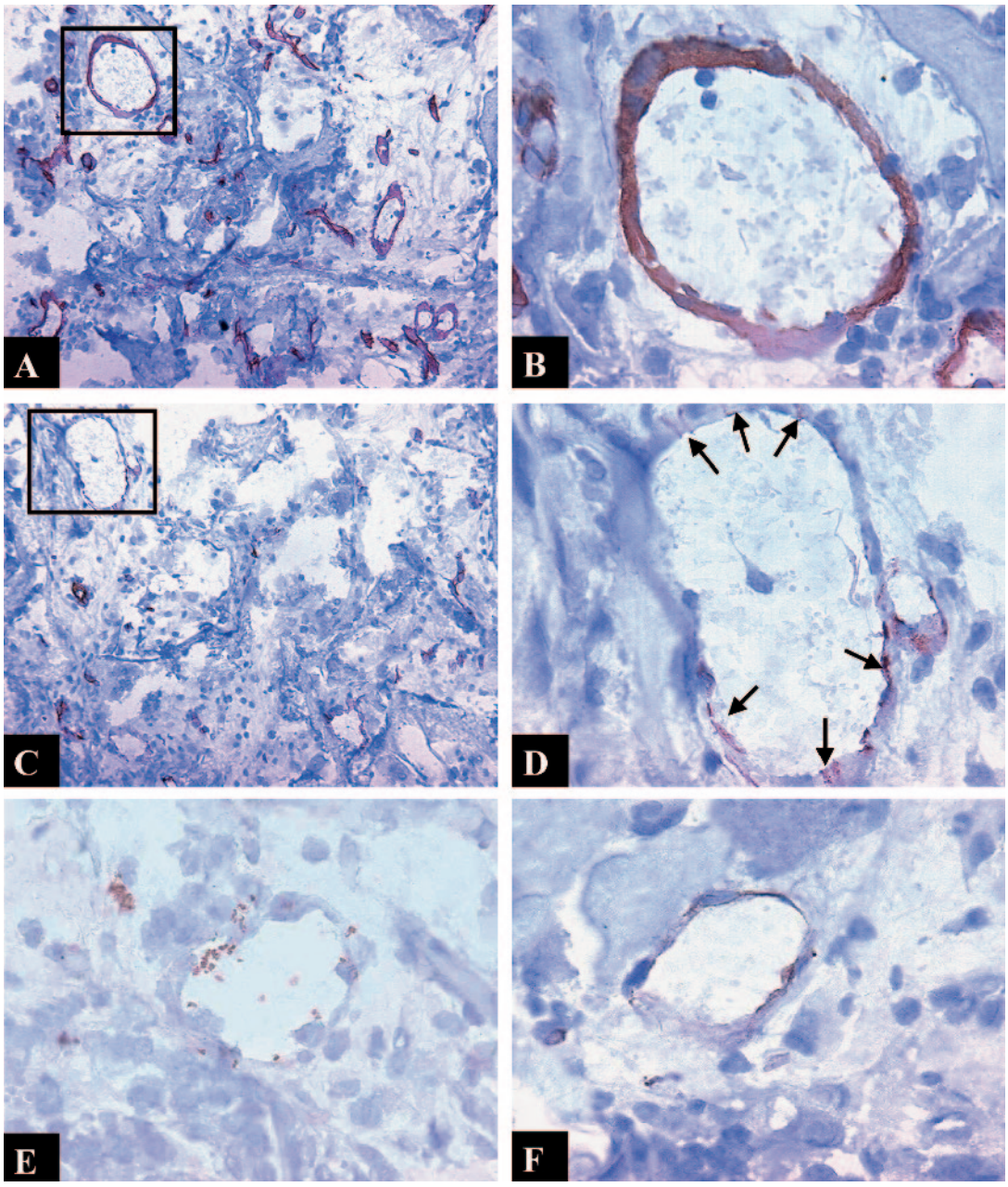

Figure 6. Light micrographs of COL-I sections at day 21 stained with antibodies for Coll IV (A, B), CD31 (C, D) and endomucin (F) and at day 28 after staining with an antibody for vWF (E). (A, B) The basal lamina of all vessels in the COL-I discs stained markedly using Coll IV. (C) In comparison to the latter, less vessels could be identified by CD31 detection. (D) Represents the irregular distribution pattern of CD31 (indicated by arrows). (E) The granular staining of vessels for vWF was sporadically observed. (F) Low intensity staining of vessels for endomucin was seen throughout the COL-I discs. Original magnification A, C: ×100; B, D: ×400; E, F: ×200.

CD31/PECAM-1

Up to day 14, the number of vessels observed by CD31 staining was comparable to the number of vessels observed by Coll IV staining (Figure 5). However, the intensity of the staining by the CD31 specific antibody was far less than the intensity of the staining by the Coll IV specific antibody. Furthermore, the CD31 specific antibody did not always stain all endothelial cells making up a vessel, but rather presented an irregular distribution pattern. At day 21 and 28, CD31 expression decreased or was even absent in endothelium of vessels in the COL-I disc. As a result, the total number of vessels identified by CD31 detection was less than that observed using Coll IV staining (Figures 6C and D).

von Willebrand factor

At day 7, high background staining was observed in all COL-I discs when using vWF specific antibody. This phenomenon made it hard to evaluate the degree of neovascularization at this time point, and is possibly a result of the surgical procedure leading to extensive vWF release at the site of implantation. vWF antibody failed to stain most of the endothelial cells of the vessels present in the COL-I discs at days 14 and 21. At day 28 a granular staining of vessels growing into the periphery of the COL-I discs was observed and sporadically vessels in the middle of the COL-I disc were stained (Figure 6E). At all time points, the capsule was often diffusely positive.

Endomucin

At day 14, endomucin expression was detected as a faint endothelial cell staining at the outer rim of the COL-I discs. At days 21 and 28, staining of vessels was seen throughout the COL-I discs (Figure 6F). The pattern of staining was comparable with that observed for Coll IV and CD31, but the staining was much less intense. Furthermore, the total number of vessels identified using the endomucin specific antibody was far less than when using the CD31 or Coll IV specific antibody.

MECA-32 antigens

At days 14, 21 and 28, a weak cellular staining was observed at some places in the COL-I discs. Due to the diffuse pattern of staining, identification of vessels was rather difficult to perform, although sporadically a clear endothelium associated staining of vessels was observed.

Discussion

The first goal in this study was to determine how the process of angiogenesis evolves as part of a FBR. After subcutaneous implantation of COL-I, cellular infiltration consisted for the main part of macrophages, giant cells and fibroblasts. This infiltration was followed by a strong neovascularization in time. Both angiogenic sprouting and intussusceptive growth were regularly observed throughout the observation period of 28 days. In general macrophages and giant cells play an important role in degradation of the biomaterial, although phagocytosis of the biomaterial did not occur in the 28 days of observation [11]. Fibroblasts form a fibrous capsule around the biomaterial, which shields it from the rest of the body, and produce extracellular matrix components in between the COL-I bundles. The exact relation between the cellular infiltration and neovascularization is not known, but they obviously seem to depend on each other. Early in the FBR, the infiltrating cells likely encounter locally reduced oxygen pressure in the COL-I discs. Hypoxia activates macrophages to induce transcriptionally active hypoxia-inducible factors (HIFs), which induce the expression of angiogenic factors including vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), angiopoetin 2 (Ang2) and others [15, 16].

From an immunohistochemical point of view, Coll IV showed to be a useful vascular marker in this model, as all vessels in the COL-I discs were positive for Coll IV at all time points. This means that the basal lamina of the vessels was constantly present. Degradation of the basal lamina is an important step in angiogenesis to facilitate migration of endothelial cells. It was therefore expected that during angiogenesis in the COL-I discs, vessels would be present that (partly) lacked a basal membrane. Our data are however in agreement with other reports showing that in all newly formed vessels and sprouts of vessels a basal lamina was present [17, 18]. It is important to realize that positive staining for vascular markers in general does not directly relate to the functionality of the vessels. Histological assessment using toluidine blue, however, showed that almost all vessels contained erythrocytes, indicating (partly) functional, perfused vessels.

Staining for the endothelial cell marker vWF often failed to identify vessels in this model, which is in accordance with other studies [19, 20]. The low or marginal staining of some of the markers studied cannot be due to masking of the antigens by the experimental procedures, because non-fixed frozen discs were used. Endothelial cells under pro-angiogenic pressure possibly temporarily loose their vWF content. At days 21 and 28, anti-CD31 also failed to stain vessels, although to a lesser extent than anti-vWF. Furthermore, the endothelium of vessels that stained positive for CD31 showed a strong redistribution of CD31. CD31 can both be diffusely expressed on the surface of individual endothelial cells, and be enriched at sites of cell–cell contact depending on the localization in the body and within the tissue [21]. Under the influence of the inflammatory cytokines TNF-α and IFN-γ, CD31 can leave cell junctions and become diffusely localized over the cell surface *in vitro* [22]. Furthermore, IFN-γ and TNF-α can lead to downregulation of CD31 expression [23, 24].

Possibly, these cytokines, all shown to play an important role in the inflammatory reaction to biomaterials [25–27], may have played a role in the observed CD31 redistribution phenomenon. Our data indicate that CD31 and especially vWF do not represent reliable endothelial cell markers in this neovascularization model. They are however often used as vascular marker to detect vessels [28–30]. Although these latter studies exploited other models of angiogenesis (i.e., tumour and arthritis models), the results of such studies should be interpreted with caution.

Both MECA-32 and endomucin were found to be less useful vascular markers in the COL-I neovascularization model, because of the low expression of both antigens by the neovasculature. As the function of both antigens is not known, the molecular and (patho)physiological basis for the low expression remains unclear at present.

Overall, we conclude that the model described is a useful model to study angiogenesis in FBR related inflammation, because of the active neovascularization taking place for a prolonged period of time. Collagen IV is the best vascular marker in this model of FBR induced neovascularization, while CD31 is a suitable marker to combine with Coll IV. Histological analyses should, however, always be performed to get vascular marker independent information on the quantity and morphology of the neovasculature under development. In the future, full morphometric analyes are needed to confirm these results.

Acknowledgements

Dr N.F. Wieringa is kindly acknowledged for calculating inter-rater agreement values.

References

1. Patan S, Haenni B, Burri PH. Implementation of intussusceptive microvascular growth in the chicken chorioallantoic membrane (CAM): 1. Pillar formation by folding of the capillary wall. Microvasc Res 1996; 51: 80–98.
2. Colville-Nash PR, Scott DL. Angiogenesis and rheumatoid arthritis: Pathogenic and therapeutic implications. Ann Rheum Dis 1992; 51: 919–25.
3. Ross R. Atherosclerosis is an inflammatory disease. Am Heart J 1999; 138: S419–20.
4. Griffioen AW, Molema G. Angiogenesis: Potentials for pharmacologic intervention in the treatment of cancer, cardiovascular diseases, and chronic inflammation. Pharmacol Rev 2000; 52: 237–68.
5. Coleman DL, King RN, Andrade JD. The foreign body reaction: A chronic inflammatory response. J Biomed Mater Res 1974; 8: 199–211.
6. Anselme K, Bacques C, Charriere G et al. Tissue reaction to subcutaneous implantation of a collagen sponge. A histological, ultrastructural, and immunological study. J Biomed Mater Res 1990; 24: 689–703.
7. van Wachem PB, van Luyn MJ, Olde Damink LH et al. Biocompatibility and tissue regenerating capacity of crosslinked dermal sheep collagen. J Biomed Mater Res 1994; 28: 353–63.
8. Fournier N, Doillon CJ. Biological molecule-impregnated polyester: An *in vivo* angiogenesis study. Biomaterials 1996; 17: 1659–65.
9. Padera RF, Colton CK. Time course of membrane microarchitecture-driven neovascularization. Biomaterials 1996; 17: 277–84.
10. Pieper JS, van Wachem PB, van Luyn MJA et al. Attachment of glycosaminoglycans to collagenous matrices modulates the tissue response in rats. Biomaterials 2000; 21: 1689–99.
11. Khouw IM, van Wachem PB, Molema G et al. The foreign body reaction to a biodegradable biomaterial differs between rats and mice. J Biomed Mater Res 2000; 52: 439–46.
12. Altman DG. Practical Statistics for Medical Research. London: Chapman & Hall 1991.
13. Streeter PR, Berg EL, Rouse BT et al. A tissue-specific endothelial cell molecule involved in lymphocyte homing. Nature 1988; 331: 41–6.
14. Morgan SM, Samulowitz U, Darley L et al. Biochemical characterization and molecular cloning of a novel endothelial-specific sialomucin. Blood 1999; 93: 165–75.
15. Semenza GL. Hypoxia-inducible factor 1: Master regulator of O2 homeostasis. Curr Opin Genet Dev 1998; 8: 588–94.
16. Sunderkotter C, Steinbrink K, Goebeler M et al. Macrophages and angiogenesis. J Leuk Biol 1994; 55: 410–22.
17. Jerdan JA, Michels RG, Glaser BM. Extracellular matrix of newly forming vessels – an immunohistochemical study. Microvasc Res 1991; 42: 255–65.
18. Kuwashima Y, Uehara T, Kurosumi M et al. Basement membrane status in undifferentiated carcinomas of the ovary. Immunohistochemical distribution of type IV collagen and laminin. Eur J Gynaecol Oncol 1995; 16: 181–6.
19. Ruiter DJ, Schlingemann RO, Westphal JR et al. Angiogenesis in wound healing and tumor metastasis. Behring Inst Mitt 1993; 92: 258–72.
20. Schlingemann RO, Rietveld FJ, Kwaspen F et al. Differential expression of markers for endothelial cells, pericytes, and basal lamina in the microvasculature of tumors and granulation tissue. Am J Pathol 1991; 138: 1335–47.
21. Albelda SM, Muller WA, Buck CA, Newman PJ. Molecular and cellular properties of PECAM-1 (endoCAM/CD31): A novel vascular cell–cell adhesion molecule. J Cell Biol 1991; 114: 1059–68.
22. Romer LH, McLean NV, Yan HC et al. IFN-gamma and TNF-alpha induce redistribution of PECAM-1 (CD31) on human endothelial cells. J Immunol 1995; 154: 6582–92.
23. Stewart RJ, Kashxur TS, Marsden PA. Vascular endothelial platelet endothelial adhesion molecule-1 (PECAM-1) expression is decreased by TNF-alpha and IFN-gamma. Evidence for cytokine-induced destabilization of messenger ribonucleic acid transcripts in bovine endothelial cells. J Immunol 1996; 156: 1221–8.
24. Rival Y, Del Maschio A, Rabiet MJ et al. Inhibition of platelet endothelial cell adhesion molecule-1 synthesis and leukocyte transmigration in endothelial cells by the combined action of TNF-alpha and IFN-gamma. J Immunol 1996; 157: 1233–41.
25. Khouw IMSL, van Wachem PB, de Leij LFMH, van Luyn MJA. Inhibition of the tissue reaction to a biodegradable biomaterial by monoclonal antibodies to IFN-γ. J Biomed Mater Res 1998; 41: 202–10.
26. Cardona MA, Simmons RL, Kaplan SS. TNF and IL-1 generation by human monocytes in response to biomaterials. J Biomed Mater Res 1992; 26: 851–9.
27. DeFife KM, Yun JK, Azeez A et al. Adhesion and cytokine production by monocytes on poly(2-methacryloyloxyethyl phosphorylcholine-co-alkyl methacrylate)-coated polymers. J Biomed Mater Res 1995; 29: 431–9.
28. Lee AH, Happerfield LC, Bobrow LG, Millis RR. Angiogenesis and inflammation in ductal carcinoma *in situ* of the breast. J Pathol 1997; 181: 200–6.
29. Lu J, Kasama T, Kobayashi K et al. Vascular endothelial growth factor expression and regulation of murine collagen-induced arthritis. J Immunol 2000; 164: 5922–7.
30. Orre M, Rogers PA. Reduced vascular basement-membrane immunostaining in mucinous tumours of the ovary. Int J Cancer 1998; 79: 139–43.