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Journal of Autoimmunity 23 (2004) 81–91

journal of autoimmunity

www.elsevier.com/locate/issn/08968411

Cellular and molecular composition of fibrous capsules formed around silicone breast implants with special focus on local immune reactions☆

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Received 4 November 2003; revised 4 February 2004; accepted 1 March 2004

Abstract

During the past 30 years, much debate has centered around side effects of silicone breast implants. Meta-analyses rejected the presumed relationship between silicone breast implants and connective tissues diseases but, in seeming contradiction, case reports about connective tissue diseases and rheumatoid symptoms continue to be published.

We analyzed the cellular and molecular composition of fibrous capsules removed from patients at various times after surgery for diagnostic purposes (breast cancer relapse) or to relieve painful constrictive fibrosis.

Frozen sections of capsule tissue were immunohistochemically stained for subsets of lymphocytes, macrophages, dendritic cells, fibroblasts, smooth muscle cells, for collagenous and non-collagenous extracellular matrix proteins, for heat shock protein 60 (HSP60) and for adhesion molecules.

Massive deposition of fibronectin and tenascin was observed adjacent to the implant surface. The capsule/silicone implant contact zone was consistently characterized by a palisade-like single or multilayered cell accumulation consisting of HSP60+ macrophages and HSP60+ fibroblasts. Mononuclear cell infiltrates consisting of activated CD4+ T-cells, expressing CD25 and CD45RO, as well as macrophages were detected beneath the contact zone as well as perivascularly. Importantly, many Langerhans-cell like dendritic cells (DCs) were found with a predilection at the frontier layer zone abutting the silicone implant. Also, at this site, massive expression of ICAM-1, but not VCAM-1 or ELAM-1 emerged. Endothelial cells of the intracapsular neovasculature were P-Selectin+.

Our results show that silicone induces a strong local T-cell immune response and future studies will determine the specificity and function of these T-lymphocytes.

Keywords: Silicone; Breast implants; Side effects; Immunity; T-cells; Adhesion molecules; Extracellular matrix proteins

1. Introduction

Silicone breast implants have been widely used for more than 50 years in reconstructive and aesthetic surgery [1–3] and more than a million women worldwide are known to have received such implants [4]. However, the estimated number of unknown implantations raises this figure significantly.

Localized and systemic complications in silicone implants have been reported in the literature [5], and the most common general complication is constrictive fibrosis resulting in excessive firmness and pain. However, data about specific local side effects (e.g. local immune response, activity of immune cells, functional

☆ Grants and support: Udo Saldow *in memoriam* Lore Saldow, P14466 to GW from the Austrian Science Fund, Ludwig Boltzmann Institute for Quality Control in Plastic Surgery.

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doi:10.1016/j.jaut.2004.03.005

Nomenclature

APAAP	alkaline phosphatase/anti alkaline phosphatase
CD	cluster of differentiation
DC	dendritic cells
ECM	extracellular matrix
HSP	heat shock protein
ICAM-1	intercellular adhesion molecule
TBS	Tris buffered saline
TCR	T-cell receptor
VCAM-1	vascular cell adhesion molecule
vWF	von Willebrand factor

studies with lymphocytes extracted from fibrous capsules) have rarely been addressed or reported. In addition to these local phenomena, several case reports of systemic connective tissue diseases [6–10], primarily scleroderma-like or rheumatologic symptoms and musculoskeletal disorders, have been published. As a result, the United States Food and Drug Administration (FDA) banned gel-filled implants in 1992 [11] for general use, except for patients undergoing breast reconstruction after therapeutic mastectomy, until additional studies were conducted and a more definitive conclusion could be reached regarding their safety [12]. Meta-analyses were performed to clarify the controversial relation between silicone breast implants and systemic side effects [13–17], but these studies have neither confirmed nor denied the safety of silicone breast implants.

Normal tissue response to silicone breast implants consists of the formation of a fibrous capsule of varying thicknesses. This encapsulation maintains correct implant positioning, and the histological composition of this fibrous capsule has been described previously [18–22].

To provide more specific information about possible immunological processes that may be involved in capsule formation, we focussed on the distribution and activity of immune cells (CD4+/CD8+ T-cells, dendritic cells, CD44, CD45RO, CD45RA and CD25 expressing cells, macrophages), as well as collagenous and non-collagenous extracellular matrix (ECM) proteins (procollagen type I and type III, collagen type I and type III, laminin, tenascin, fibronectin), HSP60 expression, reflecting the effect of mechanical or other forms of stress exerted on the implant and capsule, respectively, and on several adhesion molecules (ICAM-1, P-selectin, VCAM-1).

The purpose of this study was to understand and characterize the previously described local inflammatory immunologic reaction [20], as a preliminary step to clarifying whether this local immunologic reaction gives rise to subsequent systemic immune reactions and to establish a baseline measurement for potential prophylactic and therapeutic interventions for silicone implant side effects.

2. Materials and methods

2.1. Peri-implant capsular tissue

During the study period (October 2000–October 2001), 15 patients from 23 to 61 years of age who had received silicone breast implants from 4 months to 20 years prior, underwent implant removal, and in 4 instances simultaneous bilateral implant replacements were performed. So, our study included a total of 19 capsules. The implant removals were prompted by development of high-grade capsule fibrosis (Baker grade 3–4) in 8 cases, suspicion of breast carcinoma relapse in 3 cases, and unaesthetic results from the original implant in 4 patients. None of the patients showed symptoms of rheumatological disorders, connective tissue disease, fibromyalgia-like symptoms, or systemic involvement (e.g. arthralgia, myalgia, lymphadenopathy).

The surgical procedures were performed at the Department of Plastic Surgery, University Innsbruck (Austria), Medical School and the Department of Plastic Surgery Bogenhausen, Munich (Germany).

The surgical incision for removal of the implants were generally made within the scar resulting from the first implantation or previous implant changes, and the entire capsule, or at least a piece of 40 × 40 mm of capsular tissue was secured for the immunohistological studies.

The fibrous capsules were cut into small pieces (20 × 10 mm) and the interior surface that had been in contact with silicone was applied to a foil for orientation before the samples were placed in liquid nitrogen (−196 °C).

Finally, the pieces were cut to 4 μm thick frozen sections, which were placed onto silane-coated slides for further immunohistological preparations and stored at −20 °C until use. Around 130–160 sections per capsule were prepared for the immunohistological stainings. After establishing the optimal dilutions of all reagents, 5–8 sections at different plants were used per antibody for the experiments.

The characteristics and sources of antibodies and conjugates used are listed in Table 1. Optimal dilutions of all reagents were determined in pilot studies.

2.2. Staining procedures

2.2.1. Immunohistochemistry on frozen sections

After the slides with frozen sections were air dried for 30–60 min at room temperature, they were fixed in pure acetone for 10 min. Only sections analyzed for procollagens and collagens in immunofluorescence and for dendritic cells (DCs) in immunohistochemistry remained unfixed. Blocking of non-specific antibody binding was

Table 1
Sources and dilutions of antibodies and conjugates

Antibody specificity	Working dilution	Source	Catalogue number
CD3	1:50	DAKO	M0835
CD4	1:25	DAKO	M0716
CD8	1:50	DAKO	M0707
CD19	1:25	DAKO	M0740
CD25	1:25	DAKO	M0731
CD68	1:100	DAKO	M0814
CD45RO (memory cells)	1:25	DAKO	M0834
CD45RA (naive cells)	1:25	DAKO	M0754
CD44	1:25	DAKO	M708
CD1a	1:25	DAKO	
CD207 (Langerin)	1:25	Beckman-Coulter	IM3449
CD208 (DC-LAMP)	1:25	Beckman-Coulter	
CD209 (DC-SIGN)	1:25	R&D Systems	MAB161
HLA DR, DQ, DP	1:50	DAKO	M0731
Fibroblasts	1:25	DAKO	M0877
HSP60	1:15	Stressgen	SPA-805
Smooth muscle actin	1:50	DAKO	M0851
TCR α/β	1:25	Biodesign	P42466M
TCR γ/δ	1:25	Serotec	MCA989
von Willebrand Faktor (vWF)	1:200	DAKO	A0082
Procollagen type I	1:10	Chemicon	MAB1913
Procollagen type III	1:10	Chemicon	AB-764
Collagen type I	1:4	Own laboratory	
Collagen type IV	1:30	DAKO	M0785
Fibronectin	1:10	DAKO	A0245
Tenascin	1:25	DAKO	M0636
Laminin	1:30	DAKO	Z0097
ICAM 1	1:10	DAKO	M7063
VCAM-1	1:10	BMA	1299.27.1
P-Selectin (CD62P)	1:10	DAKO	M7199
Swine anti-rabbit Ig	1:100	DAKO	Z196
Rabbit APAAP Ig	1:30	Sigma	A9811
Rabbit anti-mouse Ig	1:30	DAKO	Z259
Mouse monoclonal APAAP IgG1	1:60	DAKO	D0651
Swine anti-rabbit FITC-conjugated	1:64	DAKO	F205
Rabbit anti-mouse FITC-conjugated	1:64	DAKO	F261
Goat anti-mouse Alexa 568	1:200	Molecular Probes	A11019

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achieved by preincubation with 10% normal human serum (blood group AB, heat inactivated at 56 °C for 30 min) in blocking reagent (LotNo.1096176, Roche, Mannheim, Germany) for 15 min. Thereafter, the blocking reagent was blotted off and the primary antibody was applied directly for 60–90 min without any further washing procedure. Incubation took place in a moist chamber at room temperature. The sections were then rinsed 3 times in Tris buffered saline (TBS, pH 7.4), the secondary antibody was applied and the sections were incubated for another 30 min.

The sections were rinsed as described above and incubated with the alkaline phosphatase/anti alkaline phosphatase (APAAP) complex (DAKO, Glostrup, Denmark) at room temperature for 30 min. Visualisation of the APAAP complex was achieved by adding the substrate Fast Red Naphthol (Sigma–Aldrich, St. Louis, USA), and counterstaining with Mayer's hemalaun. Finally, the slides were mounted in Kayser's glycerol gelatine (Merck/VWR, Darmstadt, Germany).

2.2.2. Immunofluorescence

Indirect immunofluorescence was used to detect collagen type I and III and fibronectin, since immunohistochemical methods did not afford optimal results with these antibodies. The sections were air dried at room temperature for 30–60 min and incubated with optimally diluted antibodies in a moist chamber for 1 h. Slides were washed in phosphate buffered saline (PBS; pH 7.2), and minimal background was obtained by application on a slow shaker for 10 min and changing the washing solution twice. Swine anti-rabbit/mouse Ig FITC-conjugated (DAKO, Glostrup, Denmark) antibodies were used as secondary antibodies.

After a final rinsing step the slides were mounted in elvanol (DuPont).

2.3. Negative controls

For all experiments, negative controls were carried out by omitting the first antibody or using normal sera of the appropriate species (for polyclonal primary antibodies) and isotype matched monoclonal nonsense immunoglobulins, respectively.

2.4. Photographic documentation

The stained specimens were examined by light microscopy (Optiphon-2, Nikon) equipped with an automatic camera (UFX-DX, Nikon) and using Fujichrome Velvia 50 films for documentation (immunohistochemistry) or by confocal microscopy (Zeiss Axiovert 100M, Scanhead LSM510) and digital recording (immunofluorescence).

3. Results

3.1. Cellular components of the fibrous capsules

Fibroblasts and macrophages (Fig. 1A), which also formed the palisade-like multilayered cell wall towards

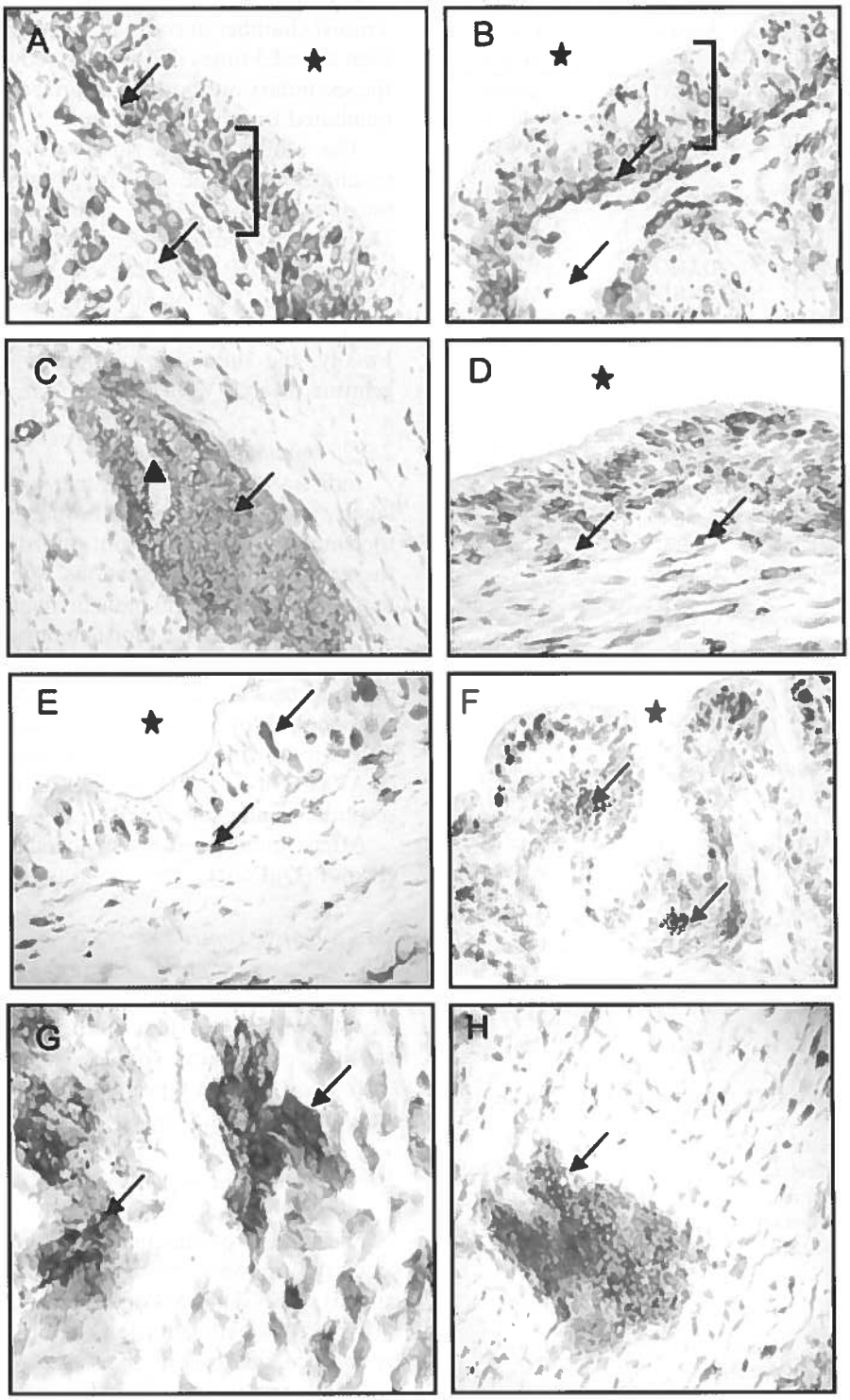

Fig. 1. A: Macrophages (CD68+ cells) in a capsule with an implant duration of 9 years. ★ Silicone implant (→) macrophages;] cells of the frontier layer. Original magnification: 400×. B: CD4+ cells in a capsule with short implant duration (1 year). ★ Silicone implant. Original magnification: 400×. C: Aggregates of CD4+ cells around a vessel. ▲ Lumen. Original magnification: 400×. D: Detection of $TCR_{\gamma/\delta}$+ cells (→) in a capsule with an implant duration of 1 year. ★ Silicone implant. Original magnification: 400×. E: Detection of dendritic cells (CD1a+ cells) in the fibrous capsule, resembling morphologically CD1a+ cells of the skin (→). Original magnification: 400×. F: Cumulative distribution of CD1a+ cells near the frontier layer. Original magnification: 400×. G, H: Accumulations of dendritic cells, expressing CD208 in a capsule with long implant duration. Original magnification: 600/400×.

the silicone implant, represented the major cell population expressed in the fibrous capsules.

In addition activated CD4+ cells (Fig. 1B) were detected beneath this frontier layer. Many of the CD4+ cells expressed the activation markers CD25 and CD45RO. Dense aggregates of activated CD4+ cells could be also demonstrated around vessels (Fig. 1C), while B-lymphocytes were rarely found. Both T-cell receptor (TCR) $\alpha/\beta+$ and $\gamma/\delta+$ cells were distributed in the specimens with an interesting preponderance of TCR $\gamma/\delta+$ cells (Fig. 1D).

The salient point of the present investigation concerned the possible presence of dendritic cells (DCs) within the peri-implant fibrous capsule. Using a panel of antibodies, recognizing markers of dendritic cells at different stages of differentiation, we were able to show abundant numbers of intra-capsular DCs scattered within the fibrous capsules or forming massive aggregates and showing characteristic Langerhans-like morphology with long branched extensions (Fig. 1F). CD1a+ DC were preferentially observed in the frontier zone abutting the silicone implant. CD207+ (Langerin+, a marker for Langerhans cell-type DCs) [23], CD208+ (DC-LAMP+, characteristic for mature DC) [24] and CD209+ (DC-SIGN+, a marker for dermal/interstitial DCs) [25] were all found to be present (Fig. 1G, H). CD208+ and CD209+ DCs prevailed over CD207+ (the latter not shown).

Finally, actin+ smooth muscle cells were not only found in vascular walls, but also in the interstitium, where they occasionally formed dense bands (Fig. 3C).

No correlation between the clinical data (e.g. duration of the implants, state of fibrosis, numbers of implant changes) and the degree of immunological reaction emerged from this limited number of specimens.

3.2. Distribution of extracellular matrix proteins

3.2.1. Collagens

Procollagen type I and procollagen type III, the precursor molecules of collagen type I and III, respectively, were more predominant in capsules with a shorter duration of implantation (Fig. 2A). Procollagen expression was taken as a marker for the mesenchymal turnover in the fibrous capsules [20]. High procollagen expression correlated with high fibrotic activity. The proportion of procollagens to collagens, showed a decrease in procollagen expression and an increase of mature collagen deposition with longer implant duration. Additionally, the specimens were analyzed for collagen type IV and laminin to determine whether the frontier layer cells, which morphologically resemble epitheloid cells, produce a basement membrane. However, collagen type IV and laminin expression could only be shown around basements of vessels (Fig. 2D). Collagens as well as fibronectin were detected by indirect immunofluorescence since immunohistochemical staining was insufficiently sensitive with the antibodies used.

3.2.2. Non-collagenous extracellular matrix proteins

All specimens showed intense positive staining for fibronectin (Fig. 2B), a prominent adhesive protein that mediates various aspects of cellular interactions with and between extracellular matrix proteins [26]. Importantly, fibronectin was primarily present in the frontier zone where the implant comes into direct contact with normal tissue. This extracellular matrix protein shows high affinity for silicone [20] on one hand and for cellular components such as macrophages, fibroblasts and T-cells on the other [27]. In addition to fibronectin, the capsules were also examined for the occurrence and distribution of tenascin (Fig. 2C), another non-collagenous ECM protein that is mainly synthesized by fibroblasts and mediates adhesion of mononuclear cells [26]. The frontier zone again showed an intense positive staining for this ECM protein, which is responsible for cell–matrix adhesion and cell migration.

3.3. Heat shock protein (HSP60) expression

Our hypothesis, that the mechanical stress to which breast implant patient tissues are exposed, is associated with heat shock protein 60 (HSP60) expression, could be verified (Fig. 3A–F). Of special interest is the fact that the cells of the frontier layer showed massive HSP60 expression (Fig. 3A) as did the endothelial cells and smooth muscle cells of the capsular vessels (Fig. 3D). Double staining experiments to clarify, which cell types express HSP60, showed, HSP60 positivity predominantly in fibroblasts (Fig. 3F), followed by macrophages (Fig. 3E) and T-cells (Fig. 3B).

3.4. Adhesion molecules

The fibrous capsules were examined for adhesion molecules with special attention to the areas in contact with the silicone implant. Significantly, the cell layers in closest proximity to the foreign material silicone showed massive expression of the intercellular adhesion molecule (ICAM-1) (Fig. 2E), while staining for other adhesion molecules, such as VCAM-1 or E-selectin, were negative. Only the endothelial cells of the neovasculative vessels in the fibrous capsules were P-selectin positive (Fig. 2F).

4. Discussion

The present study confirmed and extended our previous work [20] and detailed the composition and activity of immune cells, HSP60 expression, and the appearance of extracellular matrix proteins and adhesion molecules involved in capsule formation. One of the

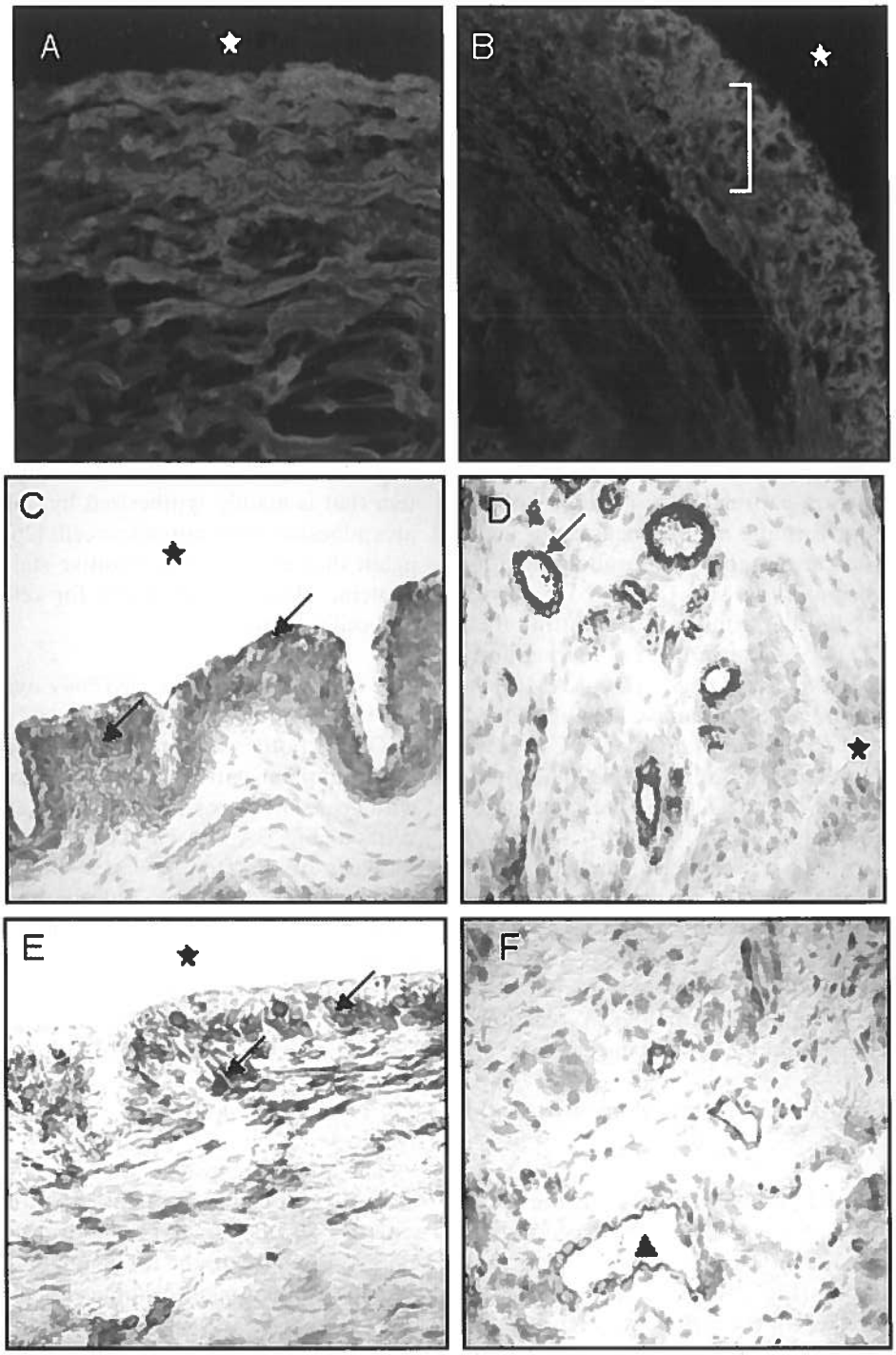

Fig. 2. A: Collagen type I expression in a capsule with an implant duration of 10 years. ★ Silicone implant. This figure shows the coarse texture of collagen type I fibrils (→). Original magnification: 400×. Excitation: Laser 488 nm. Emission: 505–530 nm (FITC). B: Fibronectin in a capsule with long implant duration (9 years). ★ Silicone implant. Cells of the frontier layer surrounded by fibronectin. Original magnification: 400×. Excitation:] Laser 488 nm. Emission: 505–530 nm (FITC). C: Massive tenascin expression by the cells of the frontier layer in a capsule with short implant duration. Original magnification: 400×. D: Internal vascular supply of the fibrous capsules stained with a-Laminin (→). Original magnification: 400×. E: Massive ICAM-1 expression by the cells of the frontier layer in a capsule with an implant duration of 10 years. Original magnification: 400×. F: P-Selectin expression only on the endothelial cells of vessels. ▲ Lumen of the vessel. Original magnification: 400×.

future aims of this analysis is the formulation of criteria concerning the design of silicone implant surfaces that should be taken into account by the manufacturers of silicone implants.

Histologically, the fibrous capsules (Fig. 4) from the above-noted patients showed a three-layered composition:

(a) The internal layer abutting the silicone surface, appeared to be single/or multilayered and was

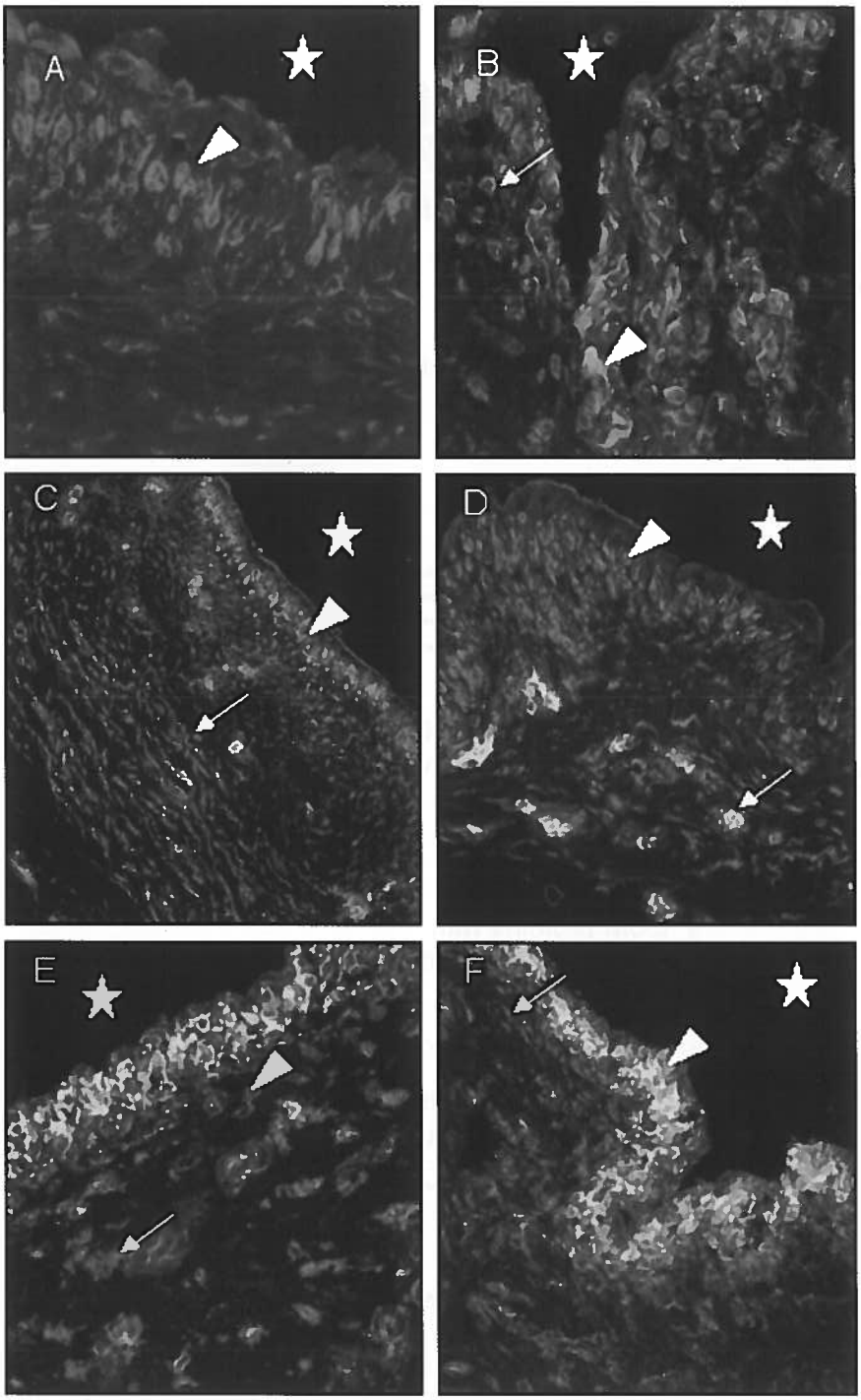

Fig. 3. A: Intensive HSP60 expression by the cells of the frontier layer (►). ★ Silicone implant. Original magnification: 400×. Excitation: Laser 488 nm. Emission: 505–530 nm (FITC). B: Double staining for HSP60 (►) and for CD3+ lymphocytes (→) in a capsule with short implant duration. Original magnification: 400×. Excitation: Laser 488 nm (FITC)/543 nm (Alexa). Emission: 505–530 nm (FITC)/LP 560 nm (Alexa). C: Double staining for HSP60 (►) and for smooth muscle actin (→). Cells expressing bot yellow. The implant duration was 14 months. Original magnification: 250×. Excitation: Laser 488 nm (FITC)/543 nm (Alexa) Emission: 505–530 nm (FITC)/LP 560 nm (Alexa). D: Double staining for HSP60 (►) and for vWF (→) expressing endothelial cells. Yellow stained cells are HSP60+ as well as vWF+. The implant duration was 1 year. Original magnification: 400×. Excitation: Laser 488 nm (FITC)/543 nm (Alexa) Emission: 505–530 nm (FITC)/LP 560 nm (Alexa). E: Double staining for CD68+ macrophages (→) and for HSP60 (►) in a capsule with an implant duration of 1 year. Yellow stained cells show CD68 and HSP60 expression. Original magnification: 400×. Excitation: Laser 488 nm (FITC)/543 nm (Alexa) Emission: 505–530 nm (FITC)/LP 560 nm (Alexa). F: Double staining for fibroblasts (→) and for HSP60 (►) in a capsule with an implant duration of 10 years. Cells expressing both antigenic determinants are stained yellow. Excitation: Laser 488 nm (FITC)/543 nm (Alexa). Emission: 505–530 nm (FITC)/LP 560 nm (Alexa).

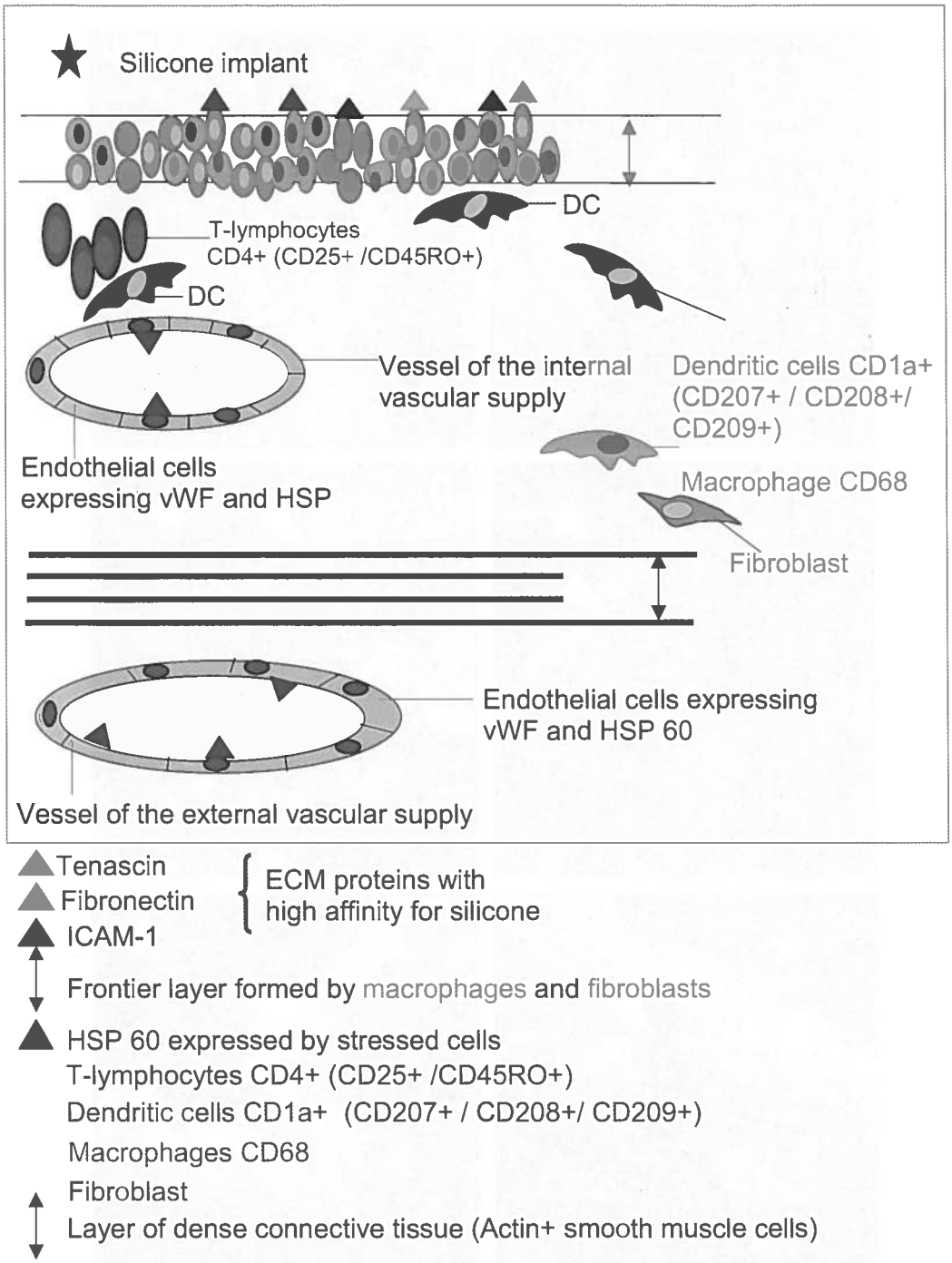

Fig. 4. The fibrous capsules show a three-layered composition: (a) The internal layer abutting the silicone surface formed by macrophages and fibroblasts, (b) the layer of loosely arranged connective tissue including the internal vascular supply and (c) the outer layer of dense connective tissue with the external vascular supply. Pronounced infiltration of activated CD4+ cells can be documented directly beneath the frontier layer as well as around vessels. Also, constant presence of CD1a+ cells in the frontier zone adjacent to the silicone implant as well as next to the accumulations of activated CD4+ cells can be shown. The ECM proteins tenascin, fibronectin, as well as the adhesion molecule ICAM-1, and HSP60 are massively expressed by the cells of the frontier layer.

previously considered to be exclusively formed by macrophages, now show to also contain abundant fibroblasts, (b) the layer of loosely arranged connective tissue including the internal vascular supply and (c) the outer layer of dense connective tissue with the external vascular supply.

Pronounced infiltration of activated CD4+ cells was documented directly beneath the frontier layer as well as around vessels. No accumulation of B-cells was found in the fibrous capsules.

These results underscore the immune response in the fibrous capsules comprised primarily of T-cells, and that

these lymphocytes may be activated by their interaction with cells of the innate immune system. The fact, that CD25 expressing T-lymphocytes formed aggregates similar to lymph follicles next to vessels (Fig. 1C) makes the possibility of a migration of these activated lymphocytes to the main blood stream very likely and might explain the occurrence of systemic side effects correlated with silicone breast implants.

Moreover, the preferential distribution of dendritic cells (CD1a/CD208+ cells) in the frontier layer zone underlines the fact that the immunological processes occurring within the fibrous capsular tissue are not identical or comparable with an unspecific local immune reaction or so called foreign body granuloma formation.

However, the constant presence of CD1a+ cells in the frontier zone adjacent to the silicone implant as well as next to the accumulations of activated CD4+ cells supports the hypothesis, that silicone is not inert, as postulated by the manufacturers [28]; but—either directly or indirectly—induces a specific T-cell dependent immune response. The peri-implant connective tissue capsule may represent a possible site of antigen processing and presentation [29]. The number of mature dendritic cells, expressing CD208, in the fibrous capsules opens new perspectives not only with respect to local immune reactions in the peri-implant tissue, but also to the occurrence of the controversial systemic side effects.

Additionally, our data implicate that the sensitisation of the lymphocytes with the foreign material silicone, that has been speculated to occur in the axillar lymph nodes, might proceed locally, i.e. directly in the capsular tissue.

Further functional studies of activated lymphocytes from fibrous capsules will identify which antigens are recognized by the activated lymphocytes and whether they are directed against native silicone, decomposition products of silicone, complexes of silicone and autologous proteins or cryptic or "altered self" epitopes. Our morphologic data, demonstrating a massive local immune response in peri-implant connective tissue, provides the basis for further functional analyses of intracapsular as well as systemic lymphocyte function in such patients.

Concerning the distribution of differential extracellular matrix proteins, our interest was focused on fibronectin and its extremely high affinity for the surface of silicone implants, which has been shown previously by our laboratory [20] and on collagenous ECM proteins involved in wound healing and general in regenerative processes.

In addition to the massive expression of fibronectin, in areas of direct fibrous capsule/silicone contact, intensive tenascin expression was detected. Fibronectin is known to interact with binding regions of cells and extracellular matrix proteins, leading to an immobilisation of immune cells [30] and to support activation of T-cells [31]. The central cell-binding domain of fibronectin is recognized by most adherent cells via the integrin receptors $\alpha 3\beta 1$, $\alpha 5\beta 1$, $\alpha V\beta 1$, $\alpha IIb\beta 3$, $\alpha V\beta 3$, $\alpha V\beta 5$ and $\alpha V\beta 6$, while the IIICS/HepII cell-binding domain is recognized by lymphoid cells [26]. These cooperative capabilities of fibronectin with cellular components seem to play a key role not only in the early stage of capsule formation, but also in the immunological processes occurring in the fibrous capsules.

The binding moieties of tenascin also seem to play a major role in capsule formation, since only the frontier layer cells showed intense positive staining for this molecule, while the other parts of the capsules were negative. Tenascin interacts with its different domains via surface receptors with cells as well as with other ECM proteins, e.g. fibronectin [32]. Tenascin receptors include members of the integrin family, cell adhesion molecules of the immunoglobulin superfamily, a transmembrane chondroitin sulfate proteoglycan/receptor (phosphacan/RPTP ζ/β), and annexin [26].

Our present results are supported by previous studies demonstrating the ability of activated T-lymphocytes to significantly increase the synthesis rate of tenascin [33] via certain cytokines (e.g. Il-4 and TNF-α). A good example in this respect is atherosclerosis, where an association between mechanical stress, to which human breast implants are also exposed, and increased expression of tenascin in arteries, was shown; the mechanical stress is transformed into a biochemical signal that induces a higher tenascin expression [34]. The massive deposition of tenascin in the frontier layer zone (Fig. 2C) supports the theory of mechanical stress dependent tenascin expression.

Our hypothesis that the mechanical stress to which breast implants are exposed to is associated with heat shock protein 60 (HSP60) expression, was verified (Fig. 3A–F). HSPs, a family of highly conserved proteins, are produced by all cells in response to various physiological and non-physiological stress-situations to protect the cells from potential lethal assaults.

Additionally, the predominance of TCR γ/δ+ lymphocytes in the fibrous capsules, which recognize only a limited number of antigens in a MHC-non restricted fashion, including HSP60 [35], underline the connection between TCR γ/δ and HSP60, which has been shown, for example, in patients with rheumatoid arthritis [36]. Future studies will clarify whether this HSP60 expression is only a hallmark of mechanical irritation, or if the occurrence of this protein is an inducer for systemic side effects, e.g. symptoms resembling those of systemic lupus erythematosus and other types of autoimmunity in general, as described in previous studies [37–40].

Another point emerging from this study concerns the distribution of adhesion molecules in the peri-implant fibrous capsules. When examined for P-selectin, VCAM and ICAM-1, only ICAM-1 could be detected in the

specimens and was mainly expressed by the macrophages and fibroblasts forming the frontier layer. The expression of this molecule in the contact zone with silicone might lead to adherence to the ECM proteins and immobilisation of lymphocytes and macrophages at this site [40]. ICAM-1 is also an important adhesion molecule in atherogenesis, being responsible for the infiltration of the arterial intima by lymphocytes and macrophages upon binding to the LFA ligand. The assumption that similar processes take part in the fibrous capsules thus seems plausible.

In our study, we were able to confirm, that silicone not only induces the formation of foreign body granulomas, but evokes a strong cellular T-cell weighted immune response that could induce systemic effects. According to the activity profile of these lymphocytes we hypothesize, that these activated lymphocytes possibly react against silicone itself, against composite neoepitopes, cryptic epitopes or altered selfproteins. Future work to clarify these assumptions, will involve isolating the T-lymphocytes from peri-implant capsules for functional studies with silicone gel and decomposition products of silicone.

In addition, we are interested in developing new preventive and therapeutic procedures to reduce or prevent the local immune reaction in the peri-implant capsular tissue and to devise new approaches for the development of implants with more biocompatible surfaces.

Acknowledgements

We would like to thank Christina Mayerl and Pia Müller for the help with immunohistochemistry and the Department of Plastic and Reconstructive Surgery Bogenhausen, Munich, as well as Prof. N. Romani from the Department of Dermatology, University of Innsbruck, for their cooperation and advice. We also would like to thank Barbara Gschirr and Reinhard Sigl for their administrative help.

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