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Immunohistochemical characterization of the perivascular infiltrate cells in tissues adjacent to stainless steel implants compared with titanium implants

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Abstract Metallic orthopaedics implants are composed of elements that are known to be skin sensitizers in the general population. In this study, we analyzed the cells of perivascular infiltration in the tissue adjacent to titanium (n = 23) and steel (n = 8) implants after explantation of the metals by immunohistochemical methods. The following panel of monoclonal antibodies were used as parameters: CD 1a (Langerhans cells), CD 4 (T-helper cells), CD 8 (T-suppressor cells), CD 11c (monocytes and macrophages), CD 45 RO (memory cells), CD 45 RA (naive cells), eosinophil cationic proteins (ECP), neutrophil elastase, and HLA-DR. The number of perivascular total cells did not differ significantly. All cells were identified in both metal subgroups, but a statistical difference was not seen in the above-mentioned parameters. We conclude that sensitization to metals is possible in the tissue adjacent to steel and titanium implants, because all cells which play an important role in allergic delayed-type hypersensitivity (type IV) reactions are present. This phenomenon may be called a 'pre-sensitization' phase, because no sensitization or allergic reactions were seen in our cases. Second, in the present study, a statistical difference was not seen in the number of infiltrate cells in the tissue adjacent to steel compared with titanium implants.

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Introduction

The role of metal sensitization due to the use of metallic implants, such as stainless steel and titanium, is coming under increased scrutiny. The reason is that steel usually contains nickel in a concentration of 8.5%–14%, but this can be as high as 35%. Nickel is known to induce the most allergic reactions in the form of allergic contact dermatitis in the general population. Furthermore, steel also contains chromium (e.g., 20%) and manganese, molybdenium, and other metals in very low concentrations. Titanium, however, contains neither chromium nor cobalt or nickel. Up to now, metal sensitivity caused by an implant with the consequence of loosening of a prosthesis or an allergic dermatological reaction is still controversial [1, 3, 4, 5, 6, 7, 8, 10].

The aims of this study were (1) to characterize the cells of perivascular infiltration in the tissue adjacent to steel and titanium implants and (2) to compare the number of total cells as well as different cell subgroups by statistical analysis.

Patients and methods

The present study included 31 patients (titanium implant n = 23, men n = 14, women n = 9; steel implant n = 8, men n = 5, women n = 3) of the Department of Trauma Surgery, Regensburg (Table 2) after random assignment. No clinical signs of allergic reactions were seen after implantation and explantation of the implants. Surgical specimens adjacent to the implants were taken during the explantation of the implant, which was done routinely 6–12 months after implantation. All specimens were immediately snap-frozen on liquid nitrogen-cooled isopentane and stored at –70°C until used.

All epicutaneous patch tests were negative before implantation and after explantation of the implants in both subgroups.

Table 1 Summary of the antibodies used in the study

Specificity	CD code	Antibody	Source	Titer
Langerhans cells, dendritic cells	CD 1a	BL-6	Dianova	1:50
T-helper cells	CD 4	13B8.2	Dianova	1:25
T-suppressor cells	CD 8	B9.11	Dianova	1:25
Monocytes, macrophages	CD 11c	KB90	DAKO	1:50
Memory cells, activated macrophages	CD 45 RO	UCHLl	DAKO	1:25
Naive T-cells	CD 45 RA	ALB11	Dianova	1:100
Neutrophil elastase	–	NP57	DAKO	1:300
Activated eosinophils	–	EG2	Pharmacia	1:200
HLA-DR	–	B8.12.2	Dianova	1:50

Immunohistochemistry

For immunohistochemistry analysis, a panel of different monoclonal antibodies (mAb), specific for well-defined differentiation antigens, were selected (Table 1) by the alkaline phosphatase antialkaline phosphatase (APAAP) technique as described previously [2]. The sections were counterstained with hematoxylin and eosin.

Negative controls included omission of primary antibody. Positive reaction was found as a specific red staining.

Quantitation

Slides were encoded and counted blindly. Five visual fields with the greatest dermal infiltrate (× 40 on a Zeiss microscope) were quantified. The results were expressed as the number of cells per high-power view (HPV × 400).

Statistical analysis

Statistically significant differences of the densities of the cellular infiltrates among the different groups were analyzed by the Mann-Whitney U-test; *P* values of less than 0.05 were regarded as significant.

Results

In the reported cases, neither local nor systemic side-effects were seen by these patients from the date of implantation to the date of explantation. The specimens consisted of granular tissue and fibrotic material. Neither bone nor marrow material was seen.

The results of the statistical analysis are shown in Table 2. The mean values of total perivascular cells are 145.2 (± 43.96) cells/HPV (c/H) in the tissue adjacent to titanium vs 151.3 (± 52.96) c/H adjacent to steel.

The specific red staining for Langerhans cell (CD 1a), which is responsible for antigen presentation in the allergic reaction, is shown Fig. 1a. The mean values of cells for CD 1a are 6.02 (± 4.16) c/H (titanium) vs 5.73 (± 3.22) c/H (steel). There are more T-helper cells (CD 4) in patients with titanium implants [12.73 (± 8.78) c/H] compared with steel implants [10.62 (± 5.48) c/H]. The mean levels for the suppressor T-cells (CD 8) are: 7.87 (± 4.98) c/H for titanium and 6.56 (± 2.79) c/H for steel. The monocytes/macrophages (CD 11c) are found as follows: 8.16 (± 4.13) c/H in the titanium subgroup vs 7.6 (± 6.22) c/H in the steel subgroup. The values of eosinophil cationic protein (ECP) are: 3.71 (± 2.88) c/H in titanium vs 4.28 (± 3.82) c/H in the steel implant subgroup. The neutrophil elastase is higher in the titanium (4.6 ± 2.66 c/H) than in the steel (3.1 ± 1.55 c/H) subgroup.

Both important components for allergic reactions, memory cells (CD 45 RO) and naive cells (CD 45 RA) (see also Fig. 1 b and c) show the following results: 17.7 ± 11.66 c/H and 8.98 ± 5.36 c/H in titanium implants versus 16.4 ± 12.59 c/H and 8.88 ± 6.59 c/H in steel implants. For the marker of cell activation (HLA-DR), we observe the mean values 27.48 ± 20.39 c/H for the titanium implant and 24.03 ± 10.6 c/H for the steel implant.

A statistical difference, however, was not found between the metal subgroups either in total cells or in the different cell subgroups using the Mann-Whitney U-test ($P < 0.05$).

Discussion

Loosening of an implant is one of the most important complications in orthopaedic as well as trauma surgery and is caused by many different possible reasons such as an infection, a mechanical failure of prosthetic components, and/or an allergic reaction to implants or to deposits. The role of allergy in the process of loosening of an implant is not entirely clear. Cutaneous reactions to orthopaedic implants are rarely seen but often reported. Furthermore, it is still being debated whether steel implants can be safely recommended for nickel-sensitive patients [1, 3, 4, 6, 7, 8].

In a prospective study of 85 patients, Waterman and Schrik investigated the relationship between the implantation of metal-to-polyethylene hip prostheses and the incidence of delayed-type allergy to components of the prostheses. They found a sensitization in 16/85 (18.8%) cases. Loosening did not occur in any of the cases of possible sensitization. Furthermore, evidence of an allergy to prosthetic components was not found in any of the 10 cases of loosening [10].

Hierholzer et al. examined 208 patients after osteosynthesis for developing allergic reactions. They found that the rates of allergy and a corresponding clinical reaction were higher, the longer the steel implant remained in situ. They concluded that the steel implant should be removed

Table 2 Summary of the patients and immunohistochemical results (*ECP* eosinophil cationic proteins, *a Neutr* neutrophil elastase)

Antibody	Gender	Age (years)	Fracture	Total cells	CD 1a	CD 4	CD 8	CD 11c	ECP	a Neutr	CD 45 RO	CD 45 RA	HLA-DR
T1	M	40	Weber B sin.	135	9	14.25	9	11.75	9	10	4.75	2.25	21.5
T2	M	53	Weber C sin.	160	12	8.5	13.5	8.25	7.25	11	28.75	19	86
T3	M	45	Radius sin.	90	7	7	13.5	12.5	6.75	8.25	9.25	6.5	15.3
T4	M	36	Weber B sin.	138	12	23	3	8	0	4.3	26	14	28.25
T5	M	64	Weber B sin.	178	16	28.25	14.6	11.75	6	6.75	47	19.6	31.75
T6	M	28	Weber B dext.	195	10	29.5	6.25	0.5	0.25	3.25	14	15.3	23
T7	F	60	Weber C dext.	238	4.25	26.25	18.5	4.25	0.75	3.75	35	7.5	64.75
T8	M	27	Humerus sin.	185	10.25	17	6.5	12	4.25	7.25	19.5	9	30
T9	M	50	Calcaneus sin.	182	11.25	28.25	18.25	13.25	4.5	5.5	35.75	11.25	55
T10	M	20	Weber C sin.	187	3.5	13.5	6.75	10.75	3.25	4.25	31.25	8	49.75
T11	F	25	Weber C sin.	189	3	13.5	2.5	14.75	4.5	2	17	4.75	42.25
T12	M	30	Weber C sin.	170	5.75	15.25	3	7.5	1.25	2.75	13.5	10.25	20.5
T13	M	35	Radius sin.	174	2.75	11.25	13.75	17.25	10.6	9.25	19.25	16.75	33.6
T14	F	64	Weber C sin.	101	3.25	3.25	7	5.75	4	3.75	6.25	3.25	12.25
T15	F	30	Weber C dext.	68	1.5	7.5	2.5	6.5	3	3.5	3.5	2.25	7
T16	F	50	Weber B sin.	134	1.5	3.75	8	7	0.75	3.25	15.75	10.75	6.5
T17	F	40	Weber B sin.	127	5.75	4.25	3.75	4.75	3.5	2.75	12.5	6.75	23.75
T18	M	28	Humerus dext.	135	0.5	3.25	7.25	4	3	1.25	10.25	1.5	9
T19	F	65	Weber B sin.	141	4.75	7.5	5	5.5	6.5	7.5	7.75	5.75	18
T20	M	33	Pilon sin.	138	3.25	6	4.75	5	0.5	2.5	24.5	10	7.75
T21	M	25	Weber B sin.	63	3	6.25	5.5	2.5	1.5	2	6.25	3	12
T22	F	35	Clavicula dext.	93	2.5	4	4.25	8	1.5	2	5.75	4.5	22
T23	F	37	Weber B dext.	118	5.75	11.25	4	6.75	2.75	3	11.75	6	12.25
S1	F	45	Radius dext.	219	4	14.75	7	2	9.75	2	14.75	12.75	12.75
S2	M	28	Weber C sin.	223	13	12	12.25	21.3	11.75	6.5	40.25	23.3	33.75
S3	M	61	Weber C sin.	151	5	20.75	6.25	8	1.5	2.5	14	4.75	30
S4	F	43	Weber B sin.	192	6.5	11.5	4.75	11.25	3.5	2	30	6.75	43.25
S5	M	30	Weber B dext.	113	5	6.25	4.5	4.5	5.25	4.25	7.25	8.75	19
S6	F	26	Weber B dext.	109	2.6	4.75	3.75	5.75	2	3	6.5	4.25	14.25
S7	M	31	Weber B sin.	104	3.5	4.75	5.25	4.25	2.25	2.75	3.5	3.5	19
S8	M	42	Weber B dext.	99	6.25	10.25	8.75	3.75	3.25	2	15	7	20.25
Median T	14M/9F	40	–	145.2	6.02	12.73	7.87	8.16	3.71	4.6	17.7	8.98	27.48
Standard T	–	–	–	43.96	4.16	8.78	4.98	4.13	2.88	2.66	11.66	5.36	20.39
Median S	5M/3F	38.25	–	151.3	5.73	10.62	6.56	7.6	4.28	3.1	16.4	8.88	24.03
Standard S	–	–	–	52.96	3.22	5.48	2.79	6.22	3.82	1.55	12.59	6.59	10.6

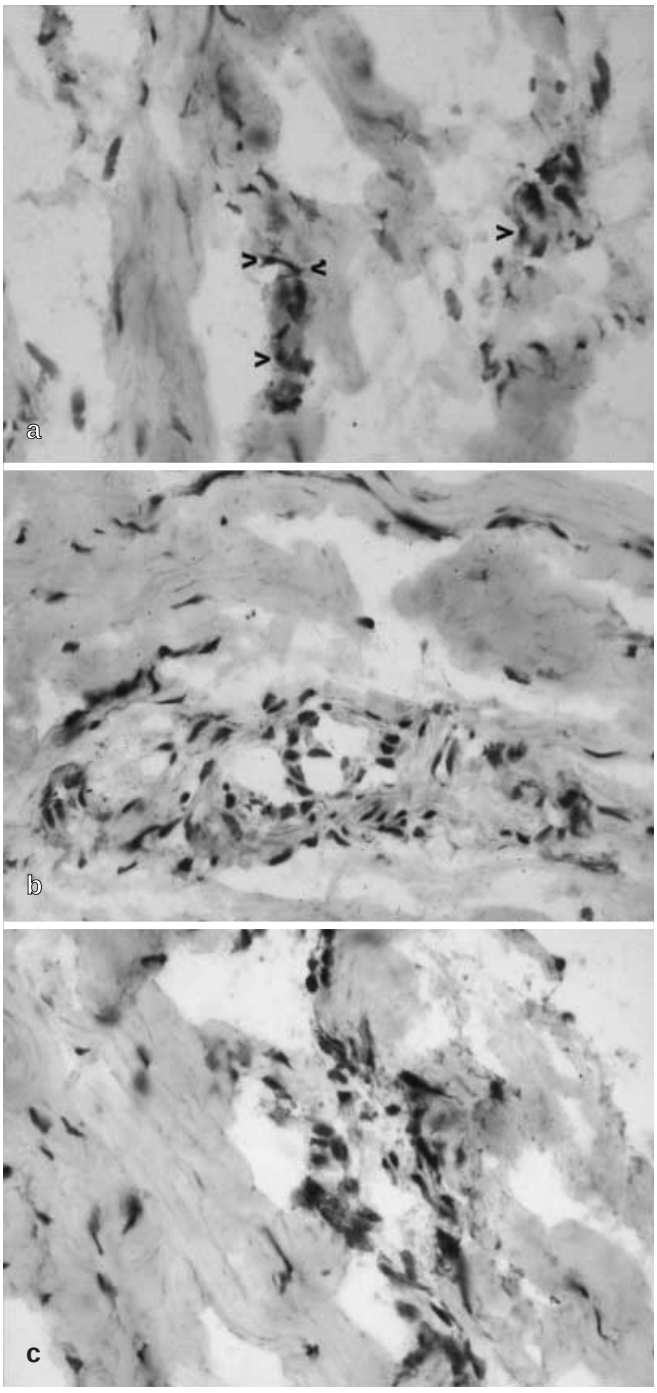

Fig. 1 Positive immunostaining for Langerhans cells (CD1a) (**a**), memory cells (CD45 RO) (**b**), and naive cells (CD45 RA) (**c**)

in some cases of allergy to nickel or chromium. Furthermore, they postulated an unknown genetic disposition in the process of sensitization [5].

In this study, we demonstrated for the first time that all cells, especially Langerhans cells, T-helper cells, memory cells, and naive cells, which are necessary for a sensitization or an allergic delayed-type hypersensitivity (type IV) reaction to an allergen as seen in cutaneous type IV reactions [9], are present in the perivascular tissue of the implant before a sensitization phase or a possible allergic reaction takes place. This phase may be called a 'pre-sensitization' phase. The quantitative and qualitative profile of the perivascular cell infiltration in the tissue adjacent to the implants did not differ between steel and titanium implants. In our patients, no sign of clinical or allergic reaction to the implants or dermatological allergic reactions were seen from the date of implantation to the date of explantation. However, an infection or another cause, leading to an increased corrosion of the implant, may upregulate the risk for a sensitization and allergic reaction to stainless steel, which contains several metal components, more so than in titanium. This hypothesis is supported by different authors, who found higher rates of sensitization after implantation of steel implants in epicutaneous patch tests against metal components, i.e., nickel and chromium [5, 8].

In summary, we conclude that it is better to remove steel implants in cases of clinical complications after osteosynthesis and after the clinical process of bone-healing to prevent an allergic reaction; titanium implants, however, may remain in situ.

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