Case Report

Cobalt-specific T Lymphocytes in Synovial Tissue After an Allergic Reaction to a Cobalt Alloy Joint Prosthesis

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ABSTRACT. Metals such as cobalt and nickel are common contact allergens. We studied the mechanisms underlying an allergic reaction with marked synovial inflammation in a patient with a cobalt alloy arthroplasty. After removing the joint prosthesis the adjacent synovial tissue was examined for cobalt-specific T lymphocytes. Synovial membrane mononuclear cells were expanded in interleukin 2 and cloned using a representative cloning protocol. T cell clones were tested for their proliferative response to cobalt and further characterized with regard to cytokine secretion, phenotype, and HLA restriction. Additionally, synovial fibroblasts were tested for their function as antigen presenting cells (APC). Almost 30% of the T cell clones reacted to cobalt, but not to the control nickel. All these T cell clones were CD4 positive. The cobalt induced proliferative response could be blocked by anti-class II antibodies. Also, synovial fibroblasts expressing class II molecules induced by interferon-γ were able to serve as APC. However, when testing a panel of APC of HLA class II mismatched donors, no requirement for a certain HLA class II molecule could be defined. Further studies are necessary to determine mechanisms of presentation and recognition of cobalt by T lymphocytes, a prerequisite for improved prevention and treatment of metal induced allergic reactions. (J Rheumatol 2001;28:1121–8)

Key Indexing Terms:
COBALT SYNOVIAL T LYMPHOCYTES ALLERGIC REACTION

Cobalt, chromium, and titanium are often used as materials for metallic joint arthroplasties[1]. As haptens, these and other metals are able to induce allergic reactions of the delayed type (type IV) in humans[2-4]. In particular, cobalt and nickel are known to cause allergic contact dermatitis that is thought to be mediated by T lymphocytes[2,4]. Generally it is assumed that contact sensitizers are bound to Langerhans cells in the skin, which migrate to the draining lymph nodes, where they prime naive T lymphocytes[5]. That T lymphocytes specific for metals have a role in the pathogenesis of contact hypersensitivity[2] is supported by *in vitro* experiments done mostly on nickel, to which 10% of the Caucasian population is sensitized[6]. It has been shown that peripheral blood mononuclear cells (PBMC) from nickel-allergic patients have higher proliferative responses to nickel than PBMC from nonallergic blood donors[3]. Nickel-specific T cell clones have been reported to be CD4 positive in peripheral blood as well as in skin lesions of patients with nickel-contact dermatitis[2-4,7,8]. Almost all nickel-specific T cell clones described so far were HLA class II restricted, with restriction elements being either HLA-DR, DQ, or DP[3,4,8].

Nickel-specific T cell clones derived from peripheral blood showed a cytokine secretion of the Th1 type[3,8]. However, from examination of T cell clones derived from skin lesions, a Th2 type cytokine pattern seems to be predominant[9,10].

The pathomechanism by which metals function as antigenic determinants has not been elucidated[4]. It is assumed that hapten-specific T lymphocytes recognize complexes of MHC molecules and peptide, which both can be modified by the metallic hapten[2].

Not much is known about the immune response induced by cobalt. We examined the mechanisms underlying an allergic reaction of the synovium to cobalt, and characterized the T lymphocytes thought to mediate this process. Our model was particularly intriguing since the dominant inflammatory reaction did not take place in the skin, but in a joint, a part of the body with different conditions of antigen presentation.

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CASE REPORT

The 47-year-old female patient had longstanding polyarthritic psoriatic arthritis, treated for many years with methotrexate. Her HLA-type was HLA-A1, 32; B8, 27; Cw2, 7; DR4, 15; DQ1, 3; DP 0301/0301. In March 1996 she underwent right wrist joint replacement with a prosthesis made of a cobalt-chromium-vanadium steel alloy (APH prosthesis, Implant Service, Hamburg, Germany; developed by H. Thabe). In April 1997 she developed a recurrent synovitis of the right wrist and the tendon sheaths of the extensor muscles. Dermatological skin testing revealed a hypersensitivity reaction against cobalt. Tenderness, erythema, and intensive swelling of the right wrist suspicious of infection required removal of the APH prosthesis, dorsal tenosynovectomy, and subsequent implantation of a Swanson arthroplasty in May 1997. The adjacent inflamed synovial tissue was transferred to culture medium (see below) and prepared for immunological investigation. At that time, bacterial or fungal infection was excluded by gram staining and by microbiological culture of the synovial fluid and tissue. One month after this second surgery she developed streptococcal infection of the skin and the scar around the implant, which was sufficiently controlled by appropriate antibiotic coverage. She has been without further complications since then.

MATERIALS AND METHODS

Separation of synovial membrane and peripheral blood mononuclear cells. Synovial tissue was obtained from both operations (DB SM II-1, March 1996; DB SM III, May 1997). The synovial membrane tissue was cut into pieces and digested with 1 mg/ml collagenase A (Sigma) and 0.15 mg/ml DNAse (Sigma, St. Louis, MO, USA) for 1.5 h at 37°C in a water bath under repeated shaking as described[11]. The digested tissue suspension was put through a mesh and synovial membrane mononuclear cells (SMNC) were separated by standard centrifugation on a Ficoll-Hypaque gradient.

PBMC from the patient as well as from different healthy donors (MHC matched or mismatched) were used as antigen presenting cells (APC). PBMC were isolated from blood by standard Ficoll-Hypaque gradient centrifugation.

Establishing T cell clones. SMNC (2×10^6) were cultured in RPMI 1640 culture medium (Gibco, Karlsruhe, Germany) containing 2 mM L-glutamine (Sigma, St. Louis, MO, USA), 10^4 U/ml penicillin, 100 μg/ml streptomycin, 5 mmol HEPES (all Gibco), and 10% normal, heat inactivated human AB serum. Recombinant interleukin 2 (IL-2) (Boehringer-Mannheim, Mannheim, Germany) was added at 20 units/ml to select for *in vivo* activated T lymphocytes. After 14 days the expanded T cell lines (DB SM II-1 in March 1996, DB SM III in May 1997) were cloned by limiting dilution as described[12]. Briefly, 0.3 T cells/well were cultured in Terasaki plates (Nunc) in the presence of 10^4 irradiated (4500 rad) allogeneic PBMC in 20 μl complete medium supplemented with 1 μg/ml phytohemagglutinin (PHA) and 20 units/ml IL-2. Expanding T cell clones were transferred after 14 days into larger wells and restimulated with fresh complete medium, PHA, irradiated feeder cells, and IL-2.

Culture of adherent synoviocytes. Adherent fibroblast-like synoviocytes were derived from the synovial membrane and cultured in M199 (Seromed, Berlin, Germany) supplemented with 10–20% fetal calf serum (FCS), 2 mM L-glutamine (Sigma), 10^4 U/ml penicillin, 100 μg/ml streptomycin (all Gibco), and ciprofloxacin 10 μg/ml (Bayer Leverkusen, Germany). Confluent cells were detached from the flasks with trypsin 0.05%/EDTA 0.02% (Seromed) and passaged at a 1:3 ratio. The cells used during this study were obtained between passages 3 and 7.

Before being used as APC a part of these synoviocytes were cultured for 4 days in the presence of 500 U/ml interferon-γ (IFN-γ; Boehringer-Mannheim) and expression of MHC class II molecules was determined by flow cytometry.

Proliferation assays. Proliferation assays of T cell clones were performed in triplicate cultures (200 μl complete medium) of 1×10^4 T cells in the presence of 3×10^4 irradiated, autologous, or heterologous PBMC and cobalt (optimum concentration 12.5 μmol $CoCl_2$; ICN Biomedicals, Aurora, OH, USA). Nickel (optimum concentration 50 μM $NiSO_4$) was used as a control. The optimal cobalt concentration was determined by a dose-response curve for the T cell line obtained from the synovial membrane DB SM III described above (Figure 1). As for nickel, no proliferative response of this T cell line was seen with varying concentrations. Therefore, the optimal concentration for nickel was determined using PBMC from a nickel sensitized blood donor (data not shown). For some experiments, monoclonal anti-class II antibody (supernatant of hybridoma DA6.231, a gift of Prof. B. Fleischer, University of Hamburg) was added at the optimal concentration.

Alternatively, synovial fibroblasts (5×10^3), untreated or pretreated with IFN-γ as described above, were used as APC. ^{3}H-TdR (0.2 μCi; Amersham UK) was added for the last 18 h and the assay harvested after 3 days onto fiberglass filters. ^{3}H-TdR incorporation was measured by standard liquid scintillation counting. To determine the cutoff for positive responses the stimulation indices (SI) of 22 T cell clones tested in 4 different assays in one experiment were grouped logarithmically as follows: SI 0.2–0.499 (0 clones), SI 0.5–0.999 (16 clones), SI 1.0–1.999 (31 clones), SI 2.0–3.999 (12 clones), SI 4.0–7.999 (2 clones), SI 8.0–15.999 (1 clone), SI 16.0–31.999 (4 clones), SI 32.0–63.999 (11 clones), SI 64.0–127.999 (9 clones), and SI ≥ 128.0 (2 clones). This grouping clearly separated 2 populations of T cell clones: one with a SI < 8 and one with a SI > 16. Thus a SI of 10 was chosen as the cutoff between negative and positive reactions.

Cytokine profiles of T cell clones. T cell clones were stimulated in complete medium at 1×10^6/ml neat or supplemented with 0.5 μg/ml anti-CD3 (Immunotech) and 10 ng/ml phorbol-12-myristate-13-acetate (PMA; Sigma). After 24 h the cells were spun down and the supernatants were stored at –20°C until use. IFN-γ, IL-4, and IL-10 levels were measured in the supernatants by ELISA as described[13]. All assays were performed in duplicate. A standard curve was prepared for each assay. The lowest detectable levels of cytokine production were 31.25 pg/ml for IFN-γ and 62.5 pg/ml for IL-4 and IL-10.

Cell surface phenotype analysis. The expression of CD4, CD8, and T cell receptor γδ was determined by an immunocytoassay as described[14]. Briefly, T cell clones were attached to Terasaki plates by means of poly-L-lysin (Sigma) and incubated with murine monoclonal antibodies against human CD4, CD8, and γδ (Immunotech). After intense washing with phosphate buffered saline (PBS) a horseradish peroxidase conjugated rabbit anti-mouse antibody was added (Dako P161). The color reaction was induced by addition of the substrate carbazol (Sigma) according to the manufacturer's guidelines. As this is a qualitative method useful in testing a large number of clones in one experiment, the results were determined by microscopic examination.

The expression of surface molecules on synoviocytes was examined by flow cytometry analysis. Briefly, 1×10^5 cells were incubated 45 min on ice with optimal concentrations of the following mouse anti-human antibodies: HB55 (anti-HLA-DR), SPVL3 (anti-HLA-DQ; Immunotech), HI43 (anti-HLA-DP; Pharmingen, San Diego, CA, USA), and anti-ICAM (Dianova). Cells were then washed in PBS containing 2% FCS and 0.2% sodium azide. Phycoerythrin conjugated goat anti-mouse IgG (Southern Biotechnology, Birmingham, AL, USA) was added at a concentration of 10 μg/ml and left for 20 min on ice. Cells were washed and fixed in 3% formaldehyde. The analysis was done with a FACScan (Becton Dickinson, Heidelberg, Germany).

RESULTS

Specificity of synovial membrane T cell lines and clones. Inflamed synovium was removed together with the cobalt alloy arthroplasty. SMNC derived from this synovial membrane were cultured in IL-2 supplemented medium to establish a T cell line (DB SM III) of *in vivo* activated T lymphocytes. After 14 days of culture this T cell line proliferated in response to cobalt, but not to nickel (Figure 1).

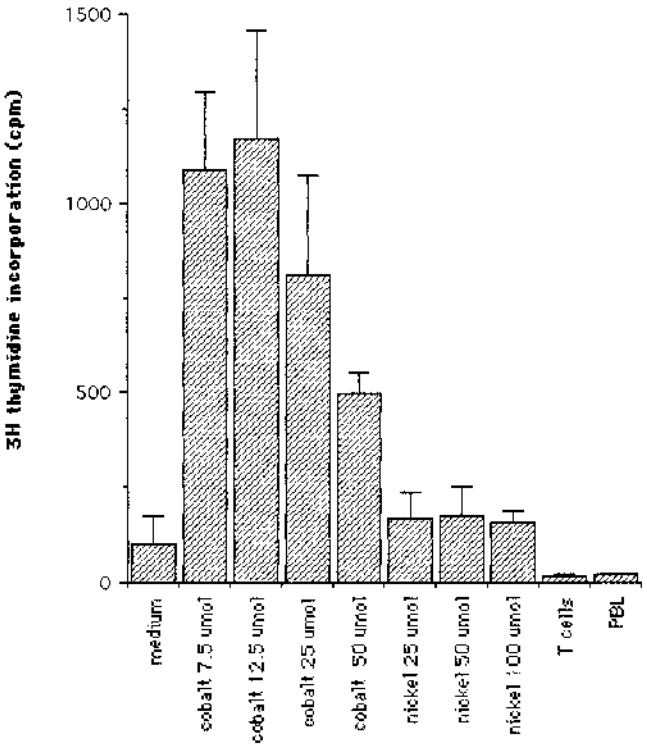

Figure 1. Dose response curve to cobalt for the T cell line DB SM III; 10^4 T cells were incubated 3 days in the presence of 3×10^4 irradiated autologous APC and cobalt or nickel at the indicated concentrations. The results show the mean ^{3}H-thymidine incorporation ± standard deviation of cultures in triplicate.

Table 1. Proliferative responses of cobalt-specific T cell clones.

No. of Clone	^{3}H-Thymidine Incorporation in Response to Medium cpm (SD)	$CoCl_2$ cpm (SD)	SI	$NiCl_2$ cpm (SD)
2	211 (120)	3888 (91)	18	257 (169)
3	26 (10)	1592 (447)	61	652 (49)*
4	61 (19)	7807 (481)	128	194 (107)
5	34 (11)	1224 (151)	36	213 (55)
10	23 (7)	886 (164)	39	50 (7)
13	105 (30)	2815 (182)	27	307 (156)
14	244 (109)	3600 (136)	15	230 (100)
16	194 (80)	2314 (379)	12	281 (118)
20	50 (5)	1302 (390)	50	69 (54)
29	35 (5)	1569 (656)	45	64 (49)
37	214 (62)	14886 (50)	70	440 (148)
40	54 (17)	2183 (359)	40	68 (15)
41	326 (156)	5927 (340)	18	871 (357)
44	70 (22)	5359 (83)	77	244 (119)
50	40 (5)	1999 (390)	50	111 (54)
55	110 (99)	5551 (592)	50	163 (125)
58	68 (27)	2052 (239)	30	196 (116)
59	73 (9)	4476 (349)	61	194 (40)

*In contrast to the positive proliferative response to cobalt a response to nickel could not be repeated. Therefore we consider T cell clone 3 nonreactive to nickel. SI: stimulation index.

The T cell line was cloned using a nonspecific, representative cloning procedure; 60 T cell clones were derived and further expanded. All T cell clones were tested for their proliferation in response to cobalt and nickel, with PBMC as APC. Eighteen T cell clones (30%) showed a positive response to $CoCl_2$, but none showed a positive response to nickel. The experiments were repeated at least 3 times. The response of a representative clone is shown in Figure 2 and Table 1 shows the data of all 18 T cell clones found to be specific for cobalt.

As control, 25 T cell clones derived from the synovial membrane of the same patient one year earlier were tested for reactivity to cobalt. None of these T cell clones, which were also established from a T cell line (DB SM II-1) of *in vivo* activated T cells, responded to stimulation with cobalt, implying that before the implantation of the joint prosthesis no synovial T lymphocytes had been sensitized to cobalt (Table 2). Cobalt-specific T cell clones were used as positive control.

Cytokine secretion pattern and phenotype of cobalt-specific T cell clones. For further characterization of the cobalt-specific T cell clones we determined the pattern of cytokines secreted; 1×10^6 T cells were stimulated for 24 h nonspecif-

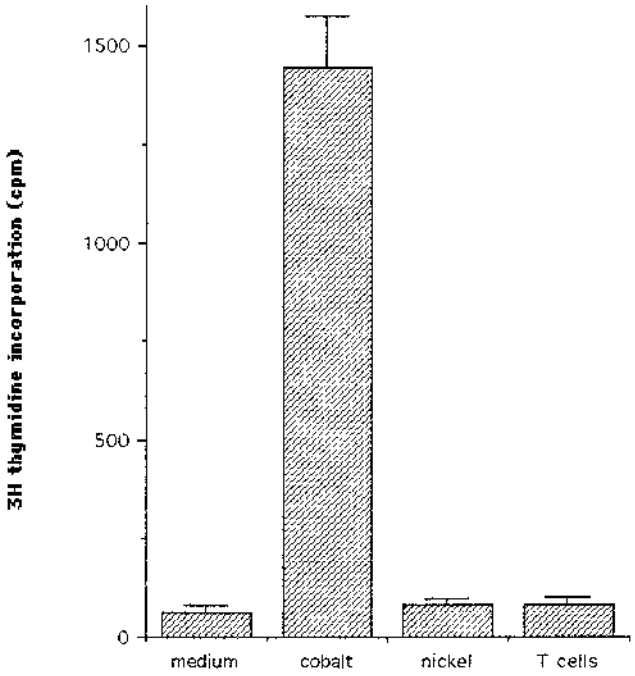

Figure 2. Proliferative response of T cell clone DB SM III-37 as a representative clone in the presence of cobalt (12.5 μM) and nickel (50 μM); 10^4 T cells were incubated in the presence of 3×10^4 irradiated autologous APC and the indicated antigens. ^{3}H-TdR (0.2 μCi) was added for the last 18 h and the assay harvested after 3 days. ^{3}H-TdR incorporation was measured by standard liquid scintillation counting. These results show the mean ^{3}H-thymidine incorporation ± standard deviation of cultures in triplicate.

Table 2. Proliferative response of 25 T cell clones derived from T cell line DB SM II-1, established before implantation of the cobalt alloy arthroplasty. None of the clones tested showed a cobalt reactive proliferative response.

No. of Clone	^{3}H-Thymidine Incorporation in Response to Medium, cpm (SD)	$CoCl_2$ cpm (SD)	PHA, cpm (SD)
2	53 (19)	97 (54)	1967 (253)
3	72 (18)	73 (61)	3098 (318)
4	29 (3)	57 (11)	2917 (712)
6	35 (4)	33 (6)	4893 (604)
8	34 (5)	22 (5)	3788 (1577)
9	56 (12)	42 (9)	2845 (196)
11	15 (5)	22 (9)	6308 (289)
12	74 (15)	55 (20)	13221 (2009)
13	52 (7)	32 (11)	3318 (505)
14	38 (12)	45 (10)	12205 (1210)
15	45 (9)	52 (8)	2968 (276)
16	46 (27)	54 (63)	5935 (839)
17	27 (8)	36 (5)	4021 (557)
18	29 (7)	34 (2)	8030 (525)
19	1077 (354)	429 (86)	2754 (59)
20	33 (6)	26 (10)	3016 (139)
21	939 (241)	439 (247)	5621 (392)
23	95 (27)	113 (29)	5419 (71)
24	16 (2)	24 (6)	2133 (370)
25	17 (6)	16 (4)	2427 (134)
35	57 (15)	26 (1)	7195 (268)
47	28 (9)	26 (4)	5275 (462)
49	133 (126)	72 (6)	9080 (825)
59	94 (8)	65 (8)	6645 (762)
63	45 (33)	37 (7)	4453 (300)

PHA: phytohemagglutinin.

ically with anti-CD3 and PMA as described above. The concentrations of IFN-γ, IL-4, and IL-10 were measured in the culture supernatants. Of 16 cobalt-specific T cell clones tested, 14 produced both IFN-γ and IL-10, while 4 secreted only IFN-γ. No T cell clone produced IL-4; however, the assay worked well, as seen on IL-4-producing control T cell clones. Thus all cobalt-specific T cell clones tested showed a Th1 type cytokine pattern (Table 3 shows a representative experiment with 9 T cell clones tested). In comparison, we determined the cytokine production of 37 out of 70 T cell clones established one year earlier from the synovial membrane of the same joint in the same patient. Of these 37 T cell clones, 8 showed a Th1 type cytokine pattern, producing IFN-γ ± IL-10; 12 clones showed a Th2 type pattern with secretion of IL-4 ± IL-10; 11 showed a Th0 pattern with production of IFN-γ, IL-10, and IL-4. Six clones could not be classified, producing IL-10 only. Of these clones, 25 were tested for a proliferative response to cobalt and had been nonreactive (see above). Table 4 gives the cytokine pattern of 20 representatives out of these 25 T cell clones.

The phenotype of all cobalt reactive T cell clones was determined by an immunocyto-ELISA staining for CD4, CD8, and T cell receptor γδ as described in Materials and Methods. All these clones were CD4 positive and γδ negative (data not shown).

Table 3. Cytokine secretion (mean value of duplicates) of 9 representative cobalt-specific T cell clones after stimulation with anti-CD3 and PMA as described in Materials and Methods.

No. of Clone	IFN-γ, pg/ml	IL-10, pg/ml	IL-4, pg/ml
2	1574	4964	0
3	2211	228	0
13	3873	4221	0
16	3002	3083	0
20	3171	4700	0
37	2067	13717	0
44	150	13322	0
50	4282	330	0
59	3289	7595	0

Table 4. Cytokine secretion (mean values of duplicates) of T cell clones derived from T cell line DB SM II-1, established before implantation of the cobalt alloy arthroplasty.

No. of Clone	IFN-γ, pg/ml	IL-10, pg/ml	IL-4, pg/ml
4	1662	12556	676
8	0	0	476
9	1966	1959	0
11	0	0	3272
12	1564	10063	446
13	1393	4116	131
15	1320	1072	2042
16	3435	3005	0
17	1014	1115	1018
18	1774	9198	566
19	892	6066	988
20	370	374	2751
21	121	15607	4982
23	479	1633	1963
24	2505	10170	0
25	286	11220	4891
35	2324	3593	0
47	0	1005	0
49	0	789	0
63	0	9877	1610

Synoviocytes as antigen presenting cells for cobalt-specific T cells. Since in inflamed synovium fibroblast-like synoviocytes can function as APC[15], we investigated whether these cells could also serve to present cobalt. Synoviocytes were used untreated or cultured for 4 days in medium supplemented with 500 U/ml IFN-γ to induce the expression of HLA class II and costimulatory molecules. The change in expression of these molecules on IFN-γ pretreated fibroblasts was determined by flow cytometry. As described[15-17],

Table 5. Proliferation of cobalt-specific T cell clones in the presence of untreated fibroblasts (–) or fibroblasts preincubated with IFN-γ (+). ^{3}H-thymidine incorporation, cpm (± SD).

	T Cell Clone			
	DB SM III.16		DB SM III.37	
	–	+	–	+
Medium	96 (± 11)	68 (± 42)	100 (± 18)	232 (± 50)
PHA	9251 (± 404)	7854 (± 315)	1033 (± 93)	838 (± 184)
Cobalt	70 (± 15)	1647 (± 169)	269 (± 154)	2461 (± 34)
Fibroblasts only	213 (± 108)	154 (± 60)	191 (± 43)	185 (± 32)

PHA: phytohemagglutinin.

HLA class II molecules DR, DQ, and DP as well as ICAM-1 were upregulated by treatment with IFN-γ (data not shown). As confirmed for 2 representative clones, cobalt-specific proliferation could be induced with fibroblasts pretreated with IFN-γ, but no reaction was seen when fibroblasts were not incubated with IFN-γ (Table 5). Thus, according to the CD4 phenotype of the T cell clones the presence of MHC class II molecules seemed to be necessary to induce a proliferative response to cobalt.

HLA dependency of cobalt-specific T cell clones. To further determine whether the proliferative response was HLA class II restricted, anti-class II antibody (DA6.231) was added in some experiments. As shown in Figure 3 this antibody could indeed induce inhibition of the proliferative response to cobalt to background levels. The PHA induced proliferation was inhibited only to a minor degree.

We then investigated the possible requirement for HLA class II compatibility by using PBMC from heterologous, HLA typed donors as APC. Cobalt-specific T lymphocyte clones were stimulated with cobalt presented by these APC. As seen in Table 6 proliferation was induced without any recognizable pattern of the expression of HLA class II molecules: a proliferative response was seen with some, but not all donors sharing an HLA class II antigen with the patient. On the other hand, when no HLA class II antigen was shared, cobalt induced activation could still take place.

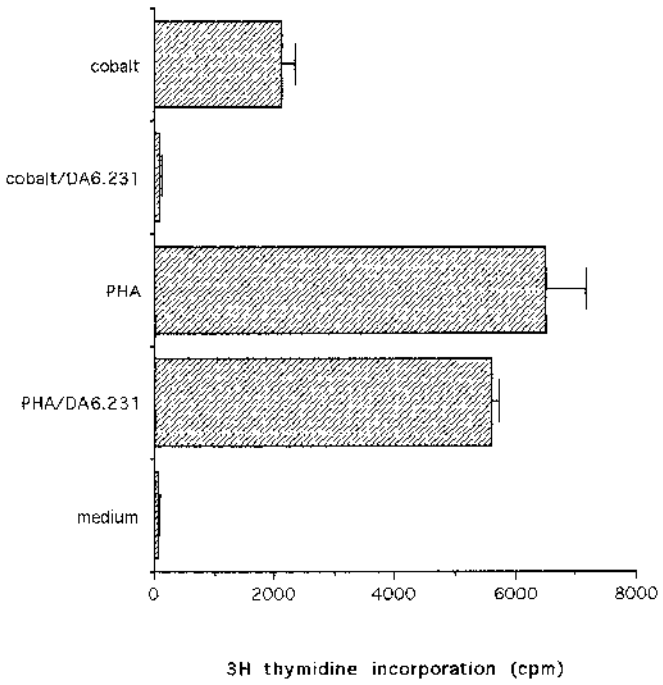

Figure 3. Cobalt induced proliferative response can be blocked by anti-class II antibody as shown for the representative clone DB SM III-5. 10^4 T cells were incubated in the presence of 3×10^4 irradiated APC and the indicated antigens with or without anti-class II antibody (DA6.231). ^{3}H-TdR (0.2 μCi) was added for the last 18 h and the assay was harvested after 3 days. ^{3}H-TdR incorporation was measured by standard liquid scintillation counting. The results show the mean ^{3}H-thymidine incorporation ± standard deviation of cultures in triplicate.

Table 6. The capacity of heterologous APC to present cobalt to cobalt-specific synovial T cell clones.

Donor	Function as APC	HLA Class II DR	DQ	DP
1 (patient)	+	4.15	1.3	0301/0301
2	+	4.17	2.3	0401/0402
3	+	11.15	6.7	0401/0402
4	+	11.15	6.7	0101/1901
5	+	13.17	1.2	0401/0401
6	–	11.13	6.7	0401/0401
7	–	15.17	ND	ND
8	–	4.8	8	0401/0401

ND: not done.

Thus, no consistent HLA class II restriction could be defined.

DISCUSSION

Metal ions such as cobalt and nickel are common allergens causing contact dermatitis, a delayed type hypersensitivity reaction. This immune response is mediated by T lymphocytes specific for the relevant metal[2]. Cobalt, together with titanium and chromium, is one of the materials most often used for joint arthroplasties[1]. Thus, allergic reactions are likely to take place within the joint capsule and its

surrounding tissue. We describe such an immune response, which developed about one year after implantation of a wrist arthroplasty necessary because of total destruction of the joint by longstanding psoriatic arthritis. While there are several studies on the characterization of nickel-specific T lymphocytes and their molecular interaction with the metal hapten nickel[3,4,8-10,18], little is known about their cobalt-specific counterparts. We examined the cobalt induced allergic response in our patient to characterize the T lymphocytes in the inflamed synovium and the process of their activation.

Both T cell lines and T cell clones established from the inflamed joint proliferated in response to cobalt, but not to the control nickel (Figures 1 and 2, Table 1). In contrast, of the T cell clones established one year earlier from the same joint none of the 25 clones could be activated by cobalt (Table 2). All T cell clones were CD4 positive and produced a Th1-like cytokine pattern. The proliferative response could be induced with synovial fibroblasts as APC, but was dependent on the presence of HLA class II molecules (Table 5). Accordingly, the proliferation could be blocked by anti-class II antibody (Figure 3). However, no consistent HLA class II restriction could be defined using HLA matched or mismatched PBMC as APC (Table 6).

T lymphocytes specific for the metal hapten nickel have been described[3,4,8-10,18]. In some of these studies nickel-specific T cell clones were examined that had been derived from PBMC after *in vitro* activation with nickel[3,4,8]. In other studies T cell clones were established from skin specimens from patients with nickel induced contact dermatitis without *in vitro* stimulation with the hapten[7,9,10]. Probst, *et al*[9] found that about 20% of the established T cell clones were specific for nickel, this being in the range of the 30% cobalt-specific T cell clones found among those from the synovial tissue in our patient. When nickel-specific T cell clones were examined for their proliferation induced by different metals no crossreaction between nickel and cobalt could be detected[4,8,10], as we could see no crossreaction of our cobalt-specific T cell clones to nickel. This finding supports the concept of cosensitization in patients with sensitivities to several metals[4]. That no T cell clone established one year earlier from the same joint reacted to cobalt points to sensitization to cobalt after implantation of the joint arthroplasty. Indeed, cobalt-chromium alloy is not biologically inert, and *in vivo* may reach biologically relevant concentrations[1].

Delayed type hypersensitivity (DTH) is a T cell dependent inflammatory reaction mediated by T lymphocytes sensitized by a prior antigen challenge[6]. The majority of T lymphocytes responsible for DTH in mice or humans expresses the CD4 molecule[3,10,19,20]. Thus, nickel-specific T lymphocytes described in patients with nickel induced contact dermatitis were found to be without exception CD4 positive[2,4,8,9] or, at a lesser frequency, also CD8 positive[3,10]. In accord with this, all the cobalt-specific T cell clones we investigated expressed the CD4 molecule. However, the culture conditions of the T lymphocytes might influence the predominance of either subset[18].

In general, DTH is thought to be an immune response driven mainly by Th1-type lymphocytes. Thus, only IFN-γ producing T lymphocytes could transfer DTH in mice[19]. Nickel-specific CD4 positive T lymphocytes from the peripheral blood of patients with nickel induced contact dermatitis were high IFN-γ producers[2,3,8], as were beryllium-specific T cell clones from the peripheral blood of patients with chronic beryllium disease[21]. However, Werfel, *et al*[10] report the presence of IL-4 producing T lymphocytes in peripheral blood as well as in the skin of patients with nickel induced contact dermatitis, with concentrations of IL-4 much higher in lymphocytes derived from the skin. Probst, *et al* could not find a single T cell clone with a clearcut Th1 profile in their skin derived T lymphocyte clones in nickel induced contact dermatitis, but found mainly Th0 and some Th2 subtypes[9]. In the latter study T lymphocytes were stimulated with nickel for the production of cytokines, while in the other studies the stimulation was either by nickel or by nonantigenic stimulation[3,8,10]. Thus, the difference in the cytokine patterns described is rather due to the different origin of the lymphocytes (i.e., skin versus peripheral blood) than to the various culture conditions. The cobalt-specific T cell clones we examined, despite being isolated from the site of the allergic reaction, consistently showed a Th1 pattern when cytokine production was induced by nonantigenic stimulation. However, T cell clones derived from the same joint one year earlier, i.e., before any contact to cobalt, showed a mixed cytokine secretion pattern, indicating that antigen specificity might also influence the cytokine production.

With respect to synoviocytes as APC, as described below, it would be interesting to see IFN-γ production induced by T cell clones after stimulation by class II positive synoviocytes presenting cobalt, and this should be investigated in further studies.

If cobalt-specific T lymphocytes had a role in the allergic reaction to the cobalt alloy prosthesis in the synovium, there should be APC capable of presenting cobalt to the synovium-infiltrating T cells. For immune reactions in synovial tissue, possible APC are the synoviocytes, which are able to present antigen to T lymphocytes, as shown by several investigators[15-17]. While no MHC class II-expressing cells are found in healthy synovial tissue, synovial fibroblasts in inflamed joints in rheumatoid arthritis show abundant expression of these molecules[15]. When examining synovial fibroblasts for their antigen-presenting capacity to the cobalt-specific T cell clones this was restricted to synoviocytes pretreated with IFN-γ, which led to expression of MHC class II and other, costimulatory molecules. These results indicated an HLA class II dependency of the proliferative response by the tested T cell clones to cobalt. The

results were supported by blocking experiments with the anti-class II antibody DA6.231, which reduced the proliferation to cobalt to background levels.

Nevertheless, we are aware that these results are not sufficient to prove this issue, although they are in keeping with reports by other authors, who also found a requirement for MHC class II molecules[3,8,10]. However, in these studies a restriction could be seen to single HLA molecules: nickel-specific T cell clones were either restricted to HLA-DR[10] or, when examined further, to HLA-DR5[8] or HLA-DR1[9]. The nickel-specific T cell clones described by Kapsenberg, *et al* showed differential restriction to either HLA-DR, DP, or DQ[3], as described by others[22]. In our experiments we could not clarify the MHC class II dependency any further. Cobalt was recognized by the cobalt-specific T cell clones when presented by heterologous APC without a consistent HLA class II pattern with regard to all 3 subclasses; however, a proliferative response was not obtained with all the heterologous APC tested. Because of these results it is unlikely that a T cell to T cell presentation could play a role. Therefore it is a result we cannot explain.

Little is known about the antigenic epitopes generated by metal salts[4]. Metal salts have a low molecular weight as a common feature[8]. It is assumed that hapten-specific T lymphocytes recognize complexes of MHC molecules and peptide, both shown to be modified by the metallic hapten[2]. Nickel, by interacting with the amino acid histidine, binds to the MHC associated peptide, rendering it not recognizable by the peptide-specific T cell[2]. However, the same is not true for all peptides. Gold, on the other side, has been shown to bind directly to the MHC class II molecule. This modification is thought to elicit gold-specific T cells responsible for the delayed type hypersensitivity reaction seen in patients treated with gold[2,23]. Apparently one single T cell can even recognize a hapten with different peptides[2]. For cobalt, it is not known yet which part of the trimolecular complex of T cell receptor, MHC molecule, and peptide is modified by the binding of this hapten.

At least for gold antigen processing is not required[23]. As for nickel, 40% of nickel-specific T cell clones were depending on the processing of the antigen, whereas 60% also showed a response to fixed APC[4].

The adverse effects of metallic corrosion products on immune response and release of immunoregulatory cytokines has not been clarified[1]. Soluble metals in particular are thought to be able to elicit clinical consequences, which, however, are thought to be reversible. Certain immune dysfunctions have been reported, as shown by an increased activation of T and B lymphocytes, plasma cell activation, and an impaired antibody response[1]. For cobalt, no direct effect on cell viability could be observed. However, inhibition of PHA induced T cell proliferation, a reduced lipopolysaccharide stimulation of B cells, and reduced IL-2, IL-6 and IFN-γ release upon PHA stimulation has been observed[1]. The effect on proliferation could possibly be mediated by reduction of the secretion of cytokines, the mechanism of which has not been elucidated[1].

There is only scarce literature about possible effects of cobalt on the immune system. Cobalt-specific T cell clones have not been reported. In this study we show that the allergic reaction to a cobalt alloy arthroplasty was most likely induced by CD4 positive, Th1 type T lymphocytes. The reaction was HLA class II dependent and could be induced by INF-γ treated, HLA class II expressing synovial fibroblasts. These results are similar to what has been described for nickel-specific T cell clones. Nevertheless, we do not know whether HLA molecules or antigenic peptides are modified by cobalt or the possible way of processing and presentation of cobalt with the effect of T cell activation. Understanding these pathways is necessary for prevention and treatment of metal induced allergic reactions.

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