

Lymphocyte responses in patients with total hip arthroplasty

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Abstract

How lymphocyte-mediated metal sensitivity affects orthopaedic implant performance remains poorly understood. Do patients with implants exhibit elevated lymphocyte reactivity to metals and is this reactivity more generalized or more implant-alloy specific? We investigated these questions by measuring lymphocyte responses to implant metals (Cr^{+3} , Co^{+2} , Ni^{+2} at 0.1 mM, and Ti^{+4} at 0.001 mM) in six subject groups: Group 1a = young controls, Group 1b = age matched controls, Group 2a = subjects with osteoarthritis (OA) and no history of metal sensitivity, Group 2b = OA subjects with history of metal sensitivity, Group 3a = total hip arthroplasty (THA) subjects with no to mild radiographic osteolysis, and Group 3b = THA subjects with moderate osteolysis. Lymphocyte proliferation, using Lymphocyte Transformation Testing (LTT), and cytokine release provided quantitative reactivity measurement, where a stimulation index of >2 indicated metal sensitivity. OA subjects with a history of metal sensitivity (Group 2b) were more metal reactive to Ni than any other group, as expected (66% incidence and Stimulation Index >20). However, THA subjects (Groups 3a & b) were >3 fold more reactive to Cr ($p < 0.04$), than were controls (Groups 1a & b) or OA subjects (Groups 2a & b). THA subjects with moderate vs mild osteolysis (Group 3b vs 3a) were more reactive to Co (43% vs 0% incidence). Only osteolytic THA subjects demonstrated increased cytokine responses with $>two$ -fold ($p < 0.05$) increases in soluble interferon- γ (IFN- γ) and interleukin-2 (IL-2) levels in response to Cr challenge. This elevated incidence and averaged level of lymphocyte reactivity supports a metal-specific adaptive immune response and suggests involvement in the pathogenesis of poor implant performance, e.g. aseptic osteolysis.

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Introduction

Sensitivity to metallic implants is well documented in case and group studies [7,9,11,12,14,31,37–40,44,47]. However, it remains a somewhat unpredictable and poorly understood phenomenon [2,11,15]. Dermal hypersensitivity to metals is common, affecting about 10% of the general population [2,11,16,41]. Dermal contact and

ingestion of metals have been reported to cause immune reactions which most typically manifest as urticaria, eczema, erythema and pruritis [2,23,30]. These reactions purportedly represent humoral secondary responses of a type IV cell mediated hypersensitivity delaying with IgA, and IgE antibodies, through mechanisms such as metal/hapten complex formation with IgG antibodies. Hypersensitivity to metallic biomaterials is difficult to diagnose because of its rare and subtle nature. Since there is no universally accepted screening test for metal sensitivity, the diagnosis is typically suspected only after aseptic adverse host response. All metals in contact with biological systems corrode [6,24] and released soluble and particulate

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metal can activate the immune system by forming complexes with native proteins [41,53,55]. These metal–protein complexes are considered candidate antigens (or more loosely termed, allergens) for eliciting hypersensitivity responses. Wooley et al. [48,51,52], have previously shown that patients with implant debris related aseptic osteolysis demonstrate elevated immune responses to metal and polyethylene particle challenge, thus dramatically extending the etiology of hypersensitivity reactions to implant materials into the realm of particulate as well as soluble debris. However, generally, the short- and long-term pharmacodynamics and bioavailability of circulating metal degradation products in vivo remains poorly characterized [5,26,29,41].

Some patients with total joint arthroplasties (TJA) can tolerate a large debris burden for long periods of time (>8 years) with relatively little peri-implant tissue response, whereas other patients with similar particulate and soluble burdens demonstrate pronounced tissue response within 2–7 years, resulting in peri-implant osteolysis [17]. Immunologic responses (e.g. cell-mediated hypersensitivity) associated with metal components may be partly responsible for this differential reactivity. The most common orthopaedic soluble metal (ions) sensitizers include nickel, Ni, [16,23,30,35] cobalt, Co, [35] and chromium, Cr, [35] while occasional responses have been reported to tantalum, Ta, [1] titanium, Ti, [33,43] and vanadium, V [1]. Nickel is the most common metal sensitizer in humans followed by Co and Cr [2,16,23,30] and cross reactivity between Ni and Co is common [2]. The incidence of metal sensitivity among the general population is approximately 10% and among patients with well functioning and poorly functioning implants is approximately 25% and 60%, respectively, in reported compiled analyses of numerous investigations [19]. What remained poorly characterized were the specific metals that mediate implant related metal hypersensitivity reactions, the incidence of THA metal hypersensitivity using modern immunological testing methods and the relation of metal hypersensitivity to implant performance.

Elevated nonspecific (innate) macrophage responses to particulate challenge have been shown in patients with THAs [34]. These THA patient macrophages were more responsive to Ti and chromium phosphate particle challenge than macrophages from individuals without implants [34]. We questioned whether this elevated reactivity is apparent in other important immunoregulatory cells. Do patients with implants exhibit elevated lymphocyte reactivity to metals and is this reactivity more generalized or more implant-alloy specific? In the present study, we hypothesize that patients with THAs will exhibit an elevated nonspecific lymphocyte response to metals, where more typically immunogenic metals (e.g. Ni) will elicit a greater response in THA patients. We tested this hypothesis by comparing

the lymphocyte responses (proliferation and cytokine production) of individuals challenged with soluble metal chlorides (Cr^{+3} , Co^{+2} , Ni^{+2} at 0.1mM, and Ti^{+4} at 0.001mM) using three basic groups of subjects: 1) healthy controls without metal implants, 2) patients with osteoarthritis (OA) without metal implants and 3) patients with THAs.

Materials and methods

Subject groups

57 individuals in 3 subject groups were investigated in this Institutional Review Board approved study: Group 1-healthy controls without implants ($n = 22$, 11 male, 11 female, average age 46 yrs, range 28–80 yrs), Group 2- subjects with OA ($n = 18$, 4 male, 14 female average age 60, range 43–75), and Group 3- subjects with THA ($n = 17$, 8 male, 9 female, average age 70, range 55–80, average implant time in-situ 13.6 years, all Harris-Galante THA, Zimmer Inc., Warsaw, IN). None of the subjects with implants had a history of metal sensitivity (Group 3). Subjects within the groups were recruited on an all-comer basis. Groups 1, 2 and 3 were each sub divided into two sub-groups (Table 1): Group 1a subjects were healthy young controls without implants, average age 28, with no history of metal allergy ($n = 10$, 5 male, 5 female); Group 1b controls without implants were age matched to Groups 2 and 3 subjects ($n = 12$ subjects 6 male 6 female, average age 63); Group 2a subjects had OA and no history of metal allergy ($n = 6$ subjects 4 male 2 female, average age 60); Group 2b subjects had OA and a clinical history of metal allergy manifested as a reported dermal reaction to metal jewellery, ($n = 12$ all female, average age 60); Group 3a subjects demonstrated no to mild osteolysis, ($n = 10$ subjects, 5 male, 5 female, average age 72); and Group 3b subjects demonstrated moderate osteolysis ($n = 7$ subjects, 3 male, 4 female average age 69). The criteria used to grade the degree of osteolysis was derived from a previous clinical study where proximal focal lesions in excess of 0.5cm^2 in total area on an anteroposterior radiograph and/or distal (diaphyseal) focal lesions greater than 1cm^2 in total area on an anteroposterior radiograph were correlated with the magnitude of articular surface wear [4]. Subjects with osteolytic lesions with total areas greater than those specified above were considered as having moderate osteolysis and those with total areas that were less were considered to have no to mild osteolysis.

Lymphocyte isolation

Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll-Hypaque (Sigma, St Louis MO) gradient separation of peripheral blood mononuclear cells (PBMC) from approximately 32 mLs of peripheral blood (from $4 \times 10\text{mL}$ heparinized vacutainers/patient). Approx $15\text{--}35 \times 10^6$ PBMCs per subject were incubated with DMEM and 10% autologous serum, and challenged with 0.01mg/ml phytohemagglutinin (PHA) as a positive control, 0.1mM CrCl_3 , 0.1mM NiCl_2 , 0.1mM CoCl_2 (Sigma, St. Louis, MO), 0.001mM Ti (using titanium-saturated DMEM produced through incubation with Ti beads) or medium alone as a negative control. Metals at were premixed in deionized water at 100x concentration and added to 0.1mM. Although Co, Cr and Ni were tested at a single high concentration (0.1mM), due to the low solubility of Ti (approx 0.001mM), higher concentrations could not be studied. The final challenge concentration of 0.1mM was chosen based on previous stimulation and toxicity studies, which indicated that 0.1mM was the highest non-toxic concentration at which lymphocytes from healthy individuals were not adversely affected by soluble metal challenge and at the same time have been reported to exist in vivo around implants [20–22]. Additionally, practical limitations of the blood collection limited the number of lymphocytes and restricted challenge to a single concentration (0.1mM). Ficoll separated PBMCs were comprised of 85–95% lymphocytes with 5–13% monocytes and <0.1% dendritic cells with limited contamination (i.e. <5% erythrocytes and <3% granulocytes). Among these PBMC

Table 1
Description of subject groups

Subject group	Number of subjects	Male	Female	Average age
<i>Group 1-Controls</i>	22	11	11	46
1a Healthy young controls	10	5	5	28
2a Age matched controls to Groups 2 and 3 subjects	12	6	6	68
<i>Group 2-Osteoarthritis</i>	18	4	14	60
2a Subjects with OA and no history of metal allergy	6	4	2	60
2b Subjects with OA and a history of metal allergy	12	0	12	60
<i>Group 3^a-Total hip arthroplasty</i>	17	8	9	70
3a Subjects with no to mild osteolysis	10	5	5	69
3b Subjects with moderate osteolysis	7	3	4	72

^a All Harris-Galante THA, Zimmer Inc., Warsaw, IN average implant time in-situ 13.6 years.

fractions, lymphocytes are the only cells capable of significant in vitro proliferation upon challenge with an allergen. Thus we used standard Lymphocyte Transformation Testing (LLT) protocol of PBMCs to measure lymphocyte proliferation, and cytokine production.

Proliferation assays

Proliferation assays of PBMC fractions, also called Lymphocyte Transformation Testing (LTT), were conducted using radioactive [³H]-thymidine (Amersham International, Arlington Heights, IL) incorporation. PBMCs (2×10^5 cells/well) were cultured in 96-well cell-culture plates (Sigma) with metal treatments, for 6 days in 150 μ L of DMEM/well, 10% autologous serum at 37°C and 0.5% CO₂ with [³H]-thymidine (1 μ Ci [³H]-thymidine/well) for the last 24 h. Each metal was tested in quadruplicate (4 wells/metal concentration) at a single concentration yielding a total of 384 samples. [³H]-thymidine was added during the last 12 h of a 6-day incubation and was measured using liquid scintillation Beta plate analysis (Wollac Gatesburg, MD). The amount of [³H]-thymidine incorporation for each metal treatment was normalized to that of the non-treated control (medium alone) producing a ratio, referred to as the stimulation index, SI (also called “proliferation factor”, “proliferation index”, or “proliferation ratio”). The stimulation index was calculated using measured radiation counts per minute (cpm) as follows: Stimulation Index, SI = (mean cpm with treatment)/(mean cpm medium alone).

Measurement of cytokines in culture media

Cytokine concentrations in supernatants of lymphocyte cultures were measured by sandwich enzyme-linked immunosorbent assays (ELISA) in 96-well microtitration plates following the manufacturer’s protocol. ELISA kits (R & D Systems, Minneapolis, MN) for IL-2, IL-4, IFN- γ , and TNF- α (assay range from 0.5 to 32 pg/ml), were conducted in triplicate for each subject at each metal treatment.

Metal analysis

Serum metal content was measured using a graphite furnace Zeeman atomic absorption spectrophotometer (GFZ-AAS) (Perkin-Elmer, Norwalk, CT), where the detection limits in serum were 0.03 ng/ml for Cr and 0.3 ng/ml for Co [25–28]. All collection containers and apparatus were triple acid-washed with Ultrex-grade chemicals (Baker, Chicago, IL) or verified to be contamination free by AAS.

Statistical analysis

Due to the relatively small numbers (approx $n = 10$) within each group, reactivity measurements were assumed normally distributed based on previous studies [20,21] and analyzed using Student’s *t*-tests. Student’s *t*-tests for independent samples with unequal or equal variances were used to test equality of the mean values at a minimum 95% confidence interval ($p < 0.05$) allowed. Comparisons between

groups were limited to individual comparison of groups or subgroups reactivity at each metal concentration. All treatment specific reactivity measurements were normally distributed.

Cytokine and Metal ion statistical analysis

By convention, to calculate group means, cytokine concentrations below the detection limit were assigned a value of one-half the method detection limit. Intergroup comparisons, independent of these means, were made using Kruskal–Wallis non-parametric analysis of variance. The Wilcoxon–Mann–Whitney test was then used if the Kruskal–Wallis test revealed significant differences at $p < 0.05$.

Results

Incidence of metal reactivity

Subjects were defined as being metal-sensitive if a statistically elevated ($p < 0.05$), stimulation index, SI demonstrated >2 fold proliferative response to Cr, Ni, Co and/or Ti. The incidences of metal-sensitive subjects within Groups 1a through 3b are listed in Table 2. None of the subjects were reactive to Ti challenge. Within Group 1 controls, 22%, 0% and 11% were reactive to Cr, Ni and Co, respectively. Of the Group 2 OA subjects the majority of those that demonstrated metal-reactivity were reactive to Ni (40%). Subgroup analysis within Group 2 indicates that subjects with a history of metal sensitivity were four times as likely to be sensitive to Ni than OA subjects with no history of metal sensitivity. This is in direct contrast to Group 3 THA subjects where Cr was the predominant metal associated with sensitivity. Group 3b subjects with moderate osteolysis demonstrated greater reactivity to Cr than Ni (i.e. no Ni sensitive subjects in Group 3b). Alternatively, THA subjects with no to mild osteolysis were twice as likely to be reactive to Cr than Ni. The lack of any sensitivity response to Ti was expected because the concentrations of Ti challenge were limited by solubility to two orders of magnitude less than those of the other metals. However, the lack of response to Ti, even at these lower (best available) concentrations, partially mitigates concerns of Ti antigenicity.

Table 2

Percent of study group found metal reactive (Metal reactive criteria: SI > 2, $p < 0.05$, t -test)

	Cr	Ni	Co	Ti ^a	Any metal
1) Controls (n = 22)	22	0	11	0	27
1a Young (n = 10)	20	0	10	0	20
1b Age Matched (n = 12)	25	0	8	0	30
2) OA No Implant (n = 18)	15	40	10	0	50
2a OA (n = 6)	0	16	16	0	33
2b OA History of Metal Sens. (n = 12)	8	66	16	0	75
3) THA (n = 17)	29	6	18	0	47
3a THA, no/mild osteolysis (n = 10)	30	0	0	0	30
3b THA moderate osteolysis (n = 7)	29	14	43	0	71

^a Challenge levels of Ti were restricted to 2000 parts per billion (approx 0.001 mM) the maximum amount of Ti that could be solubilized in DMEM solution after particle elimination using high-speed centrifugation.

Level of metal reactivity

Expectedly, differences in metal reactivity were not apparent when lymphocyte reactivity was not normalized, Fig. 1. This was expected because raw incorporated radioactivity (cpm) is dependent on many individually and environmentally variable factors including total number of isolated PBMCs, incubation time, incubator conditions, fidelity of PBMC isolation, diet, medications etc. However, this raw averaged data shows the general ranges of cpm for each group and demonstrates that typical ranges of cpm incorporation are associated with all groups and metal treatments (1000–10000 cpm), where metal reactivity is not ubiquitously as stimulatory as PHA a lymphocyte mitogen (10000–100000 cpm). Group metal-reactivity differences became evident when the amounts of incorporated [H]-thymidine (cpm) were normalized for each individual, as is standard protocol in LTT [10], Fig. 2. Intergroup comparison of lympho-

cyte reactivity to PHA (a lymphocyte mitogen) revealed no statistically significant differences between the main groups. Group 3 subjects with THA demonstrated significantly elevated reactivity to Cr ($p < 0.05$) and Group 2 subjects with OA were significantly more reactive to Ni (>10 fold greater, $p < 0.02$) when compared to Group 1 controls. Although there was elevated reactivity to Co in Group 2 OA subjects (>3 Stimulation Index) this elevation was not statistically significant.

Subjects with THA and moderate osteolysis (Group 3b) demonstrated significantly elevated proliferation to PHA and Cr when compared to controls (Groups 1a and 1b) and subjects with OA (Groups 2a and 2b, Fig. 3). While there were no differences in elevated Cr reactivity between THA subjects (Groups 3a vs 3b), subjects with moderate osteolysis were more reactive to Co than those with no to mild osteolysis. There were no metal-reactivity differences between Groups 1a, 1b and 2a.

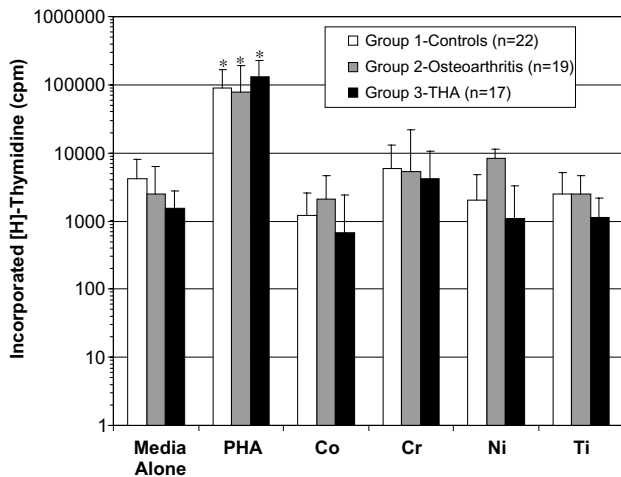


Fig. 1. The average radioactive thymidine incorporation indicates lymphocyte proliferation responses to metal challenge in Groups 1–3. Generalized elevated reactivity to PHA (a lymphocyte mitogen) is evident. Note: Bars indicate standard deviation. * $p < 0.05$, t -test.

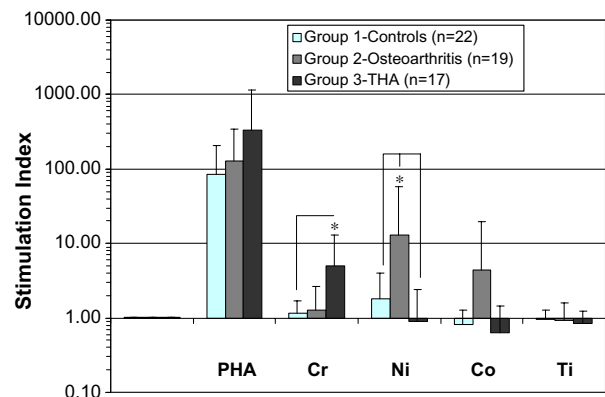


Fig. 2. The average proliferation responses of metal challenged subjects in Groups 1–3 are shown. There were no intergroup differences in reactivity to PHA (a lymphocyte mitogen). Group 3 subjects with THA demonstrated elevated reactivity to Cr ($p < 0.05$) with respect to Group 1. Group 2 subjects with OA demonstrated significantly elevated responses to Ni ($p < 0.02$) with respect to Group 1. Note: Bars indicate standard deviation. An * $p < 0.05$, t -test for intergroup comparison.

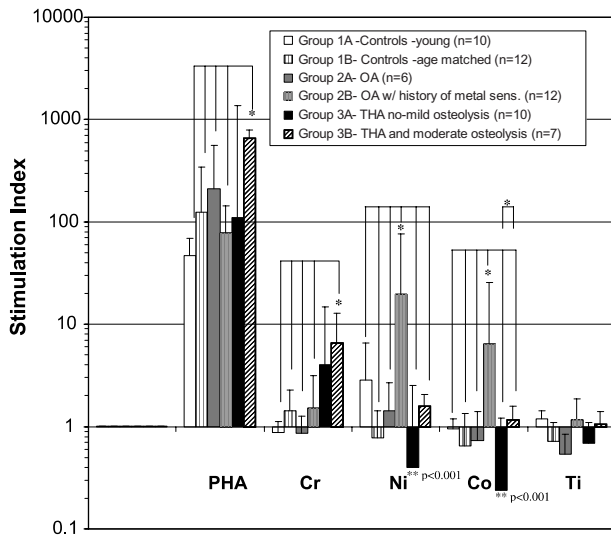


Fig. 3. Lymphocyte proliferation responses to metal challenge for all subgroups are depicted. Subjects with THA and moderate osteolysis (Group 3b) demonstrated significantly elevated proliferation in response to PHA and Cr when compared to controls (Groups 1a and 1b) and subjects with OA (Groups 2a and 2b). Subjects with OA and a history of metal sensitivity (Group 2b) demonstrate elevated proliferative responses to Ni and Co than all other subgroups. Subjects in Group 3b with moderate osteolysis had a greater reactivity to Co than subjects in Group 3a with no to moderate osteolysis. Note: Bars indicate standard deviation. An $*p < 0.05$ compared to groups indicated, $**p < 0.001$ compared to non-treated control lymphocytes, t -test.

However, Group 2b subjects (with OA and a history of metal sensitivity) were statistically elevated to Ni and Co ($p < 0.05$) when compared to all other subgroups. The greatest level of reactivity to any metal was to Ni in Group 2b subjects with OA and a history of metal sensitivity, at a proliferation level approximately 11 fold greater than untreated lymphocytes. Toxicity responses to metals were observed in Group 3a (THA subjects with mild osteolysis), where 8 of 10 subject demonstrated greater than 50% decreased proliferation to Co (SI < 0.3) and Ni (SI < 0.4) which were significant at $p < 0.001$. No other group demonstrated significantly reduced proliferation responses.

Cytokine analysis

The only significant increase in inflammatory cytokine levels produced by lymphocytes exposed to metals for any of the groups tested was that of Group 3 which demonstrated a 2 fold increase ($p < 0.05$, t -test) in IFN- γ and IL-2 levels in response to Cr over those of untreated lymphocytes (media alone), where control levels of IFN- γ and IL-2 were < 40 pg/mL and < 10 pg/mL. However, intergroup differences between Group 3a and 3b (with no to mild vs. moderate osteolysis, respectively) were not statistically significant.

Metal-ion analysis

Group 3a subjects with THA and no to mild osteolysis had Cr and Co levels of 0.39 ± 0.66 ng/mL (parts per billion) and 0.73 ± 0.36 ng/mL, respectively. Group 3b subjects with substantial osteolysis had Cr and Co levels of 0.42 ± 0.08 ng/mL and 0.47 ± 0.25 ng/mL, respectively. There were no significant differences in the Cr or Co metal content of serum between Groups 3a and 3b.

Discussion

THA subjects (Groups 3a and 3b) demonstrated elevated incidence and averaged levels of sensitivity responses to THA metals that indicated metal-specificity. THA subjects collectively exhibited a specific sensitization to Cr that exceeded that of subjects in Groups 1 and 2 in both incidence (29%) and magnitude (> 5 -fold SI), and did not respond to a more common immunogenic metal Ni. Elevation of IL-2 and IFN- γ in response to Cr challenge in subjects with THA (Group 3) also suggests specific cell mediated (adaptive) hypersensitivity responses. Furthermore, in the subgroup of THA subjects with more osteolysis (Group 3b), there was a higher prevalence of Co sensitivity in comparison to THA subjects with implants and no to mild osteolysis (43% versus 0% incidence, Table 2), suggesting that this metal-specific lymphocyte response may be etiologically linked to the phenomenon of periprosthetic bone loss [19]. Our hypothesis that lymphocytes from subjects with THA would demonstrate a nonspecific hyper-responsiveness to soluble implant alloy metals was not supported by our results.

To examine the possibility that individuals with osteoarthritis requiring a TJA may be constitutively predisposed to mononuclear cell hyper-responsiveness, this study included two groups without implants: subjects with no history of osteoarthritis (Group 1) and subjects with osteoarthritis scheduled to undergo THA (Group 2). Subjects with OA and no history of metal sensitivity had a prevalence of sensitivity to any of the metals tested of 33%, similar to Group 1 controls without OA (27%). In addition, the mean SI of Group 2a in response to Cr, Ni, Co and Ti is not different from that of subjects in Group 1. Thus, there is no evidence in this study that lymphocytes from subjects with OA without TJRs (but about to receive one) are constitutively more responsive to metal challenge. The high magnitude of Ni and Co reactivity in OA subjects with a history of metal sensitivity (SI > 11 Ni and SI > 7 Co in Group 2b) was expected and consistent with previous investigations using patch testing of metal sensitive individuals, [19] where Co reactivity is attributed to cross reactivity with Ni.

The toxicity responses associated with Co (SI < 0.3) and Ni (SI < 0.4) challenge agents in Group 3a subjects with a THA and mild osteolysis, and therefore presumably little peri-implant inflammation was both highly significant ($p < 0.001$, 8 of 10 subjects with SI < 0.5) and unexpected. Challenge concentrations of 0.1 mM were established in previous lymphocyte reactivity studies where primary lymphocytes from individuals without implants were used to determine a non-toxic yet generally antigen-capable metal concentration of 0.1 mM appropriate for testing [20–22]. If this toxicity phenomena occurs in vivo the results could range from lympho-specific toxicity resulting in localized immunosuppression to generalized cellular toxicity with a more direct role in progressive aseptic osteolysis. Alternatively, extraneous variables may account for some of these differences, such as Group 3a individuals were on average 10 years older ($p < 0.08$) than the other groups which may indicate a generalized age rather than implant sensitized toxicity response. However, individual comparison of Group 3 subjects' age vs reactivity levels as either combined (3a and 3b) or separate yielded r^2 values of 0.3 for Co and 0.001 of Ni, indicating no clear age related correlation. Larger populations and dose dependant investigations are underway to investigate more comprehensively concentration related hypersensitivity and toxicity responses in people with metallic implants. It was unexpected that such a pronounced dichotomy in lymphocyte response to metal challenge would exist between individuals with moderate and mild osteolysis when the determination of moderate or mild osteolysis was (although previously established) an arbitrary threshold. If these divergent responses are corroborated by further studies, they imply that over the long term either THA debris alarmingly cause alterations in immune system responses; or alternatively that hypersensitivity and toxicity responses measured in vitro may not be of clinical concern given that Groups 3a's and 3b's implants have been in-situ approximately 14 years and remain well functioning (pain free). Because elevated LTT reactivity and not toxicity responses are those associated with moderate osteolysis, i.e. those implants presumably closer to failure, it is this in vitro hyper-reactivity to metal challenge which may represent greater clinical concern. This elevated response may represent the onset or continued predisposition of an activated metal-related immune reaction or some form of inflammation contributing to or caused by the process of osteolysis.

Group 3a and 3b subjects with THA did not demonstrate statistically significant differences in the concentration of serum Co and Cr suggesting that the lymphocyte reactivity differences observed between the two groups were not associated with serum metal content. However, association between the magnitude of local metal release and hypersensitivity cannot be ruled

out since subtle increases in local peri-implant metal concentrations may not be discernable in peripheral serum.

While previous investigators have defined positive metal hypersensitivity using only the criteria of a statistically increased reactivity, SI > 1 [36], we have adopted a more strict criteria of SI > 2 at $p < 0.05$. This stimulation index value was chosen to partially account for the subjective and arbitrary nature of the threshold criteria, given the lack of correlative clinical information in the literature. Prospective, longitudinal investigations relating the clinical performance and outcome to LTT are critical to refine threshold criteria and determine clinical significance. LTT in this study was able to distinguish a high proportion of subjects (75%) with a history of cutaneous reactions to metal jewelry (Group 2b). Moreover our findings are consistent with past investigations of well and poorly functioning TJA populations [19], where our cumulative metal-sensitivity incidences of 30% and 71% for well (Group 3a) and more poorly functioning (Group 3b) THA subjects, respectively, are similar to previously reported multi-investigation averages of 25% and 60% incidence (primarily determined by patch test) [19]. These facts support assertions of higher sensitivity (vs dermal testing) and clinical utility for metal-LTT.

Past investigators describe a greater prevalence of reactivity using in vitro LTT when compared to dermal patch testing [8,10,13,42,46,49]. The applicability of skin testing to the study of in vivo immune responses to metal implants remains questionable. There is a lack of consensus about the most appropriate metal challenge agents when attempting to simulate biological exposure from metal implant degradation [18,53–55]. Other concerns about the applicability of patch testing include: (1) the haptenic potential of metals in dermal contact (in which dermal Langerhans cells are the primary antigen presenting cells) is likely quite different from that in the peri-implant environment [32], (2) the possible development of immunologic tolerance (i.e. suppression of dermal response to metals, anergy) [3,47], (3) the presence of an impaired host immune response [45,50], and (4) the possibility of inducing metal hypersensitivity in a previously insensitive patient [39]. For these reasons, in vitro lymphocyte proliferation testing may represent a more clinically appropriate method of assessing peripheral and peri-implant lymphocyte reactivity to metals.

However, several problems associated with contemporary LTT remain. The question of whether metal chloride solutions are appropriate challenge agents and adequately simulate soluble metal release from TJA degradation remains open. Additionally, it remains uncertain whether a stimulation index threshold of >2 ($p < 0.05$) is too stringent or too permissive. To account for this uncertainty in diagnostic threshold criteria, we

have limited our conclusions to those supported by both group incidence data (Table 2) and statistically increased levels of proliferation (Fig. 3).

Given that lymphocytes are present around implants, it is likely that metal-induced lymphocyte reactivity may contribute to the cascade of events leading to osteolysis and aseptic loosening. Activated lymphocytes release powerful cytokines such as IL-2, IFN- γ and RANKL (receptor activated NF-KB ligand), which can promote osteoclast activity (directly increasing bone resorption) and inhibit osteoblast activity (decreasing bone production).

Our findings indicate elevated metal-specific lymphocyte reactivity in individuals with THA, which was more evident in those THA subjects with osteolysis, i.e. a higher incidence and magnitude of Co-sensitivity in subjects with THA and moderate osteolysis (43% incidence in Group 3b) versus subjects with THA and no to mild osteolysis (0% incidence in Group 3a). Cr, a less immunogenic metal than Ni [19], induced the highest elevated stimulation of THA subjects' lymphocytes (30% incidence and SI > 3, $p < 0.05$), providing evidence that adaptive cell-mediated immunity responses may play a role in the etiology of osteolysis in susceptible individuals. Although these results indicate metal hypersensitivity may play a role in the clinical outcome of some TJR recipients, more evidence is needed to definitively establish this relationship. Continued follow-up investigations using variations in challenge concentration (dose), prospective and longitudinal design, and more basic science divulging mechanism(s) of metal immunogenicity will provide further information to judge and build a consensus opinion of the clinical relevance. Although supported by the current study, the utility of diagnostic screening using more elaborate multi-concentration LTT is uncertain and further evaluation using these techniques are currently underway to hopefully provide the essential knowledge for preventing and treating implant related metal hypersensitivity responses.

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