Chapter 6
Metal Sensitivity: Is It Possible to Determine Clinically?

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Introduction

Is it possible to determine metal sensitivity responses clinically? The simple answer to this question is yes, but the caveats are many and complicated, as will be discussed in this chapter. Excessive reactivity to metal implant debris or hypersensitivity to implant debris is relatively rare, where it is estimated that only 1–3 % of aseptic failures are due to hypersensitivity responses among traditional metal-on-polymer type total joint replacement designs [1–3]. Implants themselves are not known to cause hypersensitivity. Rather, implant debris (particles and ions) emanating from implant surfaces that have vastly different properties (e.g. metal ion release kinetics, specific surface areas, sizes, etc.) facilitate interaction with immune cells and elicit an immune response. This distinction is important, because when metal debris is minimized, the chances of metal hypersensitivity is also minimized [4].

This hypersensitivity is characterized by cell-mediated adaptive immune responses where conditioned lymphocytes respond to specific stimuli, as opposed to the more typical and less-specific response of macrophages to implant debris [5–7]. The slow progressive particle-induced osteolysis or "particle disease" generally refers to the process of peri-implant osteolysis, where implant loosening and inflammation are in main part due to implant particulate debris non-specifically interacting with innate immune system cells (i.e. tissue macrophages termed histiocytes) that occurs over many years (> 7 years) [8, 9]. In contrast, "metal sensitivity" or hypersensitivity has been predominantly characterized as specific, and increases in the prevalence of

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delayed type hypersensitivity (DTH) responses have been associated with the failures within the first 2–5 years from implantation of certain types of metal-on-metal (MoM) bearing implants as detected by unusual lymphocyte associated peri-implant responses and diagnostic immune metal-reactivity testing [2, 10].

To a large extent, implant materials and metals currently in use have evolved over time to the more successful candidates that wear and corrode to the smallest degree possible. Despite this optimization process, metal sensitivity is still well reported in both case and group studies [11–13]. How and why this occurs remains largely unknown. What is known is that all implant metals degrade by both corrosion and/or wear in vivo [14, 15] and the released debris (particles and ions) immediately are coated or complex with plasma proteins and interact locally and systemically [16, 17]. Released metal ions become antigenic by becoming haptens which activate the immune system by forming complexes with native serum proteins and altering their natural conformational structure [18–21]. These metal-altered-self-protein complexes are processed by antigen-presenting cells (APCs) and are recognized as foreign by lymphocytes that then become the hypersensitivity responses.

In its broadest definition metal sensitivity to implants is any aseptic (non-bacterial) material-driven "excessive" immune response that causes peri-implant pathology, such as bone loss or local inflammation of T-cells, B-cells or macrophages. The hotly debated aspect of this is just what constitutes "excessive". When an implant fails prematurely (< 7 years) due to an exuberant cell-mediated immune response to the same amount of implant debris that is typically well tolerated by most people, that response can be categorized as "metal-allergy", "implant-allergy", "implant sensitivity" or "hypersensitivity" [22]. The allergy/sensitivity/hypersensitivity terms have been liberally used as interchangeable in immunology and orthopedics despite specific nuanced differences between them. For simplicity within this discussion of metal sensitivity, any nuanced differences between them will not be discussed here.

Skin or dermal sensitivity to metals has been reported to cause skin hives, eczema, redness and itching, that affects approximately 10–15 % of people [11, 12, 21, 23–25] (Fig. 6.1), where hypersensitivity to nickel is the most common (approximately 14 %) [11], followed by cobalt and chromium [11, 21]. Other sensitizing metals include beryllium [26], nickel [23–26], cobalt [26] and chromium [26], and to a lesser degree tantalum [27], titanium [28, 29] and vanadium [27]. Although much still remains unknown about these biological steps and responses, this chapter will present an overview about what is known about how these metals elicit sensitivity in patients with implants.

Metal Sensitivity Mechanism

In general, hypersensitivity responses can take one of two central forms: (1) a humoral immediate (within minutes) type of response that is initiated by antibody–antigen complexes of Types I, II and III reactions, or (2) a cell-mediated delayed (hours to days) type of response [30, 31]. The metal hypersensitivity reactions currently

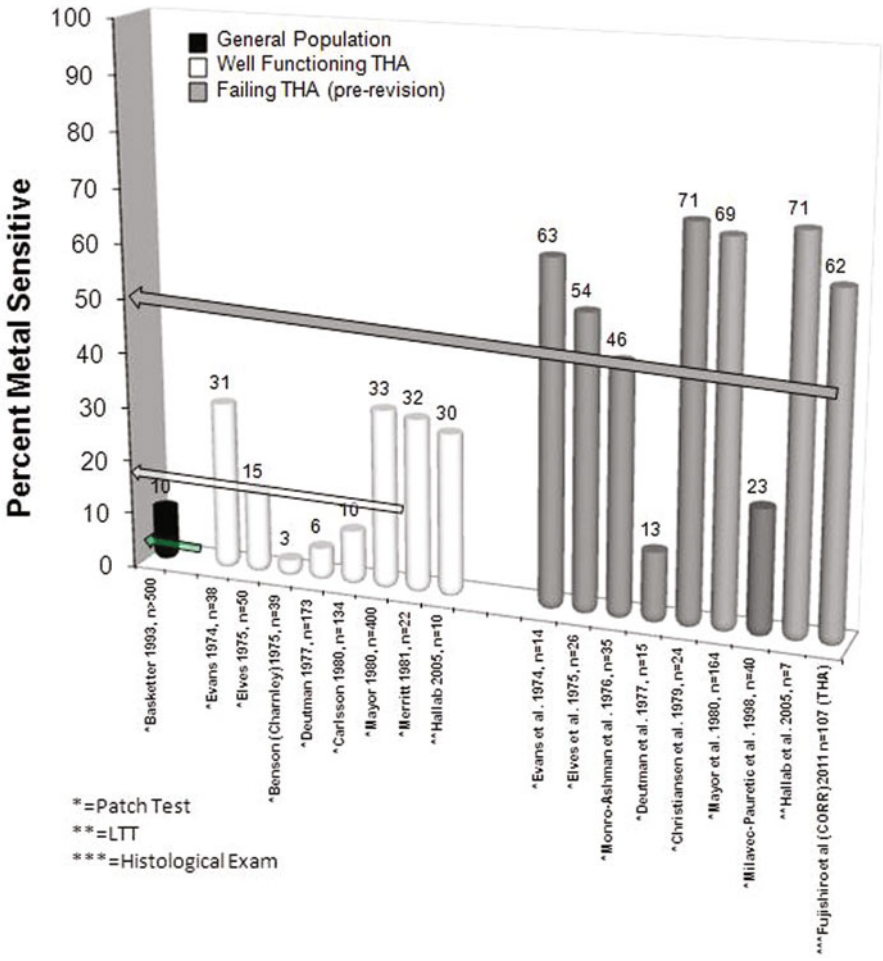

Fig. 6.1 A compilation of investigations show the averaged percentages of metal sensitivity among the general population for nickel, cobalt and chromium, among patients after receiving a metal-containing implant, and among patient populations with failed implants. All subjects were tested by means of a patch test, metal lymphocyte transformation test (LTT) or histological diagnosis

recognized are almost exclusively delayed type responses mediated by antigen-activated lymphocytes that have been classically categorized as Type IV Delayed Type Hypersensitivity responses (DTH).

This specific cell-mediated delayed type of hypersensitivity response is characterized by T-helper lymphocytes of the T_H1 subset. These T_H1 cells release a unique pattern of inflammatory cytokines, including interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1) and interleukin-2 (IL-2). Although these T_{H-1} cells are needed to combat intracellular pathogens, T_{H-1} when they are erroneously released and activated can result in autoimmune diseases [32, 33].

In this fashion, metal-sensitized and activated T-cells, in conjunction with primed/recruited APC's, will secrete a variety of pro-inflammatory cytokines that recruit and activate other innate immune cells, e.g. macrophages, monocytes and neutrophils [22]. These signature cytokines include IFN-γ and TNF-β which, of the many pro-inflammatory effects on local cells (e.g. endothelial cell), induce migration inhibitory factor (MIF)—which prevents the migration of recruited macrophages away from the site of the metal-DTH reaction (see Table 6.1). The hallmarks of a DTH response are infiltration, activation and eventual migration inhibition of innate immune cells (e.g. macrophages). These recruited and activated macrophages have an increased ability to phagocytize, process and then present pieces of the phagocytized metal–protein complexes (immune epitopes) on their surface for T-cell recognition (in class II Major Histocompatibility complexes (MHCs) for interaction with T-cell receptors (TCRs)). The release of cytokines from the recruited APCs (such as IL-1), can trigger the recruitment/activation of more T-cells, which in turn activates more macrophages in a vicious cycle. Under certain circumstances, and in some auto-immune diseases where there is an inability to turn off this DTH self-perpetuating response, the runaway results can be extensive tissue damage. Thus, the current strategies to mitigate these types of responses in people are geared towards immunosuppressive therapies that clip or temporarily stop this vicious cycle and allows the response to abate [34, 35].

However, targeted therapy for selected immunosuppressive therapies has not been developed yet due to the many things that remain unknown about metal sensitivity, including (1) how to address the fact that different specific lymphocyte populations are activated in different individuals [36], (2) the specific cellular mechanisms of recognition and activation and (3) how serum metal–protein complexes become antigenic. Dermal sensitivity is more easily studied and thus dermal metal allergy has been better characterized to some extent [37]. Skin is the primary immune barrier and the APCs of the skin, Langerhans cells, are exquisitely good at gathering and presenting antigen. Each dendritic Langerhans cell is responsible for the immuno-surveillance of 53 epidermal cells, in an amazing consistency from person to person [38]. Unfortunately these cells differ in several ways from the APC's in the periprosthetic region. Peri-implant APCs include macrophages, endothelial cells, lymphocytes, dendritic cells and, to lesser extent, parenchymal tissue cells. Tissue macrophages (histiocytes) are considered the primary APCs around implants and are involved in implant debris phagocytosis. The highly variable regions of TCRs that recognize the metal–protein complex presented by APCs have been widely acknowledged as central to metal sensitivity [39, 40, 41]. To complicate matters, metals such as nickel have also been shown to act in both classical and non-traditional ways to activate T-cells, one of which is to simply cross-link TCRs and co-stimulatory receptors on T-cells (e.g. VB17 of CDR1 TCR) to create what is termed a "superantigen" activation of TCRs [40, 42]. Despite the identification of ways by which non-typical metal induced lymphocyte activation can occur, the traditional DTH response remains the dominant mechanism associated with implant-related hypersensitivity responses [43–45], where one group of clonally specific/sensitized lymphocytes respond to metal challenge.

Table 6.1 Selection of important cytokine involved in innate and adaptive immune responses to implant debris (source and mechanisms of action) [6, 32–34, 125]

Cytokine	Principle source	Principal activities
Macrophages (innate immunity)		
IL-1β	Macrophages/monocytes	T, B-cell activation; pro-inflammation
TNFα	Macrophages, TH-1 cells	Pro-Inflammation; tumor killing
MCP-1	Monocytes, endothelial cells	Chemotactic for monocytes but not neutrophils
IL-1ra	Macrophage/monocytes	IL-1 receptor antagonist blocks action of IL-1
IL-6	Macrophages, T cells	B cell stimulation, inflammation
IL-8	Macrophages	Meutrophil (PMN) attraction
IL-12	All APCs	Stimulates T-cells into Th1-cells and IFN-g
IL-18	Macrophages/monocytes	Stimulates IFN-gamma production
GM-CSF	Macrophages/T-cells	Proliferation/differentiation macrophages
Lymphocytes (adaptive immunity)		
IFNγ	T-cells, macrophages	Inflammation, activates macrophages (induces Th1)
IL-2	T-cells	Inflammation, activates macrophages (induces Th1)
IL-4	T-cells	Inflammation, activates macrophages (induces Th2)
IL-10	Th2 and macrophages	Inhibits Th1 cytokines, enhances B-cells survival/proliferation, and can block NF-κB

Testing for Metal Sensitivity

Currently approved methods for human diagnostic testing for metal allergy include both skin testing (patch testing) and in vitro blood testing using LTT. There are commercially available assays for physicians that contain some of the metals in orthopedic implants [30, 46].

Dermal Testing While general patch testing protocols and commercial kits do exist for a variety of common metals [30, 46] there are questions regarding the applicability of skin testing to diagnose in vivo immune responses to orthopedic implant debris. In particular, there are questions regarding the location-specific APCs and skin vs serum challenge of metal challenge agents [1, 18–20]. It is hard to imagine that the exquisite specificity of myriad immune responses are not dramatically affected by both the haptenic potential of metals in a dermal environment (in which dermal Langerhans cells are the primary effector cells) vs that of an in vivo closed peri-implant environment [31, 47]. This difference is highlighted by the amazing APC's of the skin, where unique antigen-processing/endosomal-recycling organelles, called Birbeck granules, are present in Langerhans cells but are not found in the dominant

peri-implant APCs such as macrophages [48, 49]. There are other important limitations to dermal testing for implant-related metal sensitivity including the following: (1) The rudimentary and relatively subjective nature involved with grading a dermal reaction from 0 to + 3 which precludes detection of more subtle but statistically significant group differences and incorporates the wildly different opinions of clinicians on what constitutes a + 1, + 2 or + 3 response. (2) Dermal testing may be affected by site-specific immunological tolerance (i.e. suppressed skin reactivity to implants) [46, 50]. (3) There may be impaired host immune responses that are genetic, or environmental, e.g. concurrent medications [51, 52]. (4) The biggest risk associated with patch testing is the possible sensitization of metal sensitivity in a previously non-sensitive individual [53]. (5) The conditions of immune challenge during patch testing are also highly variable (i.e. non-standardized), where the environment of a patch test placed on a hairless area of the skin (typically the upper back) for 48–72 h is highly inconsistent from patient to patient and uncomfortable, where such aspects as cleanliness of the area and home environment is not standard. (6) Finally, there are no well-established challenge concentrations/doses and methods for several orthopedic metals available in commercially available/approved patch test kits (e.g. Al, Mo, V and Zr, Table 6.2).

Lymphocyte Transformation Testing Less risky from an induction perspective is LTT, which measures the proliferative responses of blood drawn lymphocytes after they are exposed to specific antigens or haptens for 3–6 days. These lymphocytes are obtained from a regular blood draw where the mononuclear cell fraction is isolated after centrifuging the heparinized blood on a layer of Ficoll (density gradient separation). Proliferation is measured using a radioactive marker and is added to cultured lymphocytes with challenge agents. The incorporation of radioactive [^{3}H]-thymidine into cellular DNA upon mitosis facilitates the quantification of a proliferation response through the measurement of incorporated radioactivity after a set time period, typically after 5–6 days of challenge (with 0.001–0.1 mM Al^{+3}, Co^{+2}, Cr^{+3}, Mo^{+5}, Ni^{+2}, V^{+3} and Zr^{+4} chloride solutions). During the last day of 12–24 h of antigen exposure, radiolabeled [^{3}H]-thymidine treatment is used to measure proliferation by measuring the amount to which it is incorporated into dividing cells DNA after "harvesting" (collecting) cells onto a paper membrane and then using liquid scintillation measurement of radiation counts per minute (cpm). This method of measuring cell proliferation is highly precise because of the ability to measure a small subset of antigen-activated dividing cells amongst the many other in a culture well, due to incorporation of radioactive Thymidine into cell DNA upon mitosis. A proliferation or stimulation index is calculated:

Proliferation Index (Factor) = (mean cpm with treatment)/(mean cpm without treatment).

The use of LTT in the assessment of orthopedic implant-related metal sensitivity is growing and although less popular and less available than patch testing (due to the highly complex nature of the immune test: culturing, challenging and measuring proliferation), it has been well established as a method for testing hyper-sensitivity in a variety of clinical settings [54–59]. Some reports seem to indicate LTT may

Table 6.2 The percentages of metals in different orthopedic alloys

Alloy	Ni	N	Co	Cr	Ti	Mo	Al	Fe	Mn	Cu	W	C	Si	V
Stainless steel (ASTM F138)	10–15.5	< 0.5	< 0.05	17–19	< 0.05	2–4	< 0.05	61–68	< 0.05	< 0.5	< 2.0	< 0.06	< 1.0	< 0.05
CoCrMo alloys														
(ASTM F75)	< 2.0	< 0.05	61–66	27–30	< 0.05	4.5–7.0	< 0.05	< 1.5	< 1.0	< 0.05	< 0.05	< 0.35	< 1.0	< 0.05
(ASTM F90)	9–11	< 0.05	46–51	19–20	< 0.05	< 0.05	< 0.05	< 3.0	< 2.5	< 0.05	14–16	< 0.15	< 1.0	< 0.05
(ASTM F562)	33–37	< 0.05	35	19–21	< 1	9.0–11	< 0.05	< 1	< 0.15	< 0.05	< 0.05	< 0.05	< 0.15	< 0.05
Ti alloys														
CPTi (ASTM F67)	< 0.05	< 0.05	< 0.05	< 0.05	99	< 0.05	< 0.05	0.2–0.5	< 0.05	< 0.05	< 0.05	< 0.1	< 0.05	< 0.05
Ti-6Al-4V (ASTM F136)	< 0.05	< 0.05	< 0.05	< 0.05	89–91	< 0.05	5.5–6.5	< 0.05	< 0.05	< 0.05	< 0.05	< 0.08	< 0.05	3.5–4.5
45TiNi	55	< 0.05	< 0.05	< 0.05	45	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Zr alloy (95 % Zr, 5 % Nb)	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05

Alloy compositions are standardized by the American Society for Testing and Materials (ASTM vol. 13.01)

be equally or better suited for the testing of implant-related sensitivity than dermal patch testing [55]. Other investigations show that metal sensitivity can be more readily detected by LTT than by dermal patch testing [60–62]. This increased sensitivity (minimized false negative) may be more important than high specificity (minimized false positives). Why? Because there is a choice of commercially available implants made from different metals and these different implants are generally equally successful, thus it is more important to be able to determine everyone who has metal sensitivity (at the expense of some false positives) because the risk of choosing a different better appropriate implant material carries little to no risk. In comparison missing the diagnosis of metal sensitivity for better specificity (minimized false positives) carries with it the spector of early failure and revision surgery for the patient.

One potential benefit of metal LTT is the use of mixed mononuclear cells derived from a blood draw (i.e. T-cells, B-cells and other more rare lymphocyte populations) that are directly exposed to metal challenge and thus may more closely mimic that of the local implant environment (compared to the dermal metal challenge). Additionally, soluble metal chloride challenge agents are able to complex with serum proteins from the same individual that is tested, i.e. autologous serum [63–65]. These artificially created metal–protein challenge agents have been shown to be similar to those produced in vivo [17, 66, 67]. However, the precise metal–protein complexes that are produced on and in the dermal tissue remain uncharacterized [17, 22]. LTT is also both highly quantitative and not technician/operator dependant (vs patch testing) [2]. A quantitative stimulation index is produced from multi-well replicates that enables calculation of an average and standard deviation for each metal challenge agent at each concentration. This increased sampling size enables the study of different patient cohorts, metal challenge agents, dose responses, different implant types, etc. An advantage of LTT over dermal testing of metal coupons is the ability to test several known concentrations (dose responses) for each metal agent (e.g. > 10) at (e.g. 0.01, 0.1 and 0.5 mM). Most immune responses are dose dependent especially in individual patients. Too little or too much immune challenge may not induce a response or simply induce toxicity, respectively. Thus, using different challenge doses is of central importance for current LTT. This provides a means to assess those people who are sensitive at lower than normal (e.g. 0.01 mM) or higher than normal (e.g. 1 mM) challenge concentrations of metal challenge. This scenario is illustrated in Fig. 6.2 where LTT results of a metal sensitive individual demonstrate dose dependent increased reactivity to Ni. Additionally advantageous is that LTT has reported greater sensitivity than dermal patch testing [62, 68–72]. While this greater sensitivity may increase the likelihood of false positives (decreased specificity), it more importantly minimizes the occurrence of false negatives, which in the authors' opinion, as stated earlier, is in the best interests of the patient, given the little to no additional risk of choosing a more biologically suited implant material for the patient. This testing is gaining popularity and is more relevant than ever, due to the increasing numbers of implants going into patients and the increasing numbers of surgeons [73] that have the technical ability and expertise to put in different implants that are made of different alloys (e.g. titanium alloy vs cobalt alloy vs zirconium alloy).

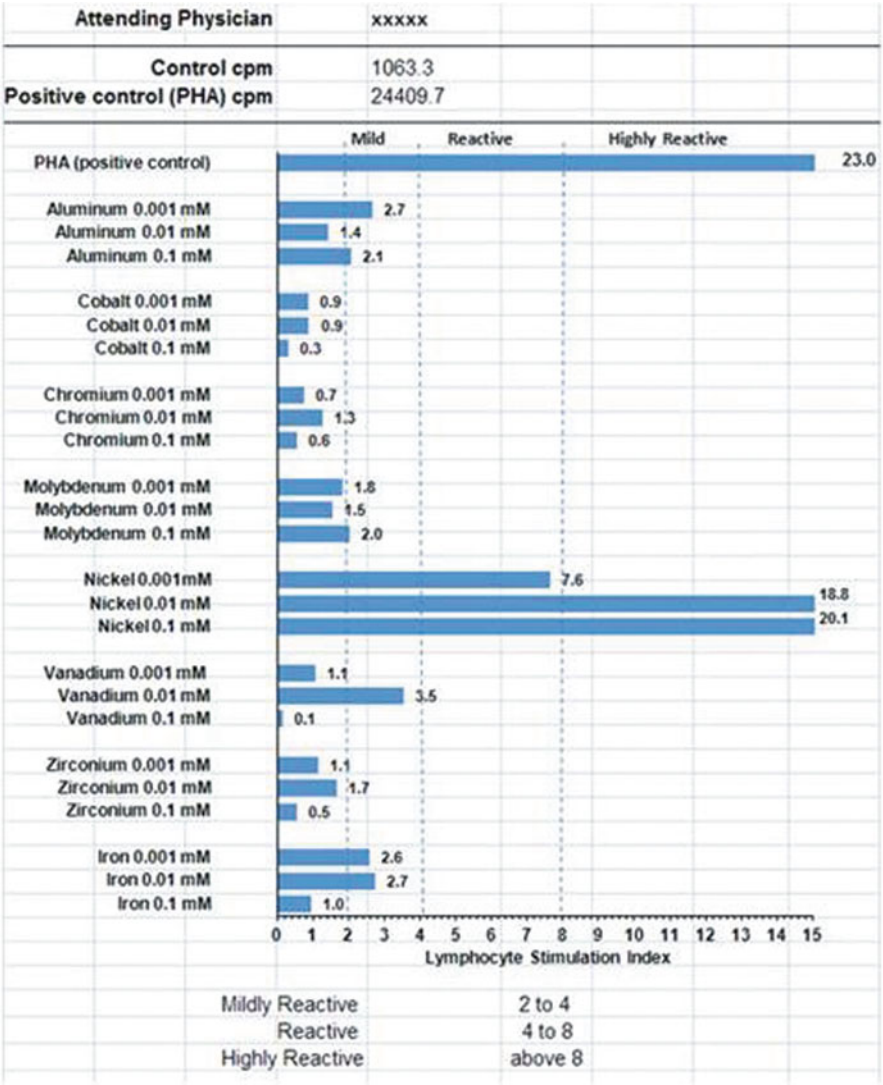

Fig. 6.2 Sample results of a metal LTT indicate high reactivity to Nickel at all 3 concentrations tested. Metals are generally used at 3 different concentrations of 0.001, 0.01 and 0.1 mM. (Courtesy of Orthopedic Analysis LLC)

Contemporary LTT does still require more enhancements. Metal solutions allowed to complex with proteins only approximate the kinds of products generated by corrosion and wear during metal implant degradation [17, 65, 67], and the degree to which lymphocyte reactivity is affected by any subtle differences remains unanswered. Additionally, it is unclear what the lower bound of stimulation index number (i.e. threshold) best indicates a clinically relevant hypersensitivity response. In the

past our laboratory and others have used an experience-based criteria of a stimulation index threshold of > 2 ($p < 0.05$) to indicate mild metal hypersensitivity and > 8 to indicate severe metal reactivity, consistent with drug allergy literature over the last half century [39, 56, 62, 74, 75]. However, it remains unclear from these studies whether this criterion is too strict or too permissive.

More prospective, longitudinal clinical studies, such as the metal-on-metal study discussed in the following section, provide support to why LTT and patch testing are meaningful in a clinical setting even with needed enhancements. Specific types of implants with greater propensity to release allergenic metals in vivo may be more prone to induce metal sensitivity. For example, failures of total hip prostheses with MoM bearing surfaces have been associated with greater prevalence of metal sensitivity than similar designs with metal-on-ultrahigh-molecular-weight-polyethylene bearing surfaces [50, 76]. Many case and group studies indicate the clinical utility and expansion of metal sensitivity testing for total implant recipients [2, 3, 22, 77–80].

Case Studies in Metal Implant-Related Metal Sensitivity

Many reports over the past 40 years have implicated metal allergy or sensitivity type responses, where the release of implant debris was temporally connected to specific responses such as severe dermatitis, urticaria, vasculitis [81–86] and/or non-specific immune suppression [51, 87–90].

One of the first correlations of dermal metal reactions to the poor performance of a metallic orthopedic implants was made in 1966 by Foussereau and Lauggier [91] where a nickel-containing implant was accompanied by dermal hypersensitivity reactions. There have been many case reports over the past 40 years that link immune responses with adverse performance of metal implants in the cardiovascular [85, 92, 93], orthopedic [12, 81, 83, 84, 86, 94], plastic surgery [95] and dental [96–102] fields. In many instances, excessive early immunological reactions (aseptic inflammation) have necessitated device removal, and after explantation the immune reactions dissipate [81–86]. Sometimes (but not always) severe skin reactions [82, 84, 85, 92–94, 103, 104] accompany the aseptic inflammation and they have also been reported to appear in conjunction with the relatively more general phenomena of metallosis (dark metallic staining of tissue due to excessive implant debris), excessive periprosthetic fibrosis and muscular necrosis [86, 105, 106].

This dermal reaction was true in one of the earliest cases of metal implant sensitivity [83], where a 20-year-old woman had symptoms of inflammation including rashes on her chest and back, approximately 5 months after stainless steel screws were used to treat chronic patellar dislocation. Topical steroids worked to treat this condition for 1 year, after which it worsened with more generalized dermal eczema, until the implant was removed. After the stainless steel screws were removed her dermal rashes completely disappeared within 72 h [83]. “The orthopedist still doubted that the steel screws could be the cause of her dermatitis and applied a stainless steel

screw to the skin of her back. In a period of 4 h, generalized puritus and erythema developed" [83]. Dermal patch testing showed aggressive reactions to nickel and the steel screw. What is fascinating about this early case is that it satisfies Koch's Postulates, a key test for causality in medicine. An agent can be considered as causative when it is removed and the symptoms abate, and when it is returned the symptoms also return. Thus, metal sensitivity associated with implant materials was conclusively demonstrated nearly 40 years ago, albeit only in a case study. There were a number of case studies to follow that showed similar temporal and physical evidence of delayed type hypersensitivity response reactivity to orthopedic implant metals [12, 21, 81, 84, 86, 95].

Generally, among the literature there are more cases of metal sensitivity reported to stainless steel and cobalt alloy implant induced immune responses and less to titanium alloy components [12, 21, 81, 82, 84, 93, 94, 104, 107, 108]. One of these early case reports of cobalt metal sensitivity indicated that metal sensitivity type responses including periprosthetic fibrosis, patchy muscular necrosis and chronic inflammatory changes peripherally, occurred 7 years after the initial operation of cobalt alloy plates and screws used in the fracture fixation of a 45-year-old woman's left radius and ulna [43]. This patient's response demonstrated that the time to develop this kind of response is not limited to the first few years of implantation. And after the implant was removed and the symptoms (swelling) disappeared, the patient remained reactive to cobalt as indicated by patch testing [43].

Cohort Studies of Implant-Related Metal Sensitivity

Almost the entire bulk of the evidence attesting to the clinical utility of metal sensitivity testing can be attributed to the many retrospective cohort studies that indicate a strong correlation between metal exposure and the performance of a metal-containing implant and metal sensitivity [12, 46, 50, 109–117]. These studies show that the incidence of metal sensitivity among patients with elevated metal exposure with well-functioning implants is approximately 25 %, roughly twice as high as that of the general population (Fig. 6.1) [46, 50, 76, 108, 110, 112, 113, 116, 118]. This sensitivity dramatically increases to 60 % in patients with a painful or poorly functioning implant (as judged by a variety of criteria) [76, 108, 110, 112, 118]. While current evidence suggests otherwise [22, 78], these patients may be "selected" for failure due to a pre-existing metal allergy. Thus the incidence of metal sensitivity in people with painful/failing implants is about six times that of the general population and approximately more than two times that of people with pain-free well performing implants [119].

Evident from past and current group studies is that specific types of implants that release more metal ions and/or particles are more likely to induce metal sensitivity [22, 78]. Some MoM total hip prostheses designs and some surgical placement resulted in metal sensitivity to a greater extent than similar designs with metal-on-ultrahigh-molecular-weight-polyethylene bearing surfaces [22, 50, 76]. New

generations of metal-on-metal (MoM) total hip replacements generally have the advantage of lower overall wear than metal-on-polymer implants but release more metal ions and particles and have greater reports of failures attributable to excessive inflammatory reactions. Hypersensitivity-like reactions have been reported to be as high as 76–100 % of the people with failing MoM implants [120, 121]. These sensitivity responses include histological inflammation accompanied by extensive lymphocyte infiltrates [120, 121]. Recent prospective studies involving people with MoM implants showed that at least over the short term, in vivo metal sensitivity responses develop even in asymptomatic well-performing MoM implants [22]. One study reported a significant increase in metal sensitivity from 5 % pre-op to 56 % at 1–4 years post-op in people with well-performing (asymptomatic) MoM surface replacement hip arthroplasties [22]. Within the same investigation, a retrospective analysis of people with asymptomatic MoM implants in place for longer than the prospectively studies group (i.e. > 7 years on average) had an even higher average incidence of metal sensitivity at 76 %, presumably because the implants were in longer exposure to elevated levels of metal (2–11 years). These levels, while high, are less than those previously reported for painful/symptomatic MoM patients (i.e. 81 % in failing MoM implants by Thomas et al. [2]). While a pattern of increasing metal reactivity with implantation time supports a causal or contributing relationship between local adaptive immune responses and the pathogenesis of MoM failure, it may be argued that the generation of wear from a failing bearing results in an immunological response to metal/protein complexes unrelated to the pathology of the implant failure . However, regardless of the role of the immune response in implant failure (which may not be generalized to individual patients) the overall findings of recent studies [22, 78] support the use of sensitivity testing for assessing implant performance. We found that [22] lymphocyte sensitivity responses to Co and Cr were not apparent at 3 months post-operatively (when serum levels of metal were already high), but developed after 1–4 years, Fig. 6.3. However, this “slow” increase in reactivity contrasted with the relatively fast elevations in Co and Cr metal ion levels measured at 3 months post-operatively. This delay suggests that metal sensitivity responses to this type of implant may develop over time and may be related to metal ion exposure levels. Incidentally, in this same study, patch testing did not correlate at any time point with in vivo metal ion levels or other measures of metal-induced immune responses such as metal LTT, flow cytometry or cytokine analysis. This study finding also suggests that patch testing may not adequately reflect adaptive immune responses in the local implant environment.

Other studies have also shown elevated levels of circulating metal ions correspond to increased acquired metal sensitivity responses and other specific MoM pathologies. Kwon et al reported that people with MoM hip implants and radiographically identifiable pseudotumors had a nearly two times increase (80 vs 45 %) in incidence of metal reactivity to Ni (LTT, SI > 2) and had fivefold increases in both Co and Cr serum ion levels, when compared to people with MoM implants without non-pseudotumors [77]. We have reported in a current large study of pain levels compared to metal sensitivity levels in people with various orthopedic hip arthroplasty implants that the percentage of people metal sensitive (metal LTT with SI > 2) was significantly higher for people with more painful implants vs non-painful (Fig. 6.4)

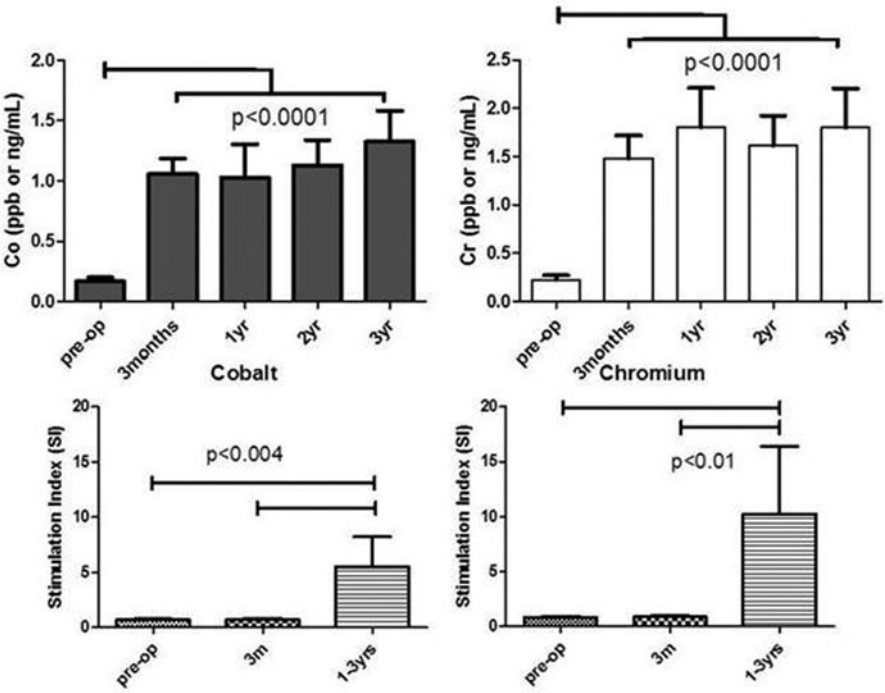

Fig. 6.3 Metal ion levels of Cobalt and Chromium are shown increased as early as 3 months in serum in people with metal-on-metal hip arthroplasty implants. However, increases in metal reactivity as measured by lymphocyte proliferations (*SI*), were only increased after 1–3 years of metal exposure in the same people with metal-on-metal hip arthroplasties. All people with metal implants used in this study were assymptomatic ($n = 21$, $p < 0.04$, Mann Whitney). (Adapted from Hallab et al. [22])

[119]. Furthermore, when the levels of metal-induced lymphocyte reactivity were categorically compared based on mild ($2 < SI < 4$), moderate ($4 < SI < 8$) or high ($SI > 8$) sensitivity with self-reported mild, moderate and high pain levels, they were significant different in pain levels between people with moderate vs high sensitivity levels. Conversely, people with Total Joint Arthroplasty (TJA) and no pain or low pain levels demonstrated a relatively low incidence of metal sensitivity (not significantly different, Fig. 6.4). This correlation suggests that pain may be connected to lymphocyte-associated immune reactivity to metal implant degradation products where higher self-reported pain levels can correlate with higher incidences of metal reactivity in vitro.

Clinical Relevance

All these past and recent studies illustrate the clinical need for sensitivity testing for two sets of people: (1) patients with a known history of metal sensitivity, and (2) patients with a painful implant where infection has not been detected through multiple

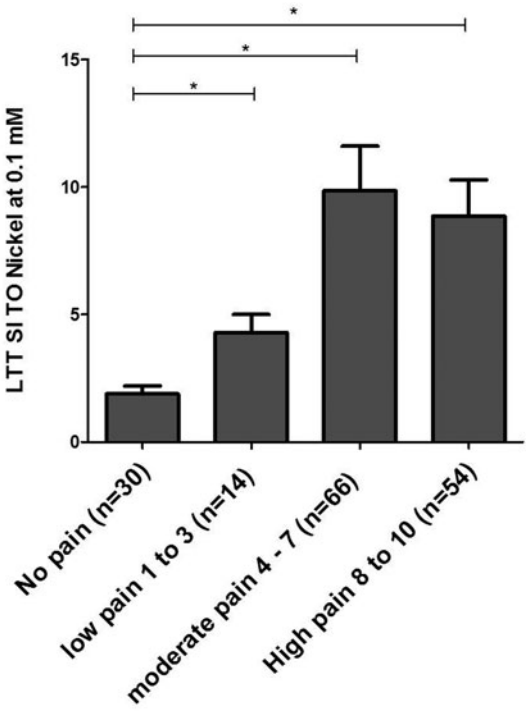

Fig. 6.4 Incidence of Nickel reactive subjects (*LTT*) according to self-reported pain levels in patients with no history of any allergy at a challenge concentration of 0.01 mM. Nickel reactivity in TJA subjects was based on their lymphocyte *SI* and was categorized as follows. Pain levels were denoted as follows in a scale of 1–10: *no pain* (0), *mild pain* (1–3), *moderate pain* (4–7), *high pain* (8–10). To obtain the incidence of metal reactivity, the percentage of subjects non-reactive, mildly reactive, reactive and highly reactive to Nickel at 0.01 mM concentration were calculated within their respective pain level group: *no pain* ($n = 30$), *mild pain* ($n = 14$), *moderate pain* ($n = 66$), *high pain* ($n = 54$). (Courtesy of Orthopedic Analysis LLC)

approaches. Although the evidence remains indirect, metal sensitivity testing is a direct measure of immune cell reactivity to implant metals, and thus represents real and heightened immune reactivity (and not simply a correlative biomarker with unknown role in the pathology). Immune reactivity to metal is well established as associated with implant performance and thus it is likely that a detectable, reproducible and quantifiable elevated immune response to an implant metal represents a clinically important phenomenon. Metal sensitivity testing is a direct test of an individual's immune response to metal challenge and the results indicate levels of immune reactivity that have been used for the past half century to measure delayed type responses drugs (such as antibiotics) and the persistence/effectiveness of vaccines such as tetanus toxin [122, 123]. Thus, it is highly likely, once a sensitivity response to metals is initiated (either before or during implant loosening or failure), that response directly plays into the etiology of further implant failure. Thus, the question of whether metal sensitivity initiates the pain, loosening, etc., is less important once sensitivity has been established and a feedback loop is formed that negatively impacts implant performance. We are currently investigating how the role metal-stimulated lymphocytes participate in the pathogenesis of aseptic osteolysis through the release of powerful cytokines such as IL-2, IFN-γ and RANKL (receptor-activated NF-KB ligand), which can directly increase bone resorption by osteoclasts and inhibit bone deposition by inhibiting osteoblast activity (Fig. 6.5) [124–127].

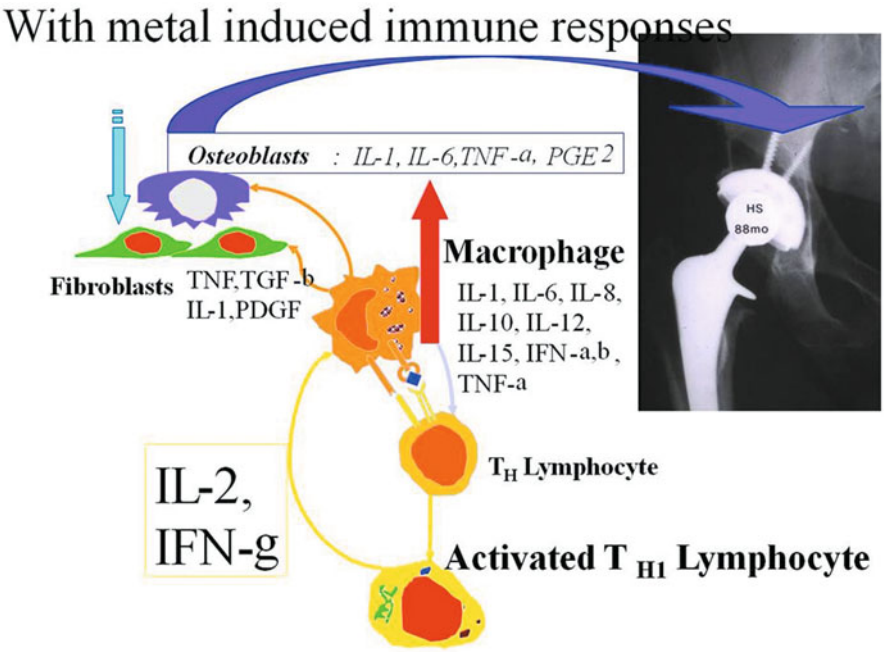

Fig. 6.5 Metal-induced immune responses can be due to both innate immune (e.g. macrophage) or adaptive (e.g. lymphocyte) immunity. Adaptive immune responses (i.e. hypersensitivity) can negatively effect bone homeostasis both directly and indirectly leading to osteolysis. (Courtesy of Orthopedic Analysis LLC)

Over the past 40 years implant-debris-induced inflammation has been characterized ad nauseam, where debris-induced localized inflammation is caused in large part by macrophages which up-regulate NFκβ and secrete inflammatory cytokines like IL-1β, TNFα, IL-6 and IL-8 [7]. Other anti-inflammatory cytokines such as IL-10 modulate the inflammatory process. Other factors involved with bone resorption include the enzymes responsible for catabolism of the organic component of bone. These include matrix metalloproteinases collagenase and stromelysin. Prostaglandins, in particular PGE_2, also are known to be important intercellular messengers in the osteolytic cascade produced by implant debris. More recently, several mediators known to be involved in stimulation or inhibition of osteoclast differentiation and maturation, such as RANKL (also referred to as osteoclast differentiation factor) and osteoprotegerin, respectively, have been suggested as key factors in the development and progression of bone loss (osteolytic lesions) produced from implant debris. Over the past 30 years we understand these mediators act to promote inflammation that decreases bone remodeling and is associated with the pathogenesis of osteolysis. However, we are only beginning to understand how implant debris could actually induce this immune system response at the cellular level.

Conclusions

When attempting to predict all of the effects of implant debris on the immune system, one of three possible outcomes could occur: (1) metal degradation products are immunogenic [39, 44, 128, 129], (2) metal degradation products are immunosuppressive [130–132] or (3) metal degradation products are immuno-neutral (i.e. non-bioreactive) [133, 134]. While all three possibilities have been shown to occur in reported case and group studies, the type of reaction and outcome that will occur in any one individual is mostly likely dependent on the individual (genetic regulation and immune status), the environment and the type of implant.

The key cell types in metal sensitivity are CD4+ lymphocytes, that traffic locally through the periprosthetic space. Upon metal exposure by APC the relevant lymphocytes proliferate and activate, which can potentially contribute to the cascade of inflammatory events leading to osteolysis and aseptic loosening. Pro-inflammatory cytokines are released such as IL-2, IFN-gamma and RANKL that can activate osteoclasts directly (increasing bone resorption) and inhibiting osteoblasts (decreasing bone production). Thus, as the number of patients receiving implants grow and the clinical specialties expected to evaluate this phenomena increases, metal sensitivity testing offers a relatively risk-free additional tool in the armamentarium of physicians/surgeons.

While positive results of sensitivity responses to metallic biomaterials which affect orthopedic implant performance in other than a few percent of patients (i.e. highly predisposed people) [135, 136] are growing, new evidence continues to demonstrate that concrete relationship and benefits of sensitivity testing may improve success rates of surgeons and satisfaction of patients [21, 30, 37].

Although the exact percentage of people that will develop metal sensitivity responses to their implant that results in early implant failure is unknown, it is clear some people experience excessive immune reactions to the metals released from implanted metallic materials [12, 81, 83, 84, 86, 94]. Metal sensitivity testing is currently the only form of testing in those individuals that are highly susceptible to excessive metal-induced immune responses (i.e. purportedly about 1 % of joint replacement recipients) [1] . Of the different forms of metal sensitivity testing, LTT may provide greater sensitivity relative to patch testing but larger clinical outcome studies that are needed to validate the sensitivity and specificity of patch testing or LTT (i.e. a clinically identifiable pathology), are still in progress [2, 22, 137]. Because there are different methods for conducting metal sensitivity testing and testing is a highly complex immune test, it is very important that any testing facility be both certified (by US law through the Clinical Laboratory Improvement Amendments (CLIA) agency, administered by the FDA), and is able to fully disclose all testing parameters to physicians, researchers and the general public. Physicians ordering this testing should be familiar with criteria such as (1) test conditions, including challenge agents (soluble and particulate), culture medium, time of incubation, etc, (2) method of proliferation detection, (3) whether autologous serum is used for culturing or if AB pooled serum is used to supplement human cell cultures, (4) if there is statistical

assessment or an acceptable level of redundancy, e.g. triplicate, duplicate, etc., (5) the pharmalogical profile of the patient at the time of testing and (6) if there is strict adherence to all patient privacy and Health Insurance Portability and Accountability Act regulations, required by law. Given that $< 1\,\%$ of the over 1 million people receiving total joint replacement implants in the USA annually are metal sensitivity tested pre-op or at revision, it is likely that implant-related metal sensitivity has been underreported [1, 3]. However, the slow and continuing improvements in sensitivity testing technology and availability will likely continue to provide accumulative clinical evidence into the utility of metal sensitivity testing along with more basic understanding into how and when metal sensitivity develops.

Recent results show that patients receiving implants who are diagnosed preoperatively by metal sensitivity testing have better outcomes than those for whom the results of sensitivity testing is not accommodated by altered surgical procedure [37]. More studies like this are needed to build a consensus and confirm the clinical utility of pre-op and/or post-op LTT, by demonstrating those tested have better outcomes when actions are taken to avoid the respective immunogenic metals compared to people tested who receive no evasive action. As these reports build scientific consensus, there is an increasing need to factor in the phenomenon of metal sensitivity and many surgeons now take this into account when planning which implant is optimal for each patient. Optimizing implant and material selection that is tailored to the immune reactivity profiles of each individual based on their genetic and environment history is paramount, as greater than 1 in 4 older Americans will eventually require a joint replacement implant [73, 138, 139] and early poor performance and revision surgery with a patient over the age of 75 can result in rates of mortality $> 10\,\%$ [140, 141]. Appropriate pre-operative testing that can extend implant performance in some cases is literally a matter of life and death and could decrease overall health costs.

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