

Palladium-based dental alloys are associated with oral disease and palladium-induced immune responses

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Summary

Background. Palladium (Pd) and gold (Au) based dental alloys have been associated with oral disease.

Objectives. This study was designed to explore possible associations between the presence of Au-based and Pd-based dental alloys, and oral lesions, systemic complaints, and specific *in vivo* and *in vitro* immune responses.

Methods. The investigated population consisted of three groups: 26 non-metal-allergic volunteers, 25 metal-allergic patients, and 20 oral disease patients. Medical histories were taken, oral examinations were carried out, and compositions of all dental alloys were determined. Then, Au and Pd patch tests and *in vitro* assays were performed, revealing cytokine production by peripheral blood mononuclear cells [T helper (Th)1, interferon- γ ; Th2, interleukin (IL)-5 and IL-13] and lymphocyte proliferation (LTT-MELISA[®]).

Results. Non-plaque-related gingivitis was associated with the presence of Pd-based dental alloys, and Pd-positive patch tests and *in vitro* assays. Collectively, participants with Pd-based dental alloys showed increased Pd patch test reactivity ($p < 0.05$) and lymphoproliferation ($p < 0.05$). In contrast, oral lichenoid lesions were associated with Au-based alloys ($p < 0.05$), but this was not reflected by Au-specific immunoreactivity.

Conclusions. Oral lesions and Pd-induced immune responses are associated with the presence of dental alloys. However, most oral disease patients did not show positive patch test results or *in vitro* signs of specific immunoreactivity, suggesting local toxic reactions or the involvement of innate immune responses.

Key words: clinical immunology; dental alloys; gold; oral disease; palladium; Th1; Th2.

Both gold (Au) and palladium (Pd) are well-known contact sensitizers, and are primarily associated with dental cast alloys and oral disease, such as oral lichenoid lesions and gingivitis (1, 2). For Au, a positive correlation was found between the number of dental Au restorations

and positive patch test results (3). One study reported that Pd monosensitization was only found in patients with oral lesions and Pd-containing dental alloys, whereas allergic contact dermatitis (ACD) patients frequently (70–90%) show concomitant reactivity to both nickel and Pd (4). In fact, cross-reactivity between the two metals has been shown both *in vivo* and *in vitro* (5, 6). It is of note that the prevalence of Pd allergy was underestimated until the introduction of a more sensitive test allergen, namely sodium tetrachloropalladate (Na_2PdCl_4) (7). Although *in vitro* immune assays to replace *in vivo* patch testing for diagnosing metal allergies are desirable, to date none has been found satisfactory, most probably because of

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the complex laboratory procedures involved and gaps in our knowledge of the exact pathogenesis. Contact allergy to nickel has historically been considered to be a T helper (Th)1-mediated immune response, but convincing evidence is accumulating suggesting mixed Th1 and Th2 responses (8–10). For Au and Pd, such evidence is scarce (11, 12).

Clinically, both Au and Pd allergies are mainly associated with oral diseases, such as lichenoid lesions and gingivitis (2, 13–15). Oral and systemic subjective complaints have also been described, albeit attributed to dental alloys in general (16). Oral complaints reportedly comprise burning mouth, dry mouth, and metal taste, whereas possibly related systemic complaints may comprise fatigue and joint and muscle pain (17). Furthermore, an association between urine Pd concentrations and thyroid gland pathology has been reported (18).

A prerequisite for adverse reactions to dental alloys is the dissolution of metal ions, mostly resulting from corrosion processes. As the corrosion properties of dental alloys depend on their composition and metallurgical structure, metal ion release may vary significantly between different alloys. Therefore, some Pd-based alloys are more prone to corrosion than others, depending on the alloying components, such as silver (Ag), copper (Cu), and Au (19). In addition, laboratory casting procedures and porcelain firing may alter the corrosion behaviour of dental alloys (20). Pd–Cu and Ag-free Pd alloys are especially associated with Pd ion release and possible subsequent adverse reactions (21).

The current study was designed to explore possible associations between the presence of various Au-based and Pd-based dental alloys and oral lesions, systemic complaints, and both Au-induced and Pd-induced immunoreactivity, as detected with *in vivo* patch testing and *in vitro* immune responses, in three well-defined patient groups. This *in vivo* study is unique, as clinical features of adverse reactions and metal-induced immunological responses are associated with specific dental cast alloys.

Materials and Methods

The investigated population consisted of three groups: (i) a group of non-metal-allergic volunteers (non-allergic); (ii) a group of patients with a history of metal-induced ACD (metal-ACD); and (iii) a group of patients with oral lesions attributable to their dental restorations (oral disease). For all participants, a medical history was taken, oral examinations were carried out, and compositions of all dental alloys were determined. Then, Au and Pd patch tests and LTT-MELISA[®] (a commercially available lymphocyte proliferation test) were performed. Metal-induced immunoreactivity was measured, in particular cytokine production

by peripheral blood mononuclear cells (PBMCs) [Th1, interferon (IFN)- γ ; Th2, interleukin (IL)-5 and IL-13].

Volunteers and patients

The participants in the non-allergic group ($n = 26$) were recruited from personnel within the local university campus on the basis of a strictly negative history of metal allergies. Participants in the metal-ACD group ($n = 25$) were selected from patients attending the Skin Allergy Unit of the Department of Dermatology from the VU University Medical Centre in Amsterdam, by taking appropriate medical histories. Patients were included if they gave a positive answer when asked whether they had ever suffered from itching, redness and/or oozing and crusting as a result of direct prolonged skin contact with jewellery or metal objects and/or as a result of wearing inexpensive, base metal-containing piercings/earrings. The oral disease group ($n = 20$) consisted of patients referred to the adverse reaction unit of the Academic Centre for Dentistry Amsterdam with oral lesions attributed to their Pd-containing and/or Au-containing dental crowns or bridges. The study was approved by the medical ethical committee of the VU University Medical Centre and the research institute of the Academic Centre for Dentistry Amsterdam. All participants gave written informed consent.

Dental alloys

Samples of all metal-based dental appliances of all participants were taken to determine their composition with scanning electron microscopy–energy-dispersive X-ray spectroscopy (SEM-EDX), a method that is used for qualitative and quantitative assessment of the composition of alloys (22). Dental alloys were first categorized as Au-based and Pd-based alloys. Au-based alloys consist of at least 45 atom percentage (At%) Au, and not more than 20 At% Pd. Subsequently, three subgroups were defined. The first subgroup is free of Pd (Au–Pd^{free}) (Au, 63–93 At%), the second contains small amounts of Pd (Au–Pd^{low}) (Au, 70–83 At%; Pd, 1–10 At%), and the third contains some Pd but less Au (Au–Pd^{med}) (Au, 45–64 At%; Pd, 2–20 At%). The Pd-based alloys contain 30–90 At% Pd, and are alloyed mainly with Au (< 45 At%), Cu (15–30 At%), or Ag (15–55 At%), or with miscellaneous metals, but contain high amounts of Pd (> 75% At%), and are referred to as Pd–Ag, Pd–Cu, Pd–Au^{med}, and Pd–Misc., respectively.

Oral lesions

The presence of oral lesions was investigated by the same clinician (J.M.), on the basis of an oral examination, and

categorized as: (i) none; (ii) oral lichen planus or oral lichenoid lesions (OLP/OLL); and (iii) gingival inflammation attributed to metal contact and not to bacterial plaque [non-plaque related gingivitis (NPG)]. Concordant oral lesions were also recorded. The putative relationship between oral lesions and exposure to dental crowns was investigated within the whole population ($n = 71$).

Systemic complaints

The medical history was taken by the same clinician (J.M.). The choice of what systemic complaints to investigate was based on earlier studies by Vamnes and Helm (17, 18): (a) thyroid gland pathology; (b) chronic fatigue; (c) joint and muscle pain; and (d) chronic sinusitis. Chronic fatigue was subjectively considered to be chronic in terms of the patients being abnormally often tired for a long period of time without obvious reasons. Owing to the relatively small sample sizes, no conclusive data were to be expected regarding possible associations between these complaints and exposure to distinct dental restorations, but trends might become visible.

Patch testing and immunological parameters

Patch testing was performed with Au [2.0% AuNa₃(S₂O₃)₂·2H₂O in pet.] (Hermal, Hamburg, Germany) and with Pd (3.0% Na₂PdCl₄ in pet.) (Sigma-Aldrich Chemie BV, Zwijndrecht, The Netherlands). Van der Bend® patch test chambers (Van der Bend BV, Brielle, The Netherlands) on Fixomull® tape were used. Patches were removed on day 2, and readings were performed on days 2 and 3 (48 and 72 hr), according to the recommendations of the International Contact Dermatitis Research Group. Additional readings were performed after 1 week (168 hr). Patch tests were regarded as positive if, at least at one time point, a positive reaction (+, ++, or +++) to the test salt was recorded. The strongest reactions were used for further analyses. Doubtful reactions, including follicular and erythematous reactions, were recorded as (+/-) but regarded as negative test results.

Furthermore, a commercial lymphocyte proliferation test, LTT-MELISA® (MEemory Lymphocyte Immuno Stimulation Assay), for Au and Pd was carried out. LTT-MELISA® is a patented modification of the lymphocyte transformation test or lymphocyte proliferation test with a standardized (23, 24) and validated (25) protocol. Blood samples, that is, 30 ml of heparinized blood per patient, were transported by private courier to arrive at the test laboratory (Department of Immunology, Laboratory Centre Bremen, Germany) within 48 hr after the blood was drawn. Metal-specific proliferation was assessed according to the stimulation index (SI)

(ratio of mean [³H]thymidine uptake in stimulated/non-stimulated cultures). Test results were considered to be positive when the SI was > 5 as defined by the LTT-MELISA® laboratory. Pokeweed mitogen and non-stimulated (Blanco's) wells were used as positive and negative controls, respectively.

Finally, to measure Au-induced and Pd-induced IFN- γ (Th1) IL-5 (Th2) and IL-13 (Th2) production, 90 ml of peripheral heparinized blood was taken directly before patch testing and subsequently transported to the laboratory within 2 hr. Culture media were supplemented with IL-12/IL-7 (0.1/0.03 ng/ml) for IFN- γ production, and with IL-4/IL-7 (3.0/0.1 ng/ml) for IL-5 and IL-13 production, as described previously (10, 26, 27). PBMCs in the three culture conditions were stimulated with or without maximum non-toxic doses and threefold dilutions of metal salts, that is, 0, 30 and 90 μ g/ml Na₂(PdCl₄) and 0, 3 and 9 μ g/ml AuNa₃(S₂O₃)₂·2H₂O, or with pokeweed mitogen as a positive control. Metal-induced cytokine increments (Δ) were assessed by subtracting the cytokine concentration of non-stimulated culture supernatants from that of stimulated culture supernatants. For practical reasons, Δ pg/ml < 1 was set as 1. Cut-off values for Au-induced and Pd-induced cytokine responses were defined as the mean cytokine increment (Δ pg/ml) plus three standard deviations (SDs) of the non-allergic patient group (control group). Note that, in the non-allergic group, 2 volunteers showed, despite their negative history of contact allergy to metals, positive patch test reactions to Pd, and were therefore excluded from the calculation of the cut-off values. For Au-induced immune responses, no positive patch test results were found. However, a few considerable outliers were found. Therefore, the highest ($n = 1$) Au cytokine response for each variable (IL-5, IL-13, and IFN- γ) was excluded from the calculation of the cut-off value.

Blinding of the data

In order to prevent the results of *in vitro* analyses being affected by knowledge of the results of *in vivo* analyses, the data were collected separately. In other words, investigators dealing with the *in vitro* assays were blinded to the patch test results and vice versa. To achieve this, all data were collected by two independent investigators (see Acknowledgements).

Statistics

IBM® SPSS® (IBM® SPSS® Inc. SPAW Statistics, New York, USA, version 21.0) was used for statistical analyses. Differences in the distributions of *in vitro* test results were analysed for statistical significance ($p < 0.05$) with a

two-sided Fisher's exact test. *p*-Values of < 0.05 were considered to be statistically significant. When *p*-values of < 0.05 were found, odds ratios were calculated, including 95% confidence intervals, to convey information about the magnitude and precision of the associations.

Results

Comparison of patient groups

First, the clinical presentation of complaints and specific immune responses were evaluated for the three selected

Table 1. Summary of data according to patient group allocation: exposure to gold (Au)-based and palladium (Pd)-based dental alloys, positive Au and Pd patch test results, positive Au and Pd *in vitro* assay results, oral lesions, and systemic complaints

	Non-allergic, % (no.) n = 26	Metal-ACD, % (no.) n = 25	Oral disease, % (no.) n = 20
Metal allergy history, n = 71	0 (0)	100 (25)	50 (10)
Oral lesions, n = 71	12 (3)	28 (7)	100 (20)
Oral lichen planus/oral lichenoid lesions	0 (0)	0 (0)	50 (10)
Non-plaque-related gingivitis	12 (3)	28 (7)	65 (13)
Age and sex correction, n = 39 (note 1)	(5)	(16)	(18)
Systemic complaints	40 (2)	50 (8)	72 (13)
Thyroid gland pathology	40 (2)	6 (1)	11 (2)
Chronic fatigue	0 (0)	13 (2)	22 (4)
Joint and muscle pain	0 (0)	50 (8)	50 (9)
Chronic sinusitis	0 (0)	6 (1)	11 (2)
Correction for age, n = 49 (note 2)	(12)	(17)	(20)
Exposure to Au or Pd crowns	75 (9)	88 (15)	100 (20)
Patients with Au-based crowns (≥ 1)	67 (8)	59 (10)	70 (14)
Patients with Pd-based crowns (≥ 1)	50 (6)	71 (12)	75 (15)
Au-induced immune responses, n = 71	(26)	(25)	(20)
Positive patch test results (+, ++, +++)	0 (0)	0 (0)	0 (0)
Positive LTT-MELISA [®] result (SI > 5)	0 (0)	4 (1)	0 (0)
Positive IL-5 production, i.e. > Mean increment + 3SD of controls, i.e. $3.4 + 3 \times 6.2 = 22.1 \Delta\text{pg/ml}$	8 (2)	28 (7)	25 (5)
Positive IL-13 production, i.e. > Mean increment + 3SD of controls, i.e. $1.3 + 3 \times 0.7 = 3.4 \Delta\text{pg/ml}$	8 (2)	24 (6)	15 (3)
Positive IFN- γ production, i.e. > Mean increment + 3SD of controls, i.e. $21.9 + 3 \times 42.1 = 148.1 \Delta\text{pg/ml}$	8 (2)	12 (3)	20 (4)
Pd-induced immune responses, n = 71	(26)	(25)	(20)
Positive patch test results (+, ++, +++)	8 (2)*,†	68 (17)*,‡	35 (7)*,‡
Positive LTT-MELISA [®] result (SI > 5)	4 (1)*	32 (8)*	20 (4)
Positive IL-5 production, i.e. > Mean increment + 3SD of controls, i.e. $4.31 + 3 \times 7.28 = 26.2 \Delta\text{pg/ml}$	4 (1)*	52 (13)*,‡	10 (2)*
Positive IL-13 production, i.e. > Mean increment + 3SD of controls, i.e. $1.42 + 3 \times 1.11 = 4.8 \Delta\text{pg/ml}$	8 (2)*	56 (14)*,‡	25 (5)*
Positive IFN- γ production, i.e. > Mean increment + 3SD of controls, i.e. $115.15 + 3 \times 233.83 = 816.6 \Delta\text{pg/ml}$	8 (2)	8 (2)	15 (3)

ACD, allergic contact dermatitis; IFN, interferon; IL, interleukin; SD, standard deviation; SI, stimulation index.

Note 1: Systemic complaints increase with age, and are thought to be related to female sex. Therefore, to compare the groups for systemic complaints, all male participants and patients aged < 39 years were excluded, to obtain groups that were equally distributed for age and sex.

Note 2: The number of dental restorations obviously increases with age. Therefore, to compare the groups for dental restorations, all patients aged < 39-years were excluded, to obtain groups that were equally distributed for age.

Grey areas represent selection criteria. Statistically significant differences are given ($p < 0.05$; two-sided Fisher's exact tests).

*Significant difference ($p < 0.05$) between the non-allergic and metal-ACD groups.

†Significant difference ($p < 0.05$) between the non-allergic and oral disease groups.

‡Significant difference ($p < 0.05$) between the metal-ACD and oral disease groups.

patient groups: non-allergic, metal-ACD, and oral disease (Table 1). Although the patients from the oral disease group were suspected of having adverse reactions to dental alloys, only half (10/20) of them reported a history of metal allergy. Evidently, oral lesions were most frequent in the oral disease group, and only NPG was found among all patient groups. Systemic complaints were not limited to the oral disease group, as similar frequencies were observed in healthy and metal-allergic patients. As systemic complaints notoriously increase with age, and are thought to be associated with female sex, the groups were corrected for both parameters. When homogeneous groups were compared by focusing on female participants aged > 38 years, systemic complaints were not associated with a particular patient group. Nevertheless, a trend was observed for an increased frequency of joint and muscle pain in both the metal-ACD and oral disease groups.

The presence of dental crowns obviously increases with age, but it is less likely that sex will play a role in the presence of dental restorations. Therefore, to compare exposure data in terms of the presence of dental alloys between the patient groups, we chose to correct for age only, that is, inclusion of participants aged > 38 years. Pd-based alloys were found more frequently in the oral disease group than in the non-allergic group, although this difference was not significant.

Regarding immune responsiveness, no positive patch test reactions to Au were observed; however, *in vitro* immune responses were found in all three patient groups. As expected, more positive Pd (17/25) patch test results were obtained in the metal-ACD group than in either the non-allergic group or the oral disease group ($p < 0.05$). As expected, oral disease patients also more frequently had positive patch test reactions to Pd (7/20) than non-allergic individuals (2/26) ($p < 0.05$), as 50% (10/20) reported contact allergy to metals. Moreover, 2 of the 10 oral disease patients without a history of metal allergy also reacted positively in Pd patch testing. Pd-induced LTT-MELISA[®] responses supported the patch test results,

although they were less frequent in all groups, and significance was only reached between the non-allergic and metal-ACD groups ($p = 0.011$). Essentially similar information was obtained with other *in vitro* Pd tests. In general, it can be concluded that, for Pd, the *in vitro* responses followed the patch test results, although positive results were obtained less often (Table 2).

Comparison according to exposure

Next, all patients were regrouped according to the presence of distinct dental alloys (see Materials and methods), regardless of the original group allocation. Au-based and Pd-based alloys were scored positive when at least one dental crown was present.

Associations between the presence of specific dental alloys and both local lesions (Table 3) and systemic complaints (Table 4) were investigated. Table 3 shows that OLP/OLL was associated with the presence of Au-based dental alloys or Pd-based alloys with considerable amounts of Au (Pd–Au^{med}), whereas NPG was clearly associated with the presence of Pd-based dental alloys. Interestingly, the presence of Au-based alloys with considerable amounts of Pd (Au–Pd^{med}) was also associated with NGP. Correction for age was also carried out for the systemic complaints (Table 4). Note that no sex correction was performed, as the number and type of dental alloys are assumed not to be sex-related. It was found that Pd–Cu alloys, in particular, were associated with systemic complaints (chronic fatigue and chronic sinusitis). For Au-based alloys, no associations were found (data not shown).

Immune responses

Both *in vivo* and *in vitro* immune response to Au and Pd were investigated. For these analyses, the metal-ACD group was excluded, as immune responsiveness might be based on patch sensitization rather than on mucosal exposure. Note that statistical analyses for systemic complaints were corrected for age. The results are summarized in Table 5. In particular, Pd–Ag and, to some extent,

Table 2. Chi-square tests for positive palladium (Pd) test results *in vivo* (patch test) versus *in vitro* [LTT-MELISA[®] (cut-off stimulation index of > 5), and Pd-induced interleukin (IL)-5 production (cut-off 26.2 Δpg/ml), IL-13 production (cut-off 4.8 Δpg/ml), and interferon (IFN)-γ production (cut-off 816.6 Δpg/ml)]. It can be concluded that, although positive patch test results outnumbered positive *in vitro* results, the latter followed the patch test results to a large extent

Patients from all groups n = 71	Pd-induced LTT-MELISA [®] -positive n = 13	Pd-induced IL-5-positive n = 16	Pd-induced IL-13-positive n = 21	Pd-induced IFN-γ-positive n = 7
Pd patch test-positive, n = 26	n = 13/13 $p < 0.001$	n = 13/16 $p < 0.001$	n = 16/21 $p < 0.001$	n = 6/7 $p = 0.008$

Table 3. Associations between the presence (≥ 1) of gold (Au)-based and palladium (Pd)-based dental crowns and local lesions in all patients, regardless of the original group allocation (non-allergic, metal-ACD, and oral disease)

Patients from all groups n = 71	OLP/OLL n = 10	NPG n = 23
Au-based dental alloys (≥ 1)		
Au-Pd ^{free} , n = 18	p = 0.002 (n = 7)	p = 0.249 (n = 8)
OR	10.6	–
95% CI	2.4–47.6	–
Au-Pd ^{low} , n = 20	p = 0.025 (n = 6)	p = 0.055 (n = 10)
OR	5.0	2.9
95% CI	1.2–20.4	1.0–8.6
Au-Pd ^{med} , n = 17	p = 0.433 (n = 1)	p = 0.015 (n = 10)
OR	–	4.5
95% CI	–	1.4–14.2
Pd-based dental alloys (≥ 1)		
Pd-Ag, n = 17	p = 0.694 (n = 3)	p < 0.001 (n = 13)
OR	–	14.3
95% CI	–	3.8–53.2
Pd-Cu, n = 16	p = 0.684 (n = 3)	p < 0.001 (n = 12)
OR	–	12.0
95% CI	–	3.2–44.5
Pd-Au ^{med} , n = 5	p = 0.018 (n = 3)	p = 1.000 (n = 1)
OR	12.6	–
95% CI	1.8–89.2	–
Pd-Misc., n = 13	p = 1.000 (n = 2)	p = 0.021 (n = 8)
OR	–	4.6
95% CI	–	1.3–16.2

ACD, allergic contact dermatitis; CI, confidence interval; OLP/OLL, oral lichen planus or oral lichenoid lesions; OR, odds ratio.

Patients with a specific type of dental alloy (≥ 1) are compared with patients without that specific type of dental alloy. Au-based subtypes are: (1) Au-based but free from Pd (Au-Pd^{free}) (Au, 63–93 At%); (2) Au-based but with small amounts of Pd (Au-Pd^{low}) (Au, 70–83 At%; Pd, 1–10 At%); and (3) Au-based with some Pd but less Au (Au-Pd^{med}) (Au, 45–64 At%; Pd, 2–20 At%). Pd-based subtypes were defined on the basis of their alloying metal, that is: (1) Pd-Ag (Ag, 15–55 At%); (2) Pd-Cu (Cu, 15–30 At%); (3) Pd-Au^{med} (Au, < 45 At%); and (4) Pd-Misc. with miscellaneous metals (Pd, > 75% At%). Statistical analyses to calculate *p*-values were performed with two-sided Fisher exact tests, and ORs were calculated including 95% CIs where appropriate. Significance was set at *p* < 0.05 (bold type).

Pd-Cu alloys were associated with Pd-induced immune responses both *in vivo* and *in vitro* (IL-5 and IL-13). It is of note that significant associations between the the presence of Pd-Ag dental alloys and most Pd-induced immune responses were also found when the metal-ACD group was included in analyses (data not shown). OLP/OLL was not associated with any of the investigated immune responses, whereas NPG was strongly associated with both Pd skin testing and Pd-induced *in vitro* assays.

Among the systemic complaints, only for joint and muscle pain were multiple associations with Pd-induced immune responses found (Table 5).

Discussion

Unexpectedly, no positive patch test reactions to Au were found, whereas Au allergy has been suggested to be frequent (approximately 15–20%) in patients with oral disease (2, 28), especially in association with OLP/OLL (13, 15). However, OLP/OLL was clearly associated with exposure to Au (Table 3), which is in line with the findings of Svedman et al. (29). Apparently, mechanisms other than classic Au-induced T cell reactivity are involved in the pathogenesis of OLP/OLL. This point of view is supported by the finding that OLP/OLL frequently diminishes after the removal of dental alloys also in the absence of allergy (30).

On the basis of the metal-allergy history, more patch test reactions to Pd were expected in oral disease patients (7/20) than in non-allergic controls (2/26) (*p* = 0.029). However, in the oral disease group, 29% (2/7) of the Pd-allergic patients did not report ACD caused by metals and were monosensitized to Pd [in the context of nickel (Ni) and Pd cross-reactivity]. In addition, significantly more Pd-monosensitized patients were found in the oral disease group (4/7) than in the metal-ACD group (1/17) (*p* = 0.014) (data not shown). These results are in line with the findings of Cristaudo et al. (4). Possibly, Pd exposure (mainly oral) may induce Pd-specific clones, whereas Ni exposure (mainly skin) may favour the development of Ni-specific clones that cross-react with Pd.

In general, receiver operating characteristic (ROC) analyses are used to calculate cut-off values for diagnostic tests. For Pd, these analyses were carried out in an earlier study with the same patient groups (non-allergic and metal-ACD) (12). In that study, positive controls (*n* = 16 for Pd) were defined more strictly and based on a history of metal-ACD in combination with positive Pd patch test results in at least two of three readings. Negative controls (*n* = 21) had a negative metal allergic anamnesis and did not show any reactivity to Pd (including doubtful, follicular, and irritant reactions) at one of the readings. For Pd, those cut-off values were essentially similar to those obtained in the current study (mean + 3SD of the non-allergic volunteers), and both methods resulted in the same associations concerning Pd-induced IL-5 and IL-13 production. It is of note that, with the ROC analysis cut-offs, associations were generally even more pronounced. In the earlier study, Ni was also investigated for comparison with Pd, because, in contrast to Pd, Ni is a well-investigated allergen in terms of immune

responses. The Ni *in vitro* assays performed similarly, although a greater spread of the data was observed, probably because of the inevitable daily exposure to Ni (12). The non-parametric Spearman correlation between Ni and Pd was as follows: $\rho = 0.735$ ($p < 0.001$; two-tailed) and $\rho = 0.731$ ($p < 0.001$) for IL-5 and IL-13, respectively. Detailed information on these patients is available in the online repository of that article. For Au, ROC analyses were not possible, owing to the lack of positive patch test results. Therefore, to enable the use of a consistent methodology for both Au and Pd in this study, the mean + 3SD in the non-allergic group was used to determine cut-off values for all *in vitro* assays.

Only few patients had positive test results for Pd-induced IFN- γ production, even among those with positive patch test results. This is attributable to the high cut-off value resulting from the mean + 3SD method. Indeed, with the ROC analyses, a much lower cut-off value was obtained (> 9.0 versus $> 816.6 \Delta\text{pg/ml}$). This is obviously attributable to the large spread in results in the non-allergic group. Interestingly, the association for IFN- γ found in this study could not be calculated with the cut-off based on ROC analyses. The role of IFN- γ in metal-induced ACD has been a matter of debate for almost two decades (8, 9, 11, 31). Perhaps IFN- γ plays a role during the sensitization phase or with high-dose exposures, whereas Th2 cytokines such as IL-4, IL-5 and IL-13 are more important during the elicitation phase and in (low-dose) chronic disease. Either way, IFN- γ was found to be a poor predictor for Ni and Pd allergy. However, the few results showing high levels of Pd-induced IFN- γ production were found in patients with Pd–Ag dental alloys, Pd–Cu dental alloys, NPG, chronic fatigue, and joint and muscle pain.

Interestingly, despite the absence of positive Au patch test results, several positive Au-induced *in vitro* immune

responses were found, suggesting better sensitivity for these *in vitro* assays, as most of them were found in the metal-ACD and oral disease groups (Table 1). However, for Pd, the opposite conclusion may be drawn, as positive Pd patch test results outnumbered the positive *in vitro* test results. However, all positive Pd *in vitro* assay results were clearly associated with Pd patch testing (Table 2), and the presence of NPG and Pd–Ag dental alloys (Table 5). Therefore, these *in vitro* assays could be of diagnostic value in patients for whom patch testing is contraindicated or for metal allergens that lack sensitivity in patch tests.

A clear association was found between exposure to Pd-based dental alloys and the presence of NPG (Table 3). Although still significant, the association between NPG and positive Pd patch test results was less obvious ($p = 0.047$; Table 5). In fact, only 38% (6/16; metal-ACD group excluded) of the patients with NPG showed positive Pd patch test results (Table 5). Possibly, this relates to a recent finding that not only Ni (32) but also Pd is able to activate Toll-like receptor 4, with subsequent non-specific inflammatory innate immune responses (33). From that perspective, NPG should be interpreted as a sign of exposure, rather than as a sign of allergy.

Pd–Cu and Pd–Ag alloys were found to be especially related to NPG (Table 4) and sensitization to Pd (Table 5). Pd–Cu alloys are known to leach considerable amounts of metal ions, including Pd, explaining the associations with immune responses and adverse reactions. Although Pd–Ag alloys are believed to be more safe in this respect (20, 21, 34, 35) our *in vivo* results do not support these *in vitro* findings.

Finally, as there is growing concern about metal-induced autoimmune diseases (36), systemic complaints,

Table 4. Associations between the presence of palladium (≥ 1) (Pd)-based dental alloys and systemic complaints in all patients regardless of the original group allocation (non-allergic, metal-ACD, and oral disease), but corrected for age (patients aged < 39 years excluded)

Patients from all groups (corrected for age) n = 49	Thyroid gland pathology n = 3	Chronic fatigue n = 7	Joint and muscle pain n = 18	Chronic sinusitis n = 5
Pd-based dental alloys (≥ 1)				
Pd–Ag, n = 17	$p = 0.542$ (n = 0)	$p = 0.397$ (n = 1)	$p = 0.122$ (n = 9)	$p = 1.000$ (n = 2)
Pd–Cu, n = 16	$p = 0.245$ (n = 2)	$p = 0.030$ (n = 5)	$p = 1.000$ (n = 6)	$p = 0.034$ (n = 4)
OR	–	7.0	–	10.1
95% CI	–	1.2–41.7	–	1.1–105.3
Pd–Au ^{med} , n = 5	$p = 1.000$ (n = 0)	$p = 1.000$ (n = 0)	$p = 0.342$ (n = 3)	$p = 0.430$ (n = 1)
Pd–Misc., n = 13	$p = 0.555$ (n = 0)	$p = 1.000$ (n = 2)	$p = 0.508$ (n = 6)	$p = 1.000$ (n = 1)

ACD, allergic contact dermatitis; CI, confidence interval; OR, odds ratio.

Patients with a specific type of dental alloy (≥ 1) are compared with patients without that specific type of dental alloy. Pd-based subtypes were defined on the basis of alloying metal, that is: (1) Pd–Ag (Ag, 15–55 At%); (2) Pd–Cu (Cu, 15–30 At%); (3) Pd–Au^{med} (Au, < 45 At%); and (4) Pd–Misc. with miscellaneous metals (Pd, $> 75\%$ At%). Calculations for systemic complaints were corrected for age. Statistical analyses to calculate p -values were performed with two-sided Fisher exact tests, and ORs were calculated including 95% CI(s) where appropriate. Significance was set at $p < 0.05$ (bold type).

Table 5. Associations between the presence (≥ 1) of palladium (Pd)-based dental alloys, the presence of oral lesions or systemic complaints and Pd-specific immunoreactivity within the non-allergic and oral disease groups

Non-allergic and oral disease n = 46	Positive* Pd patch test result n = 9	Positive [†] Pd LTT-MELISA [®] result n = 5	Positive [‡] Pd-induced IL-5 result n = 3	Positive [‡] Pd-induced IL-13 result n = 7	Positive [‡] Pd-induced IFN- γ result n = 5
Pd-Ag, n = 10	p = 0.015 /n = 5	p = 0.006 /n = 4	p = 0.008 /n = 3	p = 0.031 /n = 4	p = 0.061/n = 5
OR	8.0	23.3	NA	7.3	7.3
95% CI	1.6–40.3	2.2–246.2		1.3–41.4	1.0–52.0
Pd-Cu, n = 10	p = 0.087/n = 4	p = 0.006 /n = 4	–	–	p = 0.061/n = 5
OR	4.1	23.3			7.3
95% CI	0.9–20.0	2.2–246.2			1.0–52.0
Pd-Au ^{med} , n = 4	–	–	–	–	–
OR					
95% CI					
Pd-Misc., n = 9	–	p = 0.044 /n = 3	–	–	–
OR		8.8			
95% CI		1.2–63.9			
OLP/OLL, n = 10	–	–	–	–	–
NPG, n = 16	p = 0.047 /n = 6	p = 0.043 /n = 4	p = 0.037 /n = 3	p = 0.040 /n = 5	p = 0.043 /n = 4
OR	5.4	9.7	NA	6.4	9.7
95% CI	1.1–25.8	1.0–95.7		1.1–37.8	1.0–95.7
Age-corrected (exclusion of patients aged < 39 years) [§]					
Non-allergic and oral disease n = 32	Positive* Pd patch test result n = 8	Positive [†] Pd LTT-MELISA [®] result n = 5	Positive [‡] Pd-induced IL-5 result n = 3	Positive [‡] Pd-induced IL-13 result n = 7	Positive [‡] Pd-induced IFN- γ result n = 4
Thyroid gland pathology, n = 2	–	–	–	p = 0.042 /n = 2	–
OR				NA	
95% CI					
Chronic fatigue, n = 5	–	–	–	–	p = 0.105/n = 2
OR					OR = 8.3
95% CI					95% CI 0.8–82.9
Joint and muscle pain, n = 10	p = 0.005 /n = 6	p = 0.024 /n = 4	–	–	p = 0.079/n = 3
OR	OR = 15	OR = 14.0			OR = 9.0
95% CI	95% CI 2.2–103.0	95% CI 1.3–150.0			95% CI 0.8–101.2
Chronic sinusitis, n = 4	–	–	–	–	
OR					
95% CI					

CI, confidence interval; IFN, interferon; IL, interleukin; NPG, non-plaque-related gingivitis; OLP/OLL, oral lichen planus or oral lichenoid lesions; OR, odds ratio.

For clarity only the significant (bold) and nearly significant associations are presented. Pd exposure was subdivided for distinct alloy subtypes: (1) Pd–Ag (Ag, 15–55 At%); (2) Pd–Cu (Cu, 15–30 At%); (3) Pd–Au^{med} (Au, < 45 At%); and (4) Pd–Misc. with miscellaneous metals (Pd, > 75% At%). Calculations for systemic complaints are corrected for age, that is, patients aged < 39 years were excluded (lower part of the table). Statistical analyses to calculate *p*-values were performed with two-sided Fisher exact tests, and ORs were calculated including 95% CIs where appropriate. Significance was set at *p* < 0.05.

NA, not available, owing to empty cell in the 2 × 2 table.

*Patch test is considered to be positive when patch reactivity is marked as +, ++, or +++.

[†]LTT-MELISA[®] is considered to be positive when the stimulation index is > 5.

[‡]Cut-off values are defined as mean + three standard deviations of the non-allergic group (Table 1).

[§]Corrected for age (age < 39 years excluded); only performed for calculations of associations concerning systemic complaints.

such as joint and muscle pain and chronic fatigue, which may be associated with autoimmune disease, were also investigated. Within the clear limitations of this study, such as small sample sizes and the determination of complaints by interview, the results provide a direction for further research. Exposure to Pd–Cu alloys was associated

with chronic fatigue (Table 4). Interestingly, joint and muscle pain was clearly associated with positive Pd patch test results and positive LTT-MELISA[®] results, and a trend was found for IFN- γ production. Of course, larger studies are required to verify whether the latter results are coincidental or not.

Conclusions

The results of the present study show that oral lesions associated with dental alloys may result from concerted innate and T cell-mediated immune reactivity. OLP/OLL was strongly associated with Au exposure, and NPG was closely related to Pd exposure. Interestingly, OLP/OLL was not related to Au-induced immune responses, whereas NPG was confirmed by positive Pd patch test results and Pd-induced lymphoproliferation, IL-5 and IL-13 production, albeit in only ~40% of the cases. However, oral lesions in the absence of T cell-mediated allergy are not harmless, and need further attention. For example, constant triggering of the innate immune system as a result of protracted Pd exposure may augment destructive inflammatory processes and may enhance sensitization to other

(self)-antigens. It is clear that mechanisms other than classic T cell-mediated allergy may play an important role in the development of adverse reactions to dental materials. Finally, patch testing remains the test of choice for diagnosing oral disease resulting from Pd allergy. However, in particular cases, Au and Pd *in vitro* sensitization assays and, in particular, LTT-MELISA® may be helpful.

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