

The existence of specific antibodies to cobalt in hard metal asthma

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Summary

Twelve workers with hard metal asthma diagnosed on the basis of peak flow diaries and positive bronchial reactions to cobalt chloride (CoCl_2) were studied for sensitization by detection of specific antibodies to radioactive cobalt (^{57}Co), cobalt-conjugated human serum albumin (Co-HSA) and cobalt-conjugated exchange resin (Co-resin). Their IgE titres ranged from 73 to 1500 IU/ml and eight were atopic individuals. Sixty serum samples from asthmatic patients with IgE titres of 14–4300 IU/ml were studied as controls in all tests. Eleven of twelve subject sera that selectively bound to ^{57}Co after incubation with saturated ammonium sulphate (> 232 c.p.m., $P < 0.01$) were divided into three groups: (1) six sera showing evidence of specific IgE antibodies to Co-HSA (> 673 c.p.m., $P < 0.01$) without those to Co-resin; (2) one serum giving a positive radio-allergosorbent test (RAST) only to Co-resin (> 417 c.p.m., $P < 0.01$), and (3) four sera that were negative for two antigenic agents (Co-HSA, Co-resin). These results suggest that the subjects had occupational asthma due to hard metal exposure from cobalt sensitivity. An immuno-allergic mechanism mediated by specific IgE antibodies to cobalt was confirmed to be responsible for the development of hard metal asthma, with the possibility of some role of the reaction without reagents.

Introduction

Hard metal is an alloy consisting of tungsten (W) carbide (80–95%) with cobalt (Co) (5–20%) as a matrix. Nickel (Ni) as a matrix and other metals such as titanium (Ti), tantalum (Ta), vanadium (V) and chromium (Cr) are sometimes added in the carbide form. Occupational asthma has been demonstrated to occur in workers exposed to hard metal dust since Bruckner [1] reported the first case of an asthmatic patient in

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1967. In order to find which metal is responsible for the development of the asthmatic reaction, some researchers [2,3] performed provocation tests with cobalt and tungsten powder; the former provoked asthma, while the latter did not. Subsequent studies [4–11] gave coinciding results of workers with wheezing and occasional dyspnoea-producing positive reactions not only to powder but also to water-soluble cobalt. Further support for the provocative nature of cobalt came from studies by Demedts' group [12,13] with diamond polishers sensitive to cobalt. These findings implicated cobalt as the only causal agent in this asthma. Moreover, some investigators [7,8] have emphasized the importance of ionized cobalt in coolants as a hapten. The known ability of cobalt to sensitize the skin [14,15] and lymphocytes [16] suggests an immuno-allergic rather than an irritant mechanism of cobalt in the asthmatic reaction. However, data concerning cobalt-reactive antibodies have not yet been published [17].

The purpose of the present study was to identify specific antibodies to cobalt using the saturated ammonium sulphate precipitation test with radioactive ^{57}Co (^{57}Co -SASPT), the specific radio-allergosorbent test (RAST) with cobalt-conjugated human serum albumin (Co-HSA), cobalt-conjugated exchange resin (Co-resin) and the typical Ouchterlony method with Co-HSA. The difference in the binding natures of the subject sera to Co-HSA and Co-resin will be discussed.

Subjects and methods

Selection of subjects

Twelve workers in a hard metal plant were chosen as subjects on the basis of diagnosis of hard metal asthma according to our criteria [9–11]: (i) a positive history of wheezing and dyspnoea after starting work in a hard metal plant, (ii) no abnormal shadows in their chest X-rays and computed tomography (CT) scans, (iii) obstructive changes in lung function values in good response to the bronchodilator, (iv) positive relationship between exposure to dust and drops in their peak expiratory flow rate (PEFR) using the Wright peak flowmeters, (v) neither abnormality in bronchoalveolar lavage fluid (BALF) [12,13] nor transbronchial lung biopsy (TBLB) specimen, and (vi) positive bronchoprovocation with cobalt chloride (CoCl_2). This was done with serial dilutions from 10^{-5} to 1% using a Rosenthal dosimeter. Five subjects developed immediate asthmatic reactions (IAR), three dual (DAR) and four late (LAR), while the eight controls did not. No relationship was recorded between Co-PD₂₀ and the reaction patterns. The subject profiles are summarized in Table 1.

Work environment

The metallurgy process in the plant under study was described elsewhere [9]. Powder workers are exposed to several kinds of metal powder when they mix the powder in the mill. As all of them wear respirators, the actual uptake of cobalt is much less than the amount found in the breathing zones. Airborne dust derived from shaping pre-sintered tips may affect shaping and sintering workers on the same floor. Grinding hard metal is done mainly with cutting fluids in which cobalt is dissolved, and the resulting mist has an influence on some workers.

Subject and control sera

Subject sera were collected several days after the subjects had been away from work. The division for respiratory diseases in Takatsuki Red Cross Hospital provided 60

Table 1. Subject profiles

Subject no.	Age (years)	Onset age (years)	Work at onset	Present work	Latent period	Mean cobalt level ($\mu\text{g}/\text{m}^3$)*	Smoking (pack/day)	Medication†
1	51	47	Shaping	Shaping	3 months	158	1/3	Sa
2	52	44	Sintering	Sintering	10 years	24	1	(-)
3	52	48	Grinding	Grinding	20 years	7	1/2	Sa
4	52	41	Powder	Sintering	2 years	22	1/5	(-)
5	44	28	Powder	Inspecting	2 months	227	0	Sa
6	43	36	Grinding	Grinding	4 years	31	0	Sa
7	54	31	Grinding	Grinding	1 year	81	1/3	Tr
8	49	42	Powder	Sintering	6 months	25	0	Sa
9	46	43	Grinding	Grinding	11 years	27	0	Th, β
10	50	39	Grinding	Grinding	2 years	18	0	Th, St
11	35	28	Grinding	Grinding	3 months	n.d.	0	Sa
12	51	36	Grinding	Grinding	2 years	16	0	Th, β

* Total dust and mist were collected with personal samplers in the breathing zones and cobalt in them was determined with an atomic absorption spectrophotometer [9].

† Sa, Salbutamol inhaler; β , β -agonist; Th, Theodur; Tr, Triamcinolon; St, oral steroid.
n.d., Not done.

control sera from both intrinsic and extrinsic asthmatic patients who had not been exposed to hard metal dust. They were selected to include 20 samples of three IgE concentrations, i.e., (i) low IgE group (< 400 IU/ml), (ii) high IgE group (400–1000 IU/ml), and (iii) extra-high IgE group (> 1000 IU/ml). All tests comparing the subject and control sera were carried out in the same run.

Reagents and preparation for serological studies

Test reagents were cobalt sulphate (CoSO_4) and cobalt chloride ($\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$) of super special grade (Wako Pure Chemicals, Japan). Radioactive ^{57}Co and ^{125}I were purchased from the Japan Radioisotope Association (JRIA) and diluted at optimal concentrations. A 1 mg portion of fraction V powder HSA (Sigma, St Louis, MO, U.S.A.) included 2.4×10^{-4} mg of cobalt. Co-HSA for the Ouchterlony test was prepared in the following manner by first incubating 300 mg of HSA powder with 0.01 mm or 1.0 mm CoCl_2 in 45 ml PBS for 24 hr at 4°C . The conjugates were dialysed against phosphate-buffered saline (PBS) for 48 hr at 4°C and frozen at -70°C and dried. A 1 mg portion of conjugate included 3.4×10^{-4} or 1.2×10^{-1} mg of cobalt, respectively, which was determined by atomic absorption spectrophotometry [18]. Next, 40 mg of HSA and two types of Co-HSA were dissolved in 20 ml PBS (pH 7.4) and used for the Ouchterlony tests. Human serum for the Co-HSA RAST was dialysed against EDTA before use. The cationic exchange resin (Dowex, 50W, X-2, Bio-Rad, Richmond, CA, U.S.A.) was washed in 2.5 N HNO_3 until cobalt, nickel and chromium were not found in the elution.

Peripheral blood analysis

Total and differential white blood cell counts and immunoglobulins (IgG, IgA, IgM) were determined before each test.

Total serum IgE

This was determined by radioimmunosorbent assay with a commercial kit (Shionogi, Japan). The upper limit of the normal range in this reference was less than 400 IU/ml.

RAST scores with inhaled allergens

RAST was measured against 21 common and indigenous allergens with a common kit (Phadebas, Pharmacia, Sweden) and scored according to the Phadebas reference. A score of more than 2 was considered positive in this reference.

Intradermal skin test with cobalt chloride (Co-IDST)

Serial ten-fold dilutions from 10^{-5} to 1.0% of CoCl_2 were used for the intradermal skin test. Twenty asthmatic patients not exposed to hard metal dust were asked to undergo the same tests. The results were recorded 15 min after the injection. If a weal or flare of more than 9 mm in diameter was observed in the subjects but not in the controls, the reactions at less than that concentration were considered positive.

Saturated ammonium sulphate precipitation tests with ^{57}Co (^{57}Co -SASPT)

This test (Farr test) [19] was performed by a modification of the method of the Malo and Dolovich group [20–23] for nickel asthma, in that $^{57}\text{CoSO}_4$ was added as a radioactive antibody binding antigen. Briefly, 200 μl of 12 subject and 60 control sera were assayed in duplicate by mixing each sample with 200 μl $^{57}\text{CoSO}_4$ (0.01 $\mu\text{Ci/ml}$, 5×10^{-13} M) in a 3 ml polystyrene tube (Falcon 2003, B&D Co., NJ, U.S.A.) and incubating it overnight at 4°C, after which 1.0 ml of saturated ammonium sulphate (SAS) and 0.80 ml of borate saline buffer (0.10 M borate, 0.025 M borax, 0.075 M NaCl, pH 8.3) were pipetted into each tube, followed by centrifugation (2000 r.p.m. at 4°C for 30 min) and decantation. The sediments were washed gently with 50% SAS in borate saline buffer. The radioactivity of each tube was determined with an automatic γ -counter (Micromedex Systems Inc., PA, U.S.A.) as counts per minute.

Specific antibodies to cobalt-conjugated exchange resin (Co-resin)

This test was done in a similar fashion to the method recommended by Novey *et al.* [24]. Firstly, 250 mg net weight of exchange resin (Dowex, Bio-Rad) were incubated with 20 mg CoCl_2 in deionized water as a 1 : 4 suspension for 2 hr at room temperature (22°C). The conjugate was washed with deionized water and stored in PBS with 0.3% HSA. Assays of 50 μl of 12 subject and 60 control sera were done in duplicate by adding 1 ml volume (2 mg/ml) of Co-resin or the resin itself to each polystyrene tube (Falcon) and rotating overnight at room temperature (22°C). The samples were then washed with PBS and 50 μl ^{125}I -labelled anti-human IgE (Pharmacia RAST) were added. Each tube was again rotated overnight and washed four times with assay buffer (PBS with 0.5% Tween 20). The radioactivity was measured as counts per minute with an automatic γ -counter.

Specific antibodies to cobalt-conjugated human serum albumin (Co-HSA)

We used a slight modification of the original method recommended by Cromwell *et al.* [25] in which Sepharose-HSA, which had been incubated with CoSO_4 , served as the solid phase for specific RAST. Firstly, 15 g net weight of cyanogen bromide activated Sepharose 4B powder (Pharmacia, Sweden) was washed with 3 l of 1 mM HCl on a sintered glass filter (3G3). This was mixed with 750 mg of human serum albumin (HSA), diluted in a coupling buffer (0.1 M NaHCO_3 , 0.5 M NaCl), by overnight rotation

at 4°C. After washing the Sepharose-HSA conjugate with coupling buffer, 0.2 M glycine in 0.1 M NaHCO₃ (pH 8.0) was added, and the mixture was incubated for 2 hr at room temperature (22°C). The product was washed with at least four cycles of alternating pH using acetate buffer with 0.5 M NaCl (pH 4.0) and coupling buffer (pH 8.3).

In the next step, a 50-ml packed volume of Sepharose-HSA conjugate was mixed with 150 ml of 0.33 mM CoSO₄ or ⁵⁷CoSO₄ in 0.1 M NaHCO₃ and rotated overnight at 4°C. The Sepharose-HSA-Co complex was then washed and filtered with 2 l of 0.1 M NaHCO₃ and stored as a 1:4 suspension in PBS with 0.3% HSA.

For the Co-HSA-specific RAST, subject and control sera were assayed in duplicate by adding 50-μl samples to polystyrene tubes (Falcon 2003) and mixing them with 100 μl Sepharose-HSA-Co or Sepharose-HSA suspension in PBS with 0.3% HSA. The tubes were capped and incubated overnight at room temperature (22°C) on a horizontal shaker. The solid phase was washed three times with PBS followed by centrifugation (2000 r.p.m. for 10 min) and aspiration of the supernatant. Next, 50-μl ¹²⁵I-labelled anti-human IgE (Pharmacia RAST) were pipetted into each tube. This was incubated overnight at room temperature (22°C) before washing the complex five times with PBS plus 0.5% Tween 20. The radioactivity was determined with an automatic γ-counter as counts per minute.

Cobalt contents in insoluble matrices

Cobalt level in Co-HSA was estimated from the count of ⁵⁷Co in the elution when ⁵⁷Co-HSA-Sepharose 4B complex was washed with NaHCO₃. Also, Co-resin was washed with 2.5 N HNO₃, and Co²⁺ in it was determined according to our previously described method [9].

Ouchterlony tests with cobalt-conjugated human serum albumin (Co-HSA)

Using the typical micro-Ouchterlony method [26], 20 μl antigen (0.01 mM, 1.0 mM Co-HSA and HSA) were placed in each central hole. Serial two-fold dilutions of sample sera were placed around them, and the samples were incubated at room temperature (22°C) for a week with observation every day.

Statistical analysis

A positive reaction to cobalt was defined as the count above the highest count of the mean + 2.861 × s.d. in three control groups according to Student's *t*-distribution (*P* < 0.01).

Results

Peripheral blood analysis

No hyperglobulinaemia was observed in the subjects. Cases 5, 6 and 11 showed eosinophilia (> 200/mm³).

Total serum IgE and RAST with common allergens

The subject IgE titres ranged from 73 to 1500 IU/ml, which was within the range of the three groups of control sera. High IgE titres were observed in five subjects (Table 2). Positive RAST scores against more than three allergens were obtained from the eight subjects. Thus, they were atopic individuals (Table 3).

Table 2. Immunological results for specific antibodies to cobalt

Subject	IgE (IU/ml)	Co-resin RAST (c.p.m.)		Co-HSA RAST (c.p.m.)		⁵⁷ Co-SASPT (c.p.m.)
		Resin	Co-resin	HSA	Co-HSA	
1	217	280	396	344	749	332
2	73	229	228	328	435	315
3	1028	253	351	324	322	345
4	231	218	325	324	342	289
5	618	163	397	346	713	401
6	588	220	351	464	792	303
7	389	208	195	364	278	178
8	129	306	400	402	895	346
9	96	177	335	381	416	336
10	825	219	355	450	1291	355
11	1500	230	320	360	680	345
12	289	286	517	301	589	432
Control (upper limit, $P < 0.01$)*			417		673	232
Low IgE (mean $\pm$ s.d.)		37 $\pm$ 17	164 $\pm$ 43	139 $\pm$ 35	290 $\pm$ 44	301 $\pm$ 28
(n=20) range		14-389	118-230	93-166	223-334	271-338
High IgE (mean $\pm$ s.d.)		632 $\pm$ 191	235 $\pm$ 18	230 $\pm$ 80	349 $\pm$ 58	350 $\pm$ 25
(n=20) range		402-832	220-261	139-303	234-386	328-376
Extra-high (mean $\pm$ s.d.)		2130 $\pm$ 1830	272 $\pm$ 19	286 $\pm$ 44	425 $\pm$ 103	444 $\pm$ 80
IgE (n=20) range		1132-4300	267-303	244-326	302-594	365-589

* Upper limit was the highest value of the mean plus $2.861 \times$ s.d. in 60 control sera.

Intradermal skin tests with cobalt chloride (Co-IDST)

Skin reactions were displayed by the three controls at 1.0%. Thus, a reaction at less than $10^{-1}\%$ was positive (Table 3). Neither late nor delayed type reaction was observed in the subjects or the controls.

Cobalt contents in conjugates

Cobalt contents of the conjugates were $18 \mu\text{g/g}$ conjugate = 7×10^{-9} M/mg HSA in Co-HSA and 1.4 mg/g conjugate in Co-resin.

Specific antibodies to cobalt

Neither subject nor control sera displayed a positive reaction to HSA and Co-HSA in the Ouchterlony tests. There was more significant ⁵⁷Co precipitation by the sera from 11 subjects (> 289 c.p.m.), with the exception of case 7, than by control sera for which the mean $+ 2.861 \times$ s.d. of the 60 samples was 232 c.p.m. The mean counts of three control groups were almost equal, although that of the extra-high group was highest. Specific activity of ⁵⁷Co as ABC-33 was about 110 in this method.

Specific IgE antibodies to cobalt

In the Co-HSA RAST, significantly higher counts were presented by sera from six subjects (1, 5, 6, 8, 10, 11) whose counts ranged from 680 to 1291 c.p.m., while the upper normal limit was 673 c.p.m. The results (mean, 365 c.p.m.) in cases 2, 3, 4, 7, 9 and 12 were in the vicinity of the mean value found for the control sera (365 c.p.m.). No

Table 3. Summary of immunoallergic studies of the subjects

				Co-BPT§	Specific antibody			Specific IgE	
Mc-PC ₂₀ *				Co-PD ₂₀	Ouchterlony				
Atopy	(µg/ml)	Co-IDST†	Co-PT‡	(units)¶	⁵⁷ Co-SASPT	HSA	Co-HSA	Co-resin	Co-HSA
1 No	38	(+)	(-)	LAR (21·0)	(+)	(-)	(-)	(-)	(+)
2 No	528	(-)	(-)	LAR (45·5)	(+)	(-)	(-)	(-)	(-)
3 Yes	121	(+)	(-)	DAR (42·5)	(+)	(-)	(-)	(-)	(-)
4 No	> 25000	(-)	(-)	IAR (27·5)	(+)	(-)	(-)	(-)	(-)
5 Yes	32	(+)	(+)	DAR (44·5)	(+)	(-)	(-)	(-)	(+)
6 Yes	410	(+)	(-)	LAR (48·5)	(+)	(-)	(-)	(-)	(+)
7 Yes	74	(-)	(-)	LAR (39·5)	(-)	(-)	(-)	(-)	(-)
8 No	150	(+)	(+)	IAR (44·5)	(+)	(-)	(-)	(-)	(+)
9 Yes	150	(-)	(-)	IAR (46·0)	(+)	(-)	(-)	(-)	(-)
10 Yes	170	(+)	(-)	IAR (10·5)	(+)	(-)	(-)	(-)	(+)
11 Yes	n.d.	(+)	(-)	DAR (45·5)	(+)	(-)	(-)	(-)	(+)
12 Yes	70	(+)	(-)	IAR (4·5)	(+)	(-)	(-)	(+)	(-)

* Methacholine-PC₂₀: This was done according to Hargreave's method [37].

† (+): Flare or weal at less than 10⁻¹%.

‡ Patch test: This was done with 2% CoCl₂ in petrolatum with readings at 48 hr and 72 hr after patching, according to the method recommended by the International Contact Dermatitis Research Group (ICDRG) [38].

§ Reaction patterns of bronchial provocation test with CoCl₂, IAR: immediate asthmatic reaction, LAR: late asthmatic reaction, DAR: dual asthmatic reaction.

¶ Cobalt-PD₂₀: 1 unit as one inhalation of 2 × 10⁻²% of CoCl₂, according to the method of Chai *et al.* [39]. n.d., Not done.

significant difference was found in the HSA RAST between subject (366 c.p.m.) and control sera (355 c.p.m.). In the Co-resin RAST, only the case 12 serum had a radioactive count (517 c.p.m.) greater than the mean + 2·861 × s.d. (417 c.p.m.) of 60 control sera. Non-specific binding to exchange resin was not very high even in the control group (mean, 227 c.p.m.) and subject group (mean, 257 c.p.m.). The results of immunoallergic studies are summarized in Table 2 and the summary of allergic tests on the subjects including skin tests, provocation tests [11] and serological tests are in Table 3.

Discussion

More than 200 substances are known to cause occupational asthma [27], but metal asthma is a rare phenomenon. Metals that can cause it are platinum (Pt) [25],

chromium (Cr) [24], nickel (Ni) [20–23], vanadium (V) [28], zinc (Zn) [29], cobalt (Co) [13] and aluminium (Al) [30]. Some investigators [24, 31] have postulated that these low-weight molecules (MW < 500 daltons) act as haptens *in vivo* following conjugation with serum proteins to perform antigens, but the evidence indicating ability to sensitize workers, i.e., detection of specific IgE antibodies to metals, has been found only for platinum (Pt), nickel (Ni) and chromium (Cr).

The detection for these antibodies arose from the detailed studies by Pepys and his colleagues with platinum salts, for which Cromwell *et al.* [25] developed a specific RAST with Sepharose 4B–HSA–Pt as a solid-phase antigen. This was used to study nickel asthma by the Malo and Dolovich group [20,21] by incubating Sepharose–HSA with NiSO₄. The same group established that the antigenic determinant depends on the combination of Ni²⁺ with the Cu²⁺ transportation site on HSA [22,23], suggesting the usefulness of this method for detection of specific antibodies to nickel. However, Novey *et al.* [24] proposed a new method with an exchange resin incubated with Ni²⁺ and Cr³⁺ as solid-phase antigens. In this method, antigenic determinants are attributed to ionized metals. The serum obtained from their subject working in a metal plating factory selectively became bound to Ni-resin and Cr-resin, not to Ni-HSA or Cr-HSA. Malo *et al.* [21] found different results for two subjects; one showed a positive RAST to Ni-HSA and significant precipitation with ⁶³Ni, while the other gave a negative test result for these antigens, and both sera did not bind to Ni-resin. These findings raise doubts about the sensitivity of the method.

In hard metal asthma, cobalt has been regarded as a major agent because of its provocative nature [2–11] and lung toxicity [32,33]. A series of studies [7–11] suggest an immuno-allergic rather than an irritant mechanism of cobalt. This implies that cobalt metal in an alloy, in contrast with the cases of platinum [25], nickel [20,21] and chromium [24] where sensitization results from exposure to metal salts, has to be converted to ionized cobalt on the bronchial mucosa to act as a hapten following conjugation with proteins. This was further suggested by the higher prevalence of asthma among workers exposed to cobalt dissolved in coolants than among those breathing hard metal dust [7]. The results of skin sensitivity tests by patch and intradermal techniques, however, did not show correlation with the prevalence of asthma among our workers exposed to hard metal dust [9]. The studies of circulating antibodies specific to cobalt were required to resolve the role of cobalt in hard metal asthma.

The study of ⁵⁷Co-SASPT confirms the presence of the total binding capacity to cobalt in the subject sera, except in case 7, which was not found in the Ouchterlony method [19]. The inhibition test with non-labelled cobalt has not yet been done, but the inability of sera to bind to ⁵⁷Co when obtained just after inhalation challenge with cobalt suggested that the test was selectively blocked by blood cobalt (data not shown here). RAST with Co-HSA or Co-resin revealed specific antibodies to cobalt in seven subjects (cases 1, 5, 6, 8, 10, 11, 12) on the basis of comparison with the reactivity in control sera with not only equivalent but also higher IgE titres. The results with the HSA RAST would exclude a non-specific basis of binding by positive sera. However, RAST values for Co-HSA were in slight proportion to serum IgE titres in control sera, and some sera from asthmatic patients with extra-high IgE group showed RAST values comparable to those of several subjects. When the former were diluted to levels of total IgE comparable to those of the latter (<100 IU/ml), the former gave significantly lower RAST values (data not shown here). This dilution effect has been well documented with the Pt-HSA RAST for sera with extra-high IgE titres from patients suffering from allergic bronchopulmonary aspergillosis (ABPA). Thus, an

IgE-mediated type I allergy must be involved in those subjects. The difference in binding capacity of subject sera between Co-HSA and Co-resin may be due to ion-exchange properties of Dowex resin hindering possible antigen-antibody association. This was suggested by the higher content of cobalt in Co-resin (1.4 mg/g conjugate) than that in Co-HSA (18 µg/g conjugate). Further support for the apparent failure of this assay protocol came from our preliminary test in which the highest values of case 12 were obtained when the resin was incubated with weaker solutions. With respect to the work environment, a positive RAST tends to be associated with (i) atopic grinders exposed to mist including ionized cobalt, or (ii) workers exposed to higher levels of pre-sintered cobalt powder. Further interpretation of these findings is not possible at this time. Nevertheless, the result that IgE antibodies to Co-HSA and Co-resin were absent from four sera (cases 2, 3, 4, 9), who had no skin sensitivity to CoCl₂, may have some parallel with the findings that in occupational asthma due to TMA (Trimellitic anhydride) exposure, sera containing IgG antibodies to TMA-HSA without IgE antibodies can trigger histamine release from basophils [34], suggesting a pharmacological mechanism. Of the four subjects without IgE antibodies to cobalt, case 4 showed no bronchial hyperresponsiveness to methacholine (Mc-PC₂₀ > 25 000 µg/ml) with a positive bronchial reaction to cobalt. A direct effect of chemical mediators on bronchial muscle, triggered by non-IgE antibodies to cobalt proven by ⁵⁷Co-SASPT, may occur in those subjects. Specific IgE to TMA-HSA has been found in some individuals with IAR [35], while development of LAR as well as infiltrative lung disease has been associated with specific IgG antibodies to it [36]. Such a relationship was, however, not found in our subjects (Table 3).

Studies should be done on the antigenic determinant needed for the combination between cobalt and sensitive sera, which has been established in nickel asthma [22,23]. Moreover, as hard metal is an alloy, not a simple metal of cobalt, information is needed on the allergenicity of other components such as nickel and chromium in combination with cobalt sensitivity. Further studies for the detection of specific antibodies to these metals are under way in our laboratory.

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