In vitro Testing for Cobalt Sensitivity: An Aid to Diagnosis

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Peripheral blood lymphocytes from 45 patients with a positive patch test to cobalt chloride ($CoCl_2$) and 37 controls were stimulated with various concentrations of $CoCl_2$ and/or cobalt sulphate ($CoSO_4$) or cobalt nitrate ($Co(NO_3)_2$) or cobalt acetate ($(CH_3COO)_2Co$) for various days in culture. Lymphocytes from 35 of these patients showed a significantly greater response than that of the controls. The response occurred in the T enriched population and was monocyte dependent. The strength of the in vivo serial dilution test results did not correlate well with the height of in vitro responses. Lymphocytes from 3 nickel and/or chromium sensitive patients failed to respond to stimulation with cobalt compounds thus confirming the specificity of the reaction. The DNA synthesis test seems to be a reliable in vitro method to aid in the diagnosis of cobalt sensitivity. *Key words: Cobalt sensitivity; Lymphocyte stimulation; In vitro criteria.* (Received August 14, 1983.)

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Cobalt is allergenic like nickel to which it is closely related (1). Most cases of cobalt sensitivity occur in association with nickel sensitivity in women and with chromate sensitivity in men (2). This is often due to concomitant exposure to these metals or their compounds (1). However, Rystedt (3) found it hard to evaluate the clinical relevance of cobalt exposure. The patch testing with cobalt presents problems and even with critical evaluation, about 10% false positive and 10% false negative reactions are probable (4). In this study we tried to ascertain whether the DNA synthesis test could be used to aid in the diagnosis of cobalt sensitivity and whether the cobalt compound-induced activation of lymphocytes is specific. We also tried to determine the type of cells responding to cobalt stimulation and whether monocytes are required.

MATERIAL AND METHODS

Patients. Forty-five patients with a positive patch test (++ or more) to 1% $CoCl_2$ were included in this study. They ranged in age from 17 to 81 years. Two patients with a positive patch test to potassium dichromate ($K_2Cr_2O_7$) and one patient with a positive patch test to nickel sulphate ($NiSO_4$) were also included to test for specificity.

Controls. Thirty-seven age- and sex-matched healthy individuals without eczema or a case history of metal contact allergy were included.

Patch testing was performed according to Pirilä (5). The conditions, time of exposure and reading of the test were as previously described (6). None of the patients had had active eczema for at least 2 weeks prior to testing.

In vivo serial dilution test (SDT) was performed using the following concentrations of $CoCl_2$ in distilled water: 0.5, 0.25, 0.125, 0.0625, 0.0312, 0.0156, 0.0078 and 0.0039%. A ++ reaction or more with any of these concentrations was considered as positive.

Preparation of cobalt compounds and PHA. Stock solutions of 1% of $CoCl_2 \cdot 6H_2O$ (Merck, Germany), $CoSO_4 \cdot 7H_2O$ (Merck), $Co(NO_3)_2 \cdot 6H_2O$ (Merck) and $(CH_3COO)_2Co \cdot 4H_2O$ (Merck) were prepared in distilled water. These solutions were then diluted with modified Eagle's minimum essential medium (MEM) (Flow Laboratories, Irvine, Scotland) giving final concentrations in culture

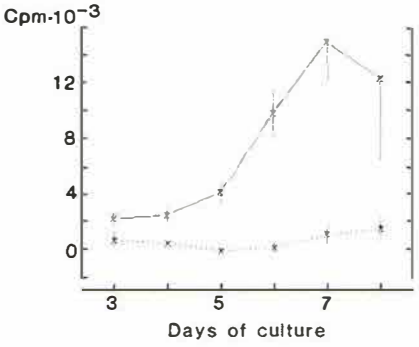

Fig. 1. Time–response curve for peripheral blood lymphocytes from 35 patients with a positive patch test to $CoCl_2$ (×——×) and 37 controls (× · · · ×) using 3.125 μg $CoCl_2$/ml culture. The mean increment counts per minute and standard error values are plotted.

of 1.5, 3.125, 6.25, 12.5, 25 and 50 μg/ml. Phytohaemagglutinin (PHA) (Wellcome) was added to a final concentration of 50 μg/ml as a positive control.

Mononuclear cell preparation and assay of DNA synthesis. This was performed as described previously (6). Monocytes were isolated by adherence to plastic and more than 90% of these cells ingested Candida. The non-adherent cell suspension was iron-treated and then fractionated into T- and B-enriched lymphocyte populations (7).

Statistical method. Student's *t*-test was used. *p*-Values <0.05 were considered significant.

RESULTS

All the 45 patients showed a ++ reaction or more when patch tested with 1% $CoCl_2$. However, the in vivo SDT was negative in 14 of the 37 patients who accepted to participate. Forty-one patients were also nickel- and/or chrome-positive on patch testing.

Lymphocytes from 35 patients (excluding 10 patients, see below) gave their highest response on day 7 of culture when stimulated with 1.5, 3.125, 6.25, 12.5 and 25 μg $CoCl_2$/ml while lymphocytes of controls showed either a negative or a weak response (Fig. 1). Responses of patients' lymphoctyes were higher ($p<0.05$ or $p<0.001$) than those of controls when stimulated with 1.5, 3.125, 6.25 and 12.5 μg $CoCl_2$/ml on days 3–7 of culture and also when stimulated with 25 μg on days 3–6 of culture. Responses of lymphocytes

Table I. *Specificity of lymphocyte responses in vitro*

Cells from patients with indicated metal allergies were tested with $CoCl_2$ and SI on day 6 of culture are shown. C = control

Expt. no.	Pat. no.	Background (cpm)	Concentration of $CoCl_2$ (μg/ml) 1.5	3.125	6.25	12.5	Clinical metal allergy	Evaluation of cobalt sensitivity in vitro
I	13	1 676	1.5	2.2	2.5	3.0	Co Cr	+
	14	2 978	1.3	2.0	2.3	1.8	Co Cr	+
	33	1 076	0.8	0.9	0.6	1.7	Cr	–
	C	1 262	0.9	1.2	0.8	0.7	–	–
	C	3 814	0.5	0.6	0.4	0.9	–	–
II	47	1 074	0.6	0.7	0.9	0.9	Ni	–
	49	4 877	2.0	1.8	2.0	3.0	Co Cr Ni	+
	50	826	1.0	0.8	0.7	0.7	Cr	–
	C	2 491	0.7	0.3	0.5	0.8	–	–
	C	1 149	0.8	0.5	0.6	0.3	–	–

from individual patients were almost always higher than those of controls simultaneously tested with any of these concentrations on days 3–8 of culture. Neither patient nor control lymphocytes showed any response when stimulated with 50 μg $CoCl_2$/ml culture.

Lymphocytes form some of these patients and controls were challenged with various concentrations of other cobalt compounds, $CoSO_4$ or $Co(NO_3)_2$ or $(CH_3COO)_2Co$ for various days in culture. The cells responded in a similar manner as when challenged with $CoCl_2$ (data not shown).

From the above data it was possible to identify certain criteria that should be fulfilled in order to define a positive response in vitro. Thus, positive reactivity is defined if lymphocytes when stimulated with 1.5, 3.125 and 6.25 μg $CoCl_2$/ml give stimulation indices of more than one and greater than those of control lymphocytes simultaneously tested on days 5 and 6 of culture. These conditions also apply when other cobalt compounds were used.

To test the specificity of cobalt-compound-induced activation, lymphocytes from 3 patients with a positive patch test to $K_2Cr_2O_7$ or $NiSO_4$ were stimulated with various concentrations of $CoCl_2$ for various days in culture. The response of lymphocytes from these 3 patients failed to fulfil the above-mentioned criteria thus confirming the specificity of the stimulation (Table I).

Lymphocytes of 9 of the 14 patients who had a negative response to serial dilutions of $CoCl_2$ in vivo responded to stimulation with $CoCl_2$ in vitro and were included in the time reponse curve, while lymphocytes from the remaining 5 patients could not be stimulated in vitro. On the other hand, lymphocytes from 4 other patients who had a positive response to the SDT in vivo failed to show a positive response in vitro. Lymphocytes from one patient who did not participate in the SDT also showed a negative response in vitro. Responses of lymphocytes from the latter 10 patients were thus excluded from the time-response curve (see above). These results were confirmed by retesting lymphocytes taken on different occasions from most of these patients. The patients' skin and lymphocyte responses are summarized in Table II.

We could not find a good correlation between the strength of in vivo SDT results and the height of in vitro lymphocyte responses (Fig. 2) to stimulation with 1.5, 3.125 and 6.25 μg $CoCl_2$/ml on days 5 and 6 of culture.

Peripheral blood mononuclear cells from 3 patients with sensitivity to cobalt and from 3 controls were fractionated into T and B enriched monocyte-depleted populations which were then stimulated with 3.125 μg $CoCl_2$/ml for 6 days in culture with or without the addition of autologous monocytes. Proliferation occurred in the T enriched population and

Table II. *Skin and lymphocyte responses of 45 patients with a positive patch test to 1% $CoCl_2$ and 37 controls*

nd = not done

Patch test (1% $CoCl_2$)	Serial dilution test	DNA synthesis test	No. of patients	No. of controls
+	+	+	19	
+	−	−	5	
+	+	−	4	
+	−	+	9	
+	nd	+	7	
+	nd	−	1	
nd	nd	−		37

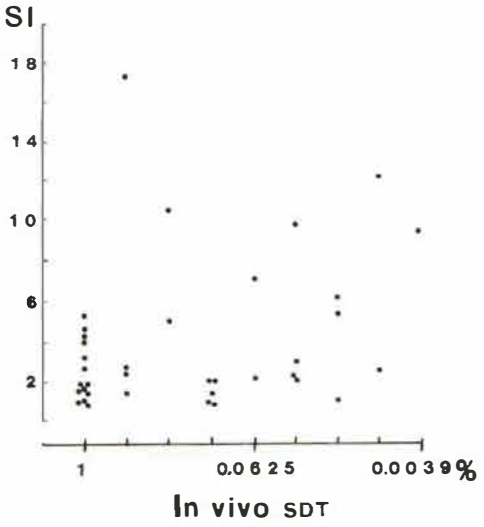

Fig. 2. Absence of correlation between the strength of patients responses to in vivo serial dilutions of $CoCl_2$ and the height of in vitro lymphocyte responses to stimulation wtih 3.125 μg $CoCl_2$/ml on day 6 of culture.

was dependent on the presence of monocytes. The results of one experiment are shown in Table III.

Lymphocytes from all patients and controls responded to PHA.

DISCUSSION

Cobalt metal itself sensitizes as do its salts and oxides (2). Cobalt sulphate was found to be a moderate sensitizer (grade 3 on a 0–5 scale) by the maximization test (8). Some investigators (9) used the leucocyte migration inhibition test to detect cobalt sensitivity in vitro and reported a 90% concordance with patch testing. Earlier, Veine and Svejgaard (10) detected transformation of lymphocytes in some cobalt sensitive patients and concluded that the clinical value of the in vitro test was limited but stated that a larger number of patients and controls must be investigated. In this study we challenged lymphocytes from

Table III. *^{3}H-thymidine uptake (cpm) by mononuclear cells of a patient with cobalt sensitivity and a control following stimulation with 3.125 μg $CoCl_2$/ml on day 6 of culture*

The response occurred in the T enriched population and was monocyte dependent

Subjects	Addition of		Cell suspension[a]		
	M[b]	$CoCl_2$	Unfractionated	T enriched	B enriched
Patient	–	–	420	130	240
	–	+	21 400	355	400
	+	–		420	350
	+	+		7 200	800
Control	–	–	140	230	410
	–	+	190	290	210
	+	–		350	320
	+	+		160	700

[a] 1×10^5 cells/well.
[b] 5×10^3 monocytes/well.

cobalt sensitive patients and from controls with different cobalt compounds and we could then define certain criteria for in vitro reactivity that can be used to assist in the diagnosis of cobalt sensitivity.

Combined reactions to cobalt, nickel and chromium are not uncommon and do not represent cross reactions but simultaneous, distinct and specific sensitizations (11). We have previously demonstrated the specificity of nickel (6) and chromium (12) induced activation of lymphocytes. Our results indicate that the cobalt induced activation of lymphocytes is also specific since the repsonse of lymphocytes form nickel or chromium (but not cobalt) sensitive patients did not fulfil the criteria for in vitro reactivity to cobalt. The role of cobalt as a primary allergen must never be overlooked (13). Four patients (3 women and 1 man) had a positive patch test reaction to cobalt but not to nickel or chrome. The in vivo SDT with $CoCl_2$ was positive in 2 of them but was not done in the other 2. Lymphocytes from all 4 patients gave a positive response when stimulated with $CoCl_2$ in vitro.

The in vivo SDT is done if a patch test reaction is suspected to be of irritant type (1). Reactions to an allergen gradually diminish in severity, the weaker the solution while reactions to an irritant stop abruptly below a certain concentration and are likely to give irregular results (2, 14). In this study, however, we could not find a good correlation between the strength of in vivo SDT results and the height of in vitro lymphocyte responses since lymphocytes from some patients with a positive skin reaction to some or all dilutions of $CoCl_2$ showed weaker or even negative responses than those of lymphocytes from patients with a weak or even negative SDT reaction. The development of contact hypersensitivity reactions (e.g. a positive patch test) require sensitized T cells assisted by Langerhans cells, mast cells and basophils with the release of various factors (15). Langerhans cells were shown to take up cobalt, nickel and chromium in sufficient amount to permit specific histochemical visualization (16). We have demonstrated that a positive in vitro response of lymphocytes to stimulation with $CoCl_2$ as measured by increased ^{3}H-thymidine incorporation is a reflection of proliferating T lymphocytes and is dependent on the presence of monocytes. However, Braathen & Thorsby (17) have shown that epidermal Langerhans cells are six times more potent than blood monocytes in inducing a $NiSO_4$-specific repsonse of T cells from sensitized individuals. Thus, the type of cells in the skin as well as their number and potency may be different from those in the peripheral blood. This may explain the absence of correlation between the in vivo and in vitro reactions.

Patch testing can give false-positive or false-negative reactions (2). Lymphocytes from 10 of the 45 patients with a positive patch test to 1% $CoCl_2$ showed a negative in vitro response. The in vivo SDT was negative in 5 of them, not done in one but positive in 1–6 dilution steps in the remaining 4 patients. The positive SDT reactions in the latter 4 patients could have been due to hyperirritability of the skin.

The DNA synthesis test is a reliable in vitro method that can be used to aid in the diagnosis of cobalt sensitivity. In industry, it can be useful in some instances where patch testing with potential sensitizers should not be performed (1). Patch testing with $CoCl_2$ has been shown to induce sensitization (18). We have demonstrated that the in vitro lymphocyte response to stimulation with $CoCl_2$ occurred within the T enriched population and was dependent on the presence of monocytes.

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