Detection of nickel cobalt and chromium contact hypersensitivity by a flow cytometric proliferation test

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Background
Allergic contact hypersensitivity to metals is a delayed type IV allergy. The gold standard for the diagnosis of delayed type IV hypersensitivity is patch testing with the suspected allergens. The patch test, however, has different disadvantages, and therefore the development of alternative or complementary *in vitro* tests is of great importance. *In vitro* tests, such as the lymphocyte transformation test (LTT), measure sensitized T-cells reactivity to the suspected antigen in culture. The possible role of the LTT as an alternative test to diagnose type IV hypersensitivity is unclear, but seems to be gaining support for use in combination with the patch test thereby improving diagnostics. More research is needed to definitively determine the validity and proper clinical use of the LTT in relation to metal allergy. The aim of this study was to establish and validate a flow cytometric LTT for the detection of delayed-type IV allergic responses to nickel, cobalt and chromium.

Methods
Thirteen patients were patch tested with the metal baseline series including nickel ($NiCl_2$), cobalt ($CoCl_2$) and chromium ($CrCl_3$) were additionally tested with flow cytometric LTT with $NiCl_2$, $CoCl_2$, and $CrCl_3$. Twelve donors without self-reported allergy (controls), were tested with flow cytometric LTT, but not patch tested. Blood (20 ml) was obtained from both healthy donors and patients. Peripheral blood mononuclear cells (PBMCs) were isolated by density centrifugation, labeled by carboxyfluorescein succinimidyl (CFSE), and cultivated with the allergen or control substances. Stimulation was performed in triplicates over a period of 7 days with the following substances: The T cell mitogen phytohemagglutinin (10 µg/ml), and tetanus toxoid (10 µg/ml) as positive control. Each metal was tested in three concentrations: Nickel chloride (NiCl2: 2.5 x 10^{-5}, 1 x 10^{-5} and 7.5 x 10^{-6} M), Cobalt chloride ($CoCl_2$: 1.01x10^{-4}, 1.01x10^{-5}, 5.05x10^{-6} M) and Chromium(III)chloride ($CrCl_3$: 1.25 x 10^{-3}, 3.75 x 10^{-4}, 9.38 x 10^{-5} M). Unstimulated cells in medium alone were used as a control. At day 7 the CFSE labeled PBMCs were harvested, labeled with monoclonal antibodies to cell-surface antigens (CD3, CD4, CD8, CD45RO+ and CLA), and analyzed by flow cytometry.

Results
The lymphocyte proliferation test was compared with the patch test in the diagnosis of nickel, cobalt and chromium contact sensitivity. Of the 13 patients, the patch test detected 7 with nickel, 4 with cobalt and 2 with chromium contact allergy. The *in vitro* stimulation with $NiCl_2$ mediated the activation and proliferation of $CD3^+$ T cells in nickel allergic patients (5/7) and in some healthy donors (4/12). The *in vitro* stimulation with $CoCl_2$ in cobalt allergic patients and healthy donors did not mediate proliferation of $CD3^+$ T cells (0/4 and 0/12 respectively). Finally, *in vitro* stimulation with $CrCl_3$ mediated the proliferation of $CD3^+$ T cells in chromium allergic patients (2/2) but not in healthy donors (0/12).
Interestingly, memory cells ($CD4^+CD45RO^+$ double positive cells) were overrepresented among the lymphoblasts of nickel and chromium allergic patients in response to all three $NiCl_2$ and $CrCl_3$ concentrations ($P \leq 0.05$) compared with healthy donors. In contrast, the $CD8^+CD45RO^+$ cells were overrepresented in response to $NiCl_2$ (1 x 10^{-5} M, $P = 0.0230$), and $CoCl_2$ (1.01x10^{-5} M, $P = 0.0130$) in healthy donors.
Finally, cells expressing the skin homing marker CLA in combination with CD4, and CD45RO are increased after $NiCl_2$, $CoCl_2$ and $CrCl_3$ *in vitro* stimulation ($P \leq 0.05$) in nickel, cobalt and chromium allergic patients compared with healthy donors.

Conclusion
Altogether, the results show that the flow cytometric LTT may be a useful method for the detection of Ni, Co and Cr sensitization. Interestingly, nickel, cobalt and chromium reactive T cells isolated from PBMCs of nickel, cobalt and chromium allergic patients are characterized by a CD4+ CD45RO+ CLA+ phenotype.