

In vitro diagnosis of contact allergy to nickel: The value of the ELISpot assay

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Background: Diagnosis of contact allergy is based on clinical data and patch tests. Among in vitro tests, lymphocyte proliferation test (LPT) is most frequently used. A disadvantage of LPT is that it is based on radiochemicals, which restricts its use only to laboratories with radionuclide facilities.

Objective: To find a cytokine secretion assay giving results that correlate best with clinical diagnosis and with LPT.

Methods: PBMC from 14 patients with ACD to nickel and 14 non-allergic controls were tested for their reactivity to nickel. In all subjects, patch tests and LPT with nickel sulphate were done. A range of non-radioactive secretion assays was performed, including ELISpot assays for IL-2, IL-5, IL-13 and IFN- γ , and ELISA for IL-5 and IFN- γ . Beside standard culture conditions, cytokine secretion was also measured in cultures favouring the development of Tc1/Th1 or Tc2/Th2 lymphocytes ("skewing" through addition of IL-7 with respectively IL-12 or IL-4).

Results: The best correlation with clinical diagnosis (patch tests and history) was observed for IL-13 ELISpot with Tc2/Th2 skewing ($r=0.654$, $P<0.001$), followed by LPT ($r=0.612$, $P<0.001$), and IL-5 ELISpot with Tc2/Th2 skewing ($r=0.551$, $P=0.002$). The non-radioactive method that correlated best with LPT was IL-2 ELISpot ($r=0.809$, $P<0.001$), followed by IL-13 ELISpot ($r=0.778$, $P<0.001$), and IL-5 ELISA ($r=0.669$, $P<0.001$). Interestingly, IFN- γ ELISpot and IFN- γ ELISA correlated very poorly with both clinical diagnosis and LPT results ($r<0.010$ in each case).

Conclusions: Results of IL-13 ELISpot with Tc2/Th2 skewing correlate best with clinical diagnosis of contact allergy to nickel, whereas IL-2 ELISpot seems a good non-radioactive alternative for lymphocyte proliferation test.

Full citation:

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IN VITRO DIAGNOSIS OF CONTACT ALLERGY TO NICKEL: THE VALUE OF THE ELISPOT ASSAY



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BACKGROUND

Diagnosis of contact allergy is based on clinical data and patch tests. Among in vitro tests, lymphocyte proliferation test (LPT) is most frequently used. A disadvantage of the LPT is that it is based on radiochemicals.



OBJECTIVE

To find a cytokine secretion assay giving results that correlate best with clinical diagnosis and with LPT.

STUDY GROUP

14 patients with ACD to nickel and 14 non-allergic sex- and age-matched controls.

METHODS

Patch tests with nickel

- LPT with nickel
- ELISpot assays for IL-2, IL-5, IL-13 and IFN-gamma
- ELISA for IL-5 and IFN-gamma

PBMC cultures

- standard conditions
- stimulation of Tc1/Th1 development (addition of IL-7 and IL-12)
- stimulation of Tc2/Th2 development (addition of IL-7 and IL-4)

RESULTS

Best correlates with clinical diagnosis (patch tests and history):

- IL-13 ELISpot with Tc2/Th2 skewing ($r=0.654$, $P<0.001$)
- LPT ($r=0.612$, $P<0.001$)
- IL-5 ELISpot with Tc2/Th2 skewing ($r=0.551$, $P=0.002$)

Best non-radioactive correlates with LPT

- IL-2 ELISpot ($r=0.809$, $P<0.001$)
- IL-13 ELISpot ($r=0.778$, $P<0.001$)
- IL-5 ELISA ($r=0.669$, $P<0.001$)

IFN-gamma ELISpot and ELISA correlated very poorly with both clinical diagnosis and LPT results ($r<0.01$ in each case).

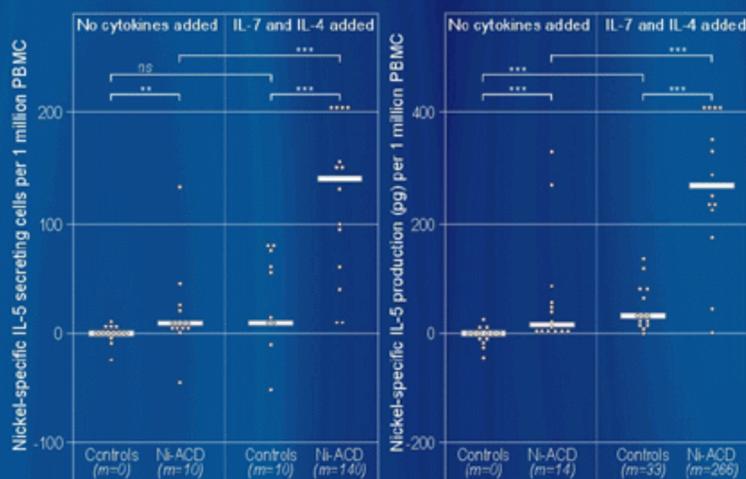


Figure 1. Analysis of nickel-specific IL-5 secretion by means of ELISpot (left) and ELISA (right). The numbers of IL-5 secreting cells (ELISpot) and the IL-5 overall production (ELISA) in response to nickel were significantly higher among Ni-ACD patients than among controls. In the Tc2/Th2 promoting conditions (addition of IL-7 + IL-4), the difference between groups further increased. Horizontal bars represent medians. Symbols: **; $p < 0.01$; ***; $p < 0.001$; ns: not significant; m: median.

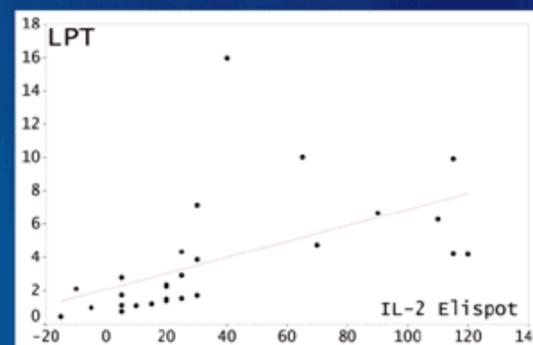


Figure 3. Analysis of lymphocyte proliferation test (LPT) and the IL-2 ELISpot assay results shows a significant correlation ($r=0.809$, $P<0.001$).

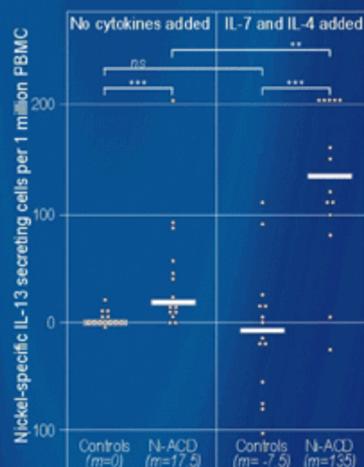


Figure 2. IL-13 secretion in response to nickel (ELISpot). The numbers of secreting cells were significantly higher among Ni-ACD patients than among controls. Addition of the Tc2/Th2 promoting cytokine cocktail (IL-7 + IL-4) to the cultures further increased the difference. Horizontal bars represent medians. Symbols: **; $p < 0.01$; ***; $p < 0.001$; ns: not significant; m: median.

CONCLUSIONS

IL-13 ELISpot with Tc2/Th2 skewing correlates best with clinical diagnosis of nickel allergy.

IL-2 ELISpot seems best non-radioactive alternative for lymphocyte proliferation test.

