Hyposensitization in nickel allergic contact dermatitis: Clinical and immunologic monitoring

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Background: In allergic contact dermatitis (ACD) previously sensitized T cells cause skin damage. If an ubiquitous allergen such as nickel is involved, no effective treatment is available. Down-regulation of this allergic response has been described after antigen presentation in the absence of adequate costimulatory signals. UV exposure can enhance such hyposensitization.

Objective: The aim of this study was to establish the capability of a hyposensitization procedure to induce antigen-specific tolerance.

Methods: Twenty-one patients with nickel ACD were randomly assigned to either a hyposensitized or control group. A schedule consisting of UVB treatment and subcutaneous nickel sulfate administration (hyposensitization) or UVB only (control) was applied. During the ensuing 2 years, several clinical and immunologic features were monitored.

Results: During UVB treatment we observed a significant clinical improvement in both groups that persisted in the hyposensitized group. Except for increased slope variances of specific lymphocyte proliferation in time, no clear changes were seen in the immunologic findings.

Conclusion: Despite significant clinical improvement induced by UVB, hyposensitization did not induce significant changes in the immunologic findings in patients with nickel ACD.
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The type of allergic contact dermatitis (ACD) most frequently diagnosed involves nickel allergy. Treatment of chronic ACD has been largely symptomatic because consistent avoidance of skin contact with and dietary intake of nickel are difficult to achieve. Allergen-specific T lymphocytes are crucial in the pathogenesis of ACD. Nickel-specific T lymphocyte clones have been isolated from peripheral blood of nickel-allergic patients.[1-4] When activated, these T lymphocytes produce interleukin 2 (IL-2) and high levels of interferon-γ[1,4] and cause inflammatory skin injury. Prevention of skin injury may be obtained through inhibition of specific T-cell activation (e.g., through hyposensitization).

A state of specific tolerance or anergy has been described after antigen presentation in the absence of costimulatory signals.[5-7] In both mice[8-21] and human beings,[5,22] UVB induces specific hyporesponsiveness when administered simultaneously with allergen. UV exposure (even suberythemagenic doses) has significant down-modulatory effects on T cell–mediated responses to contact allergens. In a previous study we demonstrated a clear effect of UVB on patch test responses to nickel, possibly through depletion of Langerhans cells (LC) and induction of $CD1a^{-}DR^{+}$ antigen-presenting cells.[5]

Until now, hyposensitization treatment has been restricted to immediate hypersensitivity reactions such as those to insect venoms and pollen. In view of the clear effects of UVB on LC and ACD, we designed a hyposensitization schedule that bypasses the highly sensitizing potential of epidermal LC by combining UVB treatment with subcutaneous injection of nickel sulfate solutions.

MATERIAL AND METHODS

Patch tests

Before the start of the study a European standard patch test series of contact allergens (van der Bend, Brielle, The

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Table I. Monoclonal antibodies used

Antibody	CD code (antigen recognized)	Source
Leu-4 (FITC/PE)	CD3	Becton Dickinson, Sunnyvale, Calif.
Leu-3 (FITC/PE)	CD4	Becton Dickinson
Leu-1	CD5	Becton Dickinson
Leu-2 (PE)	CD8	Becton Dickinson
LFA1/2	CD11a	CLB, Amsterdam, The Netherlands
My4	CD14	Coulter Clone, Hialeah, Fla.
Leu-11c (PE)	CD16	Becton Dickinson
B1 (FITC)	CD19	Coulter Clone
B4 (FITC)	CD20	Coulter Clone
2A3 (PE)	CD25	Becton Dickinson
HLe-1	CD45	Becton Dickinson
BBA4	CD54	British Biotechnology, Oxon, U.K.
Leu-19 (PE)	CD56	Becton Dickinson
L243 (PE)	HLA-DR	Becton Dickinson

CLB, Central Laboratory of the Red Cross Blood Transfusion Service; *FITC*, fluorescein isothiocyanate; *LFA*, lymphocyte function–associated antigen; *PE*, phycoerythrin.

Netherlands), including nickel sulfate, potassium dichromate, and cobalt chloride, was performed according to guidelines of the International Contact Dermatitis Research Group. After 48 hours the patch chambers were removed. At 48 and 72 hours the skin reaction was scored as described earlier.[23] At the start of the study patch tests with nickel, chromium, and cobalt were repeated to obtain baseline values. Because of the risk of further sensitization or boostering, patch tests were not repeated.

Patients

Twenty-one patients allergic to nickel as judged by history and clinical signs of contact allergy (predominantly on the hands and face) indicating nickel as a likely cause and confirmed by a patch test response of 2+ or greater to nickel sulfate were enrolled in and completed the study. All subjects were nonpregnant women.

Each patient was randomly assigned to either the hyposensitized group, scheduled for UVB plus nickel sulfate administration, or to the control group, which was to receive only UVB treatment. The protocol and informed consent documents were approved by the committee on medical ethics of our hospital. The hyposensitized group consisted of 12 women, 28 ± 5 years of age. Nine patients, 38 ± 16 years of age, formed the control group.

UVB treatment

A Waldmann UV1000 standing UV cabinet was used for UV treatment. All patients (hyposensitized and control groups) received UVB pretreatment during the first 3 to 6 months. On the basis of skin type, exposure started at 5 to 10 mJ/cm^2 three times a week and gradually increased, allowing only mild erythema. Nickel sulfate injections were started in the hyposensitized group as soon as a cumulative dose of 1.0 J/cm^2 was reached. At this point, UV exposure was continued once a week until a cumulative dose of approximately 1.25 mJ/cm^2 was reached.

Hyposensitization procedure

Sterile, pyrogen-free solutions of 10^{-6}, 10^{-5}, 10^{-4}, and 10^{-3} mol/L nickel sulfate were prepared. The lowest concentration equals the nickel concentration normally measured in body fluids.[24-27] In a manner similar to hyposensitization schedules used in type I allergy, weekly subcutaneous injections were started with 0.1 ml of the lowest nickel sulfate concentration in the arm. After each injection the patients were observed for at least 20 minutes. If possible, the next doses were subsequently 0.2, 0.4, 0.7, and 1.0 ml of the same concentration, and finally 0.1 ml of the next concentration. Doses were increased until either minimal local symptoms occurred or the highest concentration was reached. At this point the maximal dose achieved was continued and the interval was gradually prolonged up to 1 month. The total duration of the nickel sulfate hyposensitization was 2 years.

Clinical evaluation

The clinical follow-up period was 24 months. At three defined time points—at the start of the study (month 0), at maximal UVB exposure (month 6), and at the end (month 18) of the hyposensitization treatment—clinical evaluation was done. The affected area of involved skin, severity (itching, papules, vesicles, and fissure formation), frequency of symptoms, therapeutic need (use of corticosteroids and its potency), and a subjective quality of life assessment were scored on a standard evaluation form. With respect to clinical scores, the control group consisted

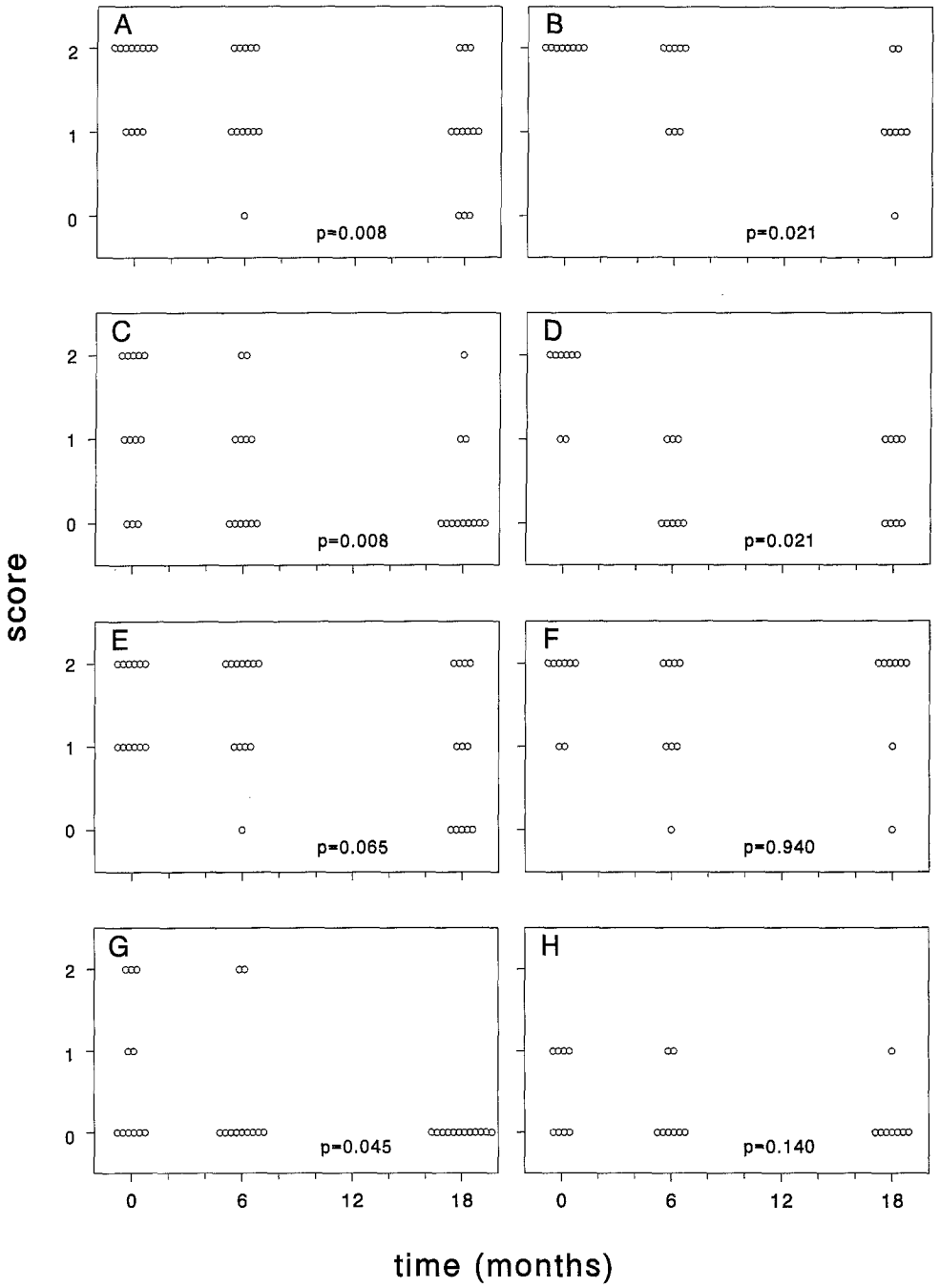

Fig. 1. Time course of disease activity scores in hyposensitized *(left panels)* and control *(right panels)* groups with regard to affected area (*A* and *B*), severity (*C* and *D*), frequency (*E* and *F*) and therapy (*G* and *H*).

of eight patients, because we excluded one patient because of lack of clinical follow-up information.

Lymphocyte proliferation assays

Lymphocyte proliferation assays (LPA) were performed as described earlier.[23] Lymphocyte proliferation was expressed as counts per minute. Stimulation indices (SI) (i.e., relative proliferation) were calculated by dividing specific by background proliferation. In our laboratory an SI greater than 3 was considered to be indicative of prior lymphocyte sensitization to nickel.

Fluorescence-activated cell sorter immunophenotyping

Peripheral blood samples were immunophenotyped with the monoclonal antibodies listed in Table I. Except

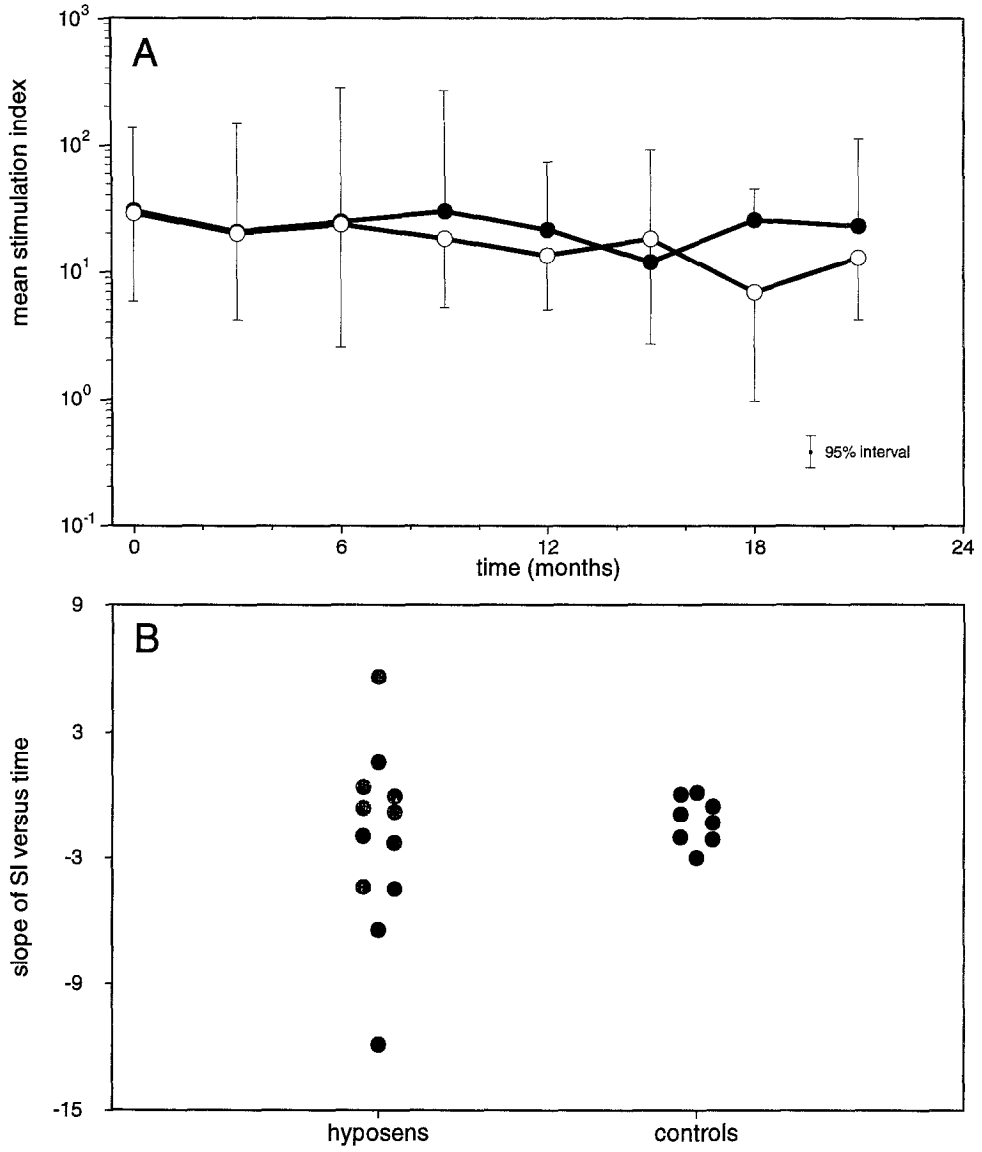

Fig. 2. Level of nickel reactivity in time as measured by nickel-specific lymphocyte proliferation. **A,** Mean SI values over time in all patients within each group. *Bars* represent 95% confidence intervals. **B,** Slopes of individual time plots as calculated by linear regression analysis. *Closed circles*, hyposensitized group; *open circles*, control group.

for lymphocyte function–associated antigen (LFA)-1/2 and BBA4, all monoclonal antibodies were directly conjugated to either fluorescein isothiocyanate or phycoerythrin. Measurement and analysis was done with a FACScan flow cytometer (Becton Dickinson, San Jose, Calif.) with Simulset, FACScan, Consort 30, and Lysis research software (Becton Dickinson, San Jose, Calif.). Both absolute cell numbers per microliter and percentages were calculated.

Statistical analysis

Professional STATA 3.0 Statistics/Data Analysis (Computing Resource Center, Santa Monica, Calif.) and EGRET (version 0.26.6, 1991, Serc and Cytel, Seattle, Wash.) were used for data analysis and statistical calculations.

The change of the disease activity scores during treatment was analyzed within each group with the Wilcoxon signed-rank test. An exact trend test was used to compare the hyposensitized and control groups with regard to the distribution of the scores at the end of the study.

The distributions of SI and cell counts were transformed to normal by taking natural log values. This enables use of parametric statistical methods. The resulting distributions were checked for normality in normal plots.

Linear regression was used to analyze time trends of

Table II. Distribution of lymphocyte phenotypes

Cell type	Nickel-allergic subjects		Nonallergic controls
	Hyposensitized	Control	
Lymphocytes	2.0 (1.4-4.1)	1.3 (1.0-2.5)	1.7 (1.1-2.6)
B cells	0.2 (0.08-0.4)	0.1 (0.05-0.2)	0.1 (0.1-0.2)
T cells	1.4 (1.1-3.2)	1.1 (0.7-2.2)	1.2 (0.8-2.2)
CD4	1.1 (0.6-2.5)	0.7 (0.5-1.4)	0.7 (0.4-1.5)
CD8	0.5 (0.3-1.0)	0.3 (0.1-0.7)	0.4 (0.2-0.7)
Natural killer cells	0.2 (0.08-0.3)	0.1 (0.1-0.2)	0.2 (0.06-0.6)

Values are median absolute cell counts per milliliter; 95% confidence intervals shown in *parentheses*.

lymphocyte proliferation and activated T-cell numbers during treatment.[28] The resulting slope values of both groups were compared in a *t* test modified for unequal variances (Welch test). Equality of variances was tested in the variance ratio test (*F* test).

RESULTS

Baseline values of patch tests and LPA

The distributions of baseline values for both patch tests and LPA in the hyposensitized and control groups were compared. Patch test scores and specific lymphocyte proliferation were comparable in both groups. In both the hyposensitized and the control groups a patient previously (before study) patch test reactive to nickel showed a negative baseline value of the patch test at the start of treatment. However, LPA results showed that lymphocyte reactivity to nickel still existed.

UVB dose

Individual UVB doses ranged between 5 and 50 mJ/cm^2. In both groups the cumulative dose of 1.0 J/cm^2 was reached within the first 4 months.

Nickel sulfate administration

Most patients tolerated a 100- to 1000-fold increase of the nickel dose within 3 months. In two patients the maximum dose of 1.0 ml of 10^{-3} mol/L nickel sulfate was reached. Adverse effects remained limited to transient local induration that developed at the site of injection within 8 to 24 hours. These lesions were histologically characterized by a perivascular accumulation of mononuclear cells. To avoid this reaction, doses were temporarily lowered. As a result, in some patients doses varied, occasionally by 10-fold, because of variable nickel tolerability.

Clinical evaluation

Fig. 1 shows dot plots of the four disease activity scores in both groups at three time points: at the start of the study (month 0), during maximal UVB exposure (month 6), and at the end of the study (month 18). Overall, scores within both groups dropped during UVB treatment. This trend continued even after UVB exposure was stopped. In the hyposensitized group this led to *p* values of 0.0076, 0.0076, 0.065, and 0.045 for the affected area, severity, frequency, and therapy scores respectively. In the control group these *p* values were larger, namely, 0.021, 0.021, 0.94, and 0.14, respectively. The change of the disease activity scores was most evident for the affected area and severity scores. However, between the hyposensitized and control groups no statistically significant score differences were found ($p = 0.84$, 0.33, 0.26, and 0.40, respectively).

Lymphocyte proliferation

Specific lymphocyte reactivity against nickel was monitored during the hyposensitization therapy. Within each patient some variation of SIs was found during the 2-year observation period. Neither SI nor net counts per minute values showed significant correlation with clinical scores.

In Fig. 2, *A*, mean SI values and 95% confidence intervals of all patients within each group are plotted against duration of treatment. Mean proliferation indices over time varied between 10 and 30. No significant difference was seen between the groups. In an attempt to characterize further the time course of lymphocyte reactivity, slopes of individual time plots were calculated by linear regression analysis. The resulting slope values indicate ascending or descending trends of lymphocyte reactivity with duration of treatment. However, neither positive nor negative slope values corresponded with clinical improvement. Fig. 2, *B*, shows approximately equivalent mean slope values but increased slope variance in the hyposensitized group as compared with control subjects. This difference of variances was highly significant ($p < 0.001$).

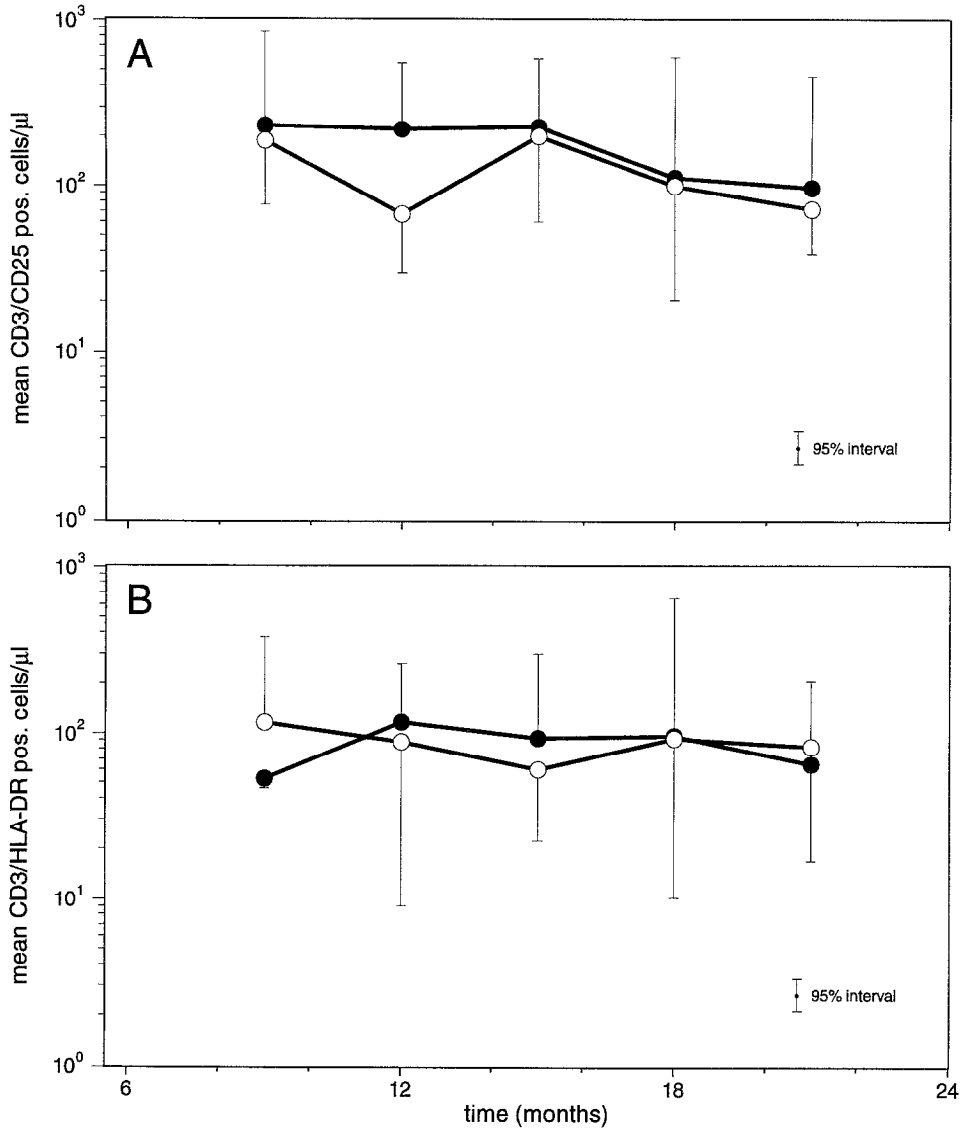

Fig. 3. Mean CD3+/IL-2 receptor+ (**A**) and CD3+/HLA-DR+ (**B**) cell numbers over time in all patients within each group. *Bars* represent 95% confidence intervals. *Closed circles*, hyposensitized group; *open circles*, control group.

Fluorescence-activated cell sorter immunophenotyping

Numbers of B cells, T cells (including the subpopulations), and natural killer cells were within normal limits, comparable in both groups, and fairly constant over time (Table II).

In an attempt to characterize further the nickel-reactive lymphocytes, we did flow cytometric analysis of peripheral blood mononuclear cells for activated T cells with CD3/HLA-DR and CD3/CD25 double staining. Actual double-positive cell numbers per microliter were calculated and plotted against time. Values ranged from 10 to 1000 cells/µl in both groups. Fig. 3 shows the time course of mean $CD3^+$/IL-2 $receptor^+$ and $CD3^+$/$HLA\text{-}DR^+$ cell counts and 95% confidence intervals of all patients within each group. On average, $CD25^+$ counts were 1.7 times higher than $HLA\text{-}DR^+$ counts. As previously described,[29] coexpression of IL-2 receptor and HLA-DR on T cells is limited. Apparently these markers represent at least partially different types or phases of activation. The average number of activated T cells was estimated at approximately 100 to 150 cells/µl (i.e., 5% to 20% of circulating T cells). These values are within normal limits.

LFA-1α and intercellular adhesion molecule–1 expression of both peripheral blood lymphocytes and monocytes was also measured. Lymphocytes showed

a bimodal LFA-1α expression, that is, normal (LFA-1^+) and high (LFA-1^{++}), mainly because of the expression of this molecule on T cells. During the study these two levels of expression appeared to be inversely correlated. These time trends, however, did not match clinical disease activity.

DISCUSSION

Hyposensitization with contact allergens has previously been shown to be effective.[30-34] The present study describes monitoring of clinical and immunologic features during such nickel hyposensitization.

Considerable clinical improvement was observed during UVB pretreatment. In both groups this trend persisted even after UVB withdrawal. No statistically significant difference between the groups was seen.

Lymphocyte reactivity to nickel appeared to fluctuate with time. Height of SI showed no correlation with clinical scores. Discordance between clinical and in vitro findings has previously been described.[35-40]

Overall, no significant change in the in vitro lymphocyte reactivity to nickel was seen during the monitored period. However, time-trend analysis of each patient revealed a significantly larger slope variability within the hyposensitized group. Its meaning is not entirely clear. Possibly the effect of hyposensitization varies in different persons.[21, 41] Activated T lymphocytes in peripheral blood, measured as $CD3^+/HLA\text{-}DR^+$ and $CD3^+/CD25^+$ cells, remained within normal limits during treatment. It is conceivable that the number of nickel-specific memory T lymphocytes is too low to allow detection of changes in the peripheral circulation.

As in type I hyposensitization, the mechanisms by which clinical improvement might be explained remain to be elucidated. Evidence is emerging that besides LC and T lymphocytes, keratinocytes are also actively involved in immunologic reactions in the skin. Insight into the process of induction of central or peripheral tolerance, clonal anergy, or active suppression is only beginning to appear.[42-45] Analysis of epidermal cells and lymphocytes for the presence and susceptibility of costimulatory factors, such as adhesion molecules[46-48] and cytokines,[7, 49, 50] and the influence of immunomodulators such as UV radiation on these, may provide more insight into this process.

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