

Identification of anti-inflammatory drugs according to their capacity to suppress type-1 and type-2 T cell profiles

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Summary

Background Down-regulation or modulation of T cell activity by immunosuppressive drugs is an effective treatment in diseases where exaggerated T cell responses play a role. A primary effect of the anti-inflammatory drugs (AIDs) is inhibition of the synthesis of growth factors, such as IL-2, thereby down-regulating T cell proliferation. However, it is still largely unknown to what extent these AIDs are able to down-regulate specifically type-1 or type-2 T cell cytokine production, and whether they can down-modulate chemokine receptor expression, thereby preventing migration of T cells to the site of inflammation.

Objective We investigated the suppressive effect of dermatologically used AID (cyclosporin A (CsA), lactoferrin (LF), 1α , 25-dihydroxyvitamin D₃ (VD₃), hydrocortisone (HC), di-methyl-fumarate (DMF), diclofenac (DF)) on both type-1 and type-2 T cells. Since allergic contact dermatitis is a skin disorder in which an exaggerated T cell response of both types of T cell subsets can be observed, we used this disorder as a model to study the capacity of AID to suppress type-1 or type-2 T cell responses.

Methods Peripheral blood mononuclear cells of nickel allergic patients were cultured in the presence of allergen and increasing concentrations of AID. Proliferation was determined by measuring ³H thymidine incorporation; chemokine receptor (CCR10, CCR4, CXCR3) expression was studied by flow cytometric analysis and IFN- γ or IL-5 cytokine production was measured by ELISA.

Results Three major patterns can be distinguished regarding the effect of AID on T cell responses. The first group, including CsA and LF, inhibited non-selectively T cell proliferation, chemokine receptor expression and cytokine production, with CsA as the most potent drug tested. A second group of AID, which included VD₃, HC and DMF, suppressed mainly type-1 T cell responses, as revealed by strong interference with IFN- γ production and CXCR3 expression, and limited effects on either or both IL-5 and CCR4 expression. The third pattern was displayed by DF, which down-regulated IL-5 production and CCR4 expression, whereas IFN- γ and CXCR3 were unaltered.

Conclusions Using a contact allergy model, we have demonstrated that various AIDs show distinct pharmacological profiles in that either type-1 or type-2 or both T cell responses are suppressed. These results should contribute to a more rational selection of AID in treating inflammatory skin diseases mediated by either or both of these T cell subsets.

Keywords allergic contact dermatitis, anti-inflammatory drugs, chemokine receptor, cytokine, down-regulation, T cell

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Introduction

Down-regulation of T cell activation by immunosuppressive drugs is known to be a primary target in the treatment of patients with inflammatory skin disorders [1–7]. The immunosuppressive drugs cyclosporin A (CsA) and hydrocortisone (HC) are widely used in clinical practice to treat

inflammatory skin diseases [3, 4]. Di-methyl-fumarate (DMF) and 1α , 25-dihydroxyvitamin D₃ (VD₃) have been shown to be effective in psoriasis [2, 6–9]. Diclofenac (DF) – a non-steroidal anti-inflammatory drug (NSAID) – is well known as a broadly active anti-inflammatory drug (AID). Lactoferrin (LF) has been shown to have primarily anti-bacterial properties but may also contribute to the regulation of immune and inflammatory responses [10].

The immunomodulatory action of these drugs is primarily based on the synthesis of cytokines, notably IL-2, and thereby suppress proliferative capacity of T cells [3, 5, 11–13].

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However, little is known about the differential effects of these drugs on type-1 or type-2 cytokine production. Patients with psoriasis, a type-1 T cell disease, would benefit from down-regulation of IFN- γ and IL-2, whereas patients with atopic dermatitis (AD), a type-2 T cell disease, might benefit from lowering of IL-4 and IL-5 levels. Another possibility to suppress hyper-reactive T cells is by suppressing a subset of T lymphocytes expressing skin specific chemokine receptors. Down-regulation of these receptors might be a reasonable strategy in the treatment of disease by preventing T cells from entering the site of inflammation.

The present study addresses the inhibitory effects of different immunosuppressive drugs on type-1 and type-2 T cells. Interestingly, allergic contact dermatitis (ACD) is characterized by the hyper-reactivity of both type-1 and type-2 T cells [14–16]. T cell reactivity to contact allergens, such as nickel, results in proliferating CD4⁺ memory T cells producing both IFN- γ and IL-4/IL-5 [15]. Moreover, effector T cells can up-regulate chemokine receptors CCR10, CXCR3 (expressed on type-1 T cells) or CCR4 (on type-2 cells), enabling preferential migration of type-1 or type-2 T cell subsets from the blood into the inflammatory skin. [15, 17–21].

Here, using nickel contact dermatitis as a model in which both type-1 and type-2 T cells can be activated, [15, 22] the capacity of AID to down-regulate nickel-induced T cell proliferation will be studied. Next to this, the capacity to modulate chemokine receptor expression on nickel-activated T cells will be determined. Finally, the potential of these drugs to suppress preferentially type-1 or type-2 T cells will be examined. In order to detect nickel-induced cytokine production, lymphocytes are cultured in the presence of either type-1 stimulating cytokines (i.e. IL-7 and IL-12) or type-2 stimulating cytokines (IL-7 and IL-4) in combination with the allergen.

Materials and methods

Patients

Seven nickel-allergic patients with an eczematous dermatitis after contacting nickel were included in this study. Nickel allergy was evaluated according to the standard patch test procedure using 5% nickel sulphate in vaseline or water; responses were read at 48, 72 and 144 h after application. To avoid the possible influence of a positive patch test, blood samples were obtained before or at least 6 weeks after patch testing. Each patient is represented as a unique symbol in all consecutive figures described in Results.

Lymphocyte proliferation assay

Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized blood by density centrifugation on Ficoll-Paque (Pharmacia Biotech, Uppsala, Sweden). PBMCs were used in a lymphocyte proliferation test. Cells were resuspended in complete medium (Iscove's Modified Dulbecco's Medium (Biowhittaker, Verviers, Belgium) supplemented with 50 IU/mL penicillin-G, 50 μ g/mL streptomycin, 50 μ M dithiotreitol (Merck, Darmstadt, Germany) and 10% human pooled serum (Biowhittaker)) and stimulated by culturing in

the presence or absence of 50 μ M nickel sulphate hexahydrate (NiSO₄, Merck, Darmstadt, Germany). Together with nickel sulphate, six different AIDs were added in varying concentrations, with the highest concentration being the maximal non-toxic concentration. Viability experiments with trypan blue exclusion were performed to determine these non-toxic concentrations for the different drugs used (in all concentrations more than 75% cells are viable, data not shown). Cells were cultured with CsA (Novartis, Arnhem, the Netherlands) or VD₃ (Leo Pharmaceutical Products, Copenhagen, Denmark) in a concentration ranging from 10^{−10} to 10^{−6} M. HC (Bufa Pharmaceutical Products, Uitgeest, the Netherlands), DMF (Kethel Pharmacy, Schiedam, the Netherlands) and DF (Bufa Pharmaceutical Products) were added in a concentration range from 10^{−8} to 10^{−4} M. LF (Agennix, Houston, TX, USA) addition ranged from 10^{−8} to 10^{−6} M. The drugs were all tested at the same time, to rule out variability between drugs because of day-to-day experimental variation.

All cell cultures were performed in quadruplicate in a final volume of 200 μ L with a cell concentration of 2 $\times$ 10⁵ cells/well in 96-well round-bottomed microtiter plates. To determine nickel-specific proliferative responses, cells were cultured for 6 days at 37 °C, 5% CO₂, 95% humidity. After 6 days, T cell proliferation was measured by ³H thymidine incorporation as follows: cells were pulsed with 0.5 μ Ci ³H thymidine (Amersham, Pharmacia, Bucks, UK) per well during the last 5 h of culture. Radioactivity was measured by a liquid scintillation counter (Wallac, Turku, Finland) and the proliferative capacity was expressed as counts per minute (c.p.m.).

Cytokine measurement

To determine cytokine production, additional cytokine supplements consisting of IL-12 (1 ng/mL; Strathmann Biotech GMBH, Hannover, Germany) and IL-7 (0.1 ng/mL; Strathmann Biotech GMBH) or IL-4 (3 ng/mL; Strathmann Biotech GMBH) and IL-7 were used during culture. These supplements were added to evaluate respectively type-1 T cell response (IFN- γ production) or type-2 response (production of IL-5) as described elsewhere [15, 22]. After 6 days of culture, supernatants were harvested and stored at −30 °C until testing. Production of IFN- γ was measured by IFN- γ PeliPair reagent set (Sanquin, Amsterdam, the Netherlands; lower detection level 40 pg/mL) following the manufacturer's instructions. IL-5 was measured by commercially available ELISA antibodies (coating antibody (JES1-39D10), detection antibody (JES1-5A10) and standard (human recombinant IL-5) from BD Pharmingen (San Diego, CA, USA); lower detection level 8 pg/mL).

Flow cytometric analysis

To study the chemokine receptor expression after nickel-specific stimulation of PBMCs with or without addition of AIDs, flow cytometric analysis (FACS) was performed. Cells were stained for 30 min with antibodies against: CD3-PeCy5 or CD3-PE (clone CLB-T3/2, from Sanquin) CXCR3-PE (clone 1C6), CCR4-PE (clone 1G1) – both from BD Pharmingen. A rabbit polyclonal antibody against the chemokine receptor CCR10 (Rabbit Ig, IMG-286; Imgenex,

San Diego, CA, USA), followed by anti-rabbit PE antibody labelling (Southern Biotechnology Associates, Inc., Birmingham, AL, USA), was used to measure the expression of CCR10. Since this antibody is directed against an epitope in the cytoplasmic domain of the receptor, cells were first fixed with formaldehyde (2%) and permeabilized with saponine (0.1%; Merck, Darmstadt, Germany). Cells were measured on a FACScan and analysed with CellQuest software (Becton Dickinson Immunocytometry Systems, Mountain View, CA, USA).

Statistical analysis

For statistical evaluation, the paired *t*-test was used (medCalc. Statistical software Version 6, Mariakerke, Belgium). *P*-values < 0.05 were considered significantly different.

Results

Cyclosporin A and lactoferrin inhibit nickel-induced T cell proliferation, chemokine receptor expression, type-1 and type-2 cytokine production

As we have previously reported [15], addition of NiSO₄ to peripheral blood T cells from nickel-allergic patients resulted in an increased proliferative response (Fig. 1a). In addition, the number of cells expressing chemokine receptors involved in T cell migration to the skin (CCR10, CCR4 and CXCR3) were significantly up-regulated when stimulated with nickel (Figs 1b, c and d respectively). Moreover, both type-1 (IFN- γ) and type-2 (IL-5) cytokines were increased in the nickel-stimulated cultures (Figs 1e and f). In this study, various concentrations of immunosuppressive drugs were added in combination with the allergen nickel, and the down-regulatory/modulatory effects of different drugs on nickel-induced T cell activation were examined.

The calcineurin inhibitor CsA completely suppressed the nickel-induced augmentation of T cell proliferation in all individuals at all concentrations tested in the range of 10^{-10} – 10^{-6} M (Fig. 1a). Likewise, increased chemokine receptor expression was completely suppressed to basal expression by CsA (10^{-10} M, CCR10: Fig. 1b, CCR4: Fig. 1c, CXCR3: Fig. 1d). IL-5 and IFN- γ production were below detection limits when CsA was added to the culture (10^{-10} M, Figs 1e and f). Taken together, these findings clearly demonstrate that CsA completely blocks nickel-induced T cell activation.

The effect of the anti-bacterial compound LF on nickel-activated T cells was studied in a concentration range varying from 10^{-8} to 10^{-6} M (Fig. 1a). LF suppressed the increased proliferation, chemokine receptor expression and cytokine production similar to the CsA, although less effective. Proliferation (Fig. 1a) and chemokine receptor (Figs 1b–d) expression could significantly be suppressed by the highest concentration of LF (10^{-6} M). However, IL-5 and IFN- γ production could only partly be suppressed (Figs 1e and f).

Vitamin D₃, hydrocortisone and di-methyl-fumarate suppress mainly type-1 T cell responses

Addition of VD₃ to PBMC at a concentration range of 10^{-10} – 10^{-6} M resulted in the suppression of nickel-induced T

cell proliferation (significant at 10^{-6} M, Fig. 2a). Comparable with proliferation, increased chemokine receptor expression can be reduced by VD₃ to basal expression (10^{-6} M, Figs 2b–d). Allergen-induced IL-5 secretion could not significantly be reduced in response to VD₃ (Fig. 2e). Increased IFN- γ production, however, could be suppressed to basal levels after addition of VD₃ (Fig. 2f). Thus, although the effect of VD₃ on proliferation and chemokine receptor expression is reduced as it is in CsA-added cultures, the down-regulatory effect of VD₃ on cytokine production is different. Only type-1 and not type-2 cytokine production can completely be suppressed, which might help to restore the type-1/type-2 T cell balance.

The corticosteroid HC suppressed nickel-activated T cell proliferation in a dose-dependent way (concentration range from 10^{-10} to 10^{-6} M, Fig. 2a). Chemokine receptor expression analysis demonstrated that only CCR10 and CXCR3 (mainly expressed on type-1 T cells) but not CCR4 (preferentially on type-2 cells) could be down-regulated to basal expression (Figs 2b–d). Type-1 (IFN- γ) and type-2 (IL-5) cytokines are equally affected by HC; they are both strongly suppressed.

Culturing nickel-activated T cells in combination with the anti-psoriatic drug DMF resulted in the suppression of proliferation only in the highest concentration tested (10^{-4} M, Fig. 2a). CCR10 and the type-1-related receptor CXCR3 were down-regulated, whereas the type-2 associated receptor CCR4 was unaffected (Figs 2b–d). In line with the receptor expression, we found that only IFN- γ and not IL-5 can completely be blocked by DMF (Figs 2e and f). Together, these results indicate that also DMF has the highest down-regulatory effect on type-1 T cells, whereas type-2 cells are unaffected.

Diclofenac modulates nickel-induced T cell response away from a type-2 response

Nickel-stimulated T cells were exposed to various concentrations of DF ranging from 10^{-8} to 10^{-4} M. Figure 3a demonstrates that only the highest concentration of 10^{-4} M gives a significant reduction of the proliferative response. In parallel, the chemokine receptors CCR10 and CCR4 were significantly reduced when DF was added (Figs 3b and c). However, the chemokine receptor CXCR3 could not be down-regulated by addition of DF (Fig. 3d). In accordance with the down-regulation of the type-2 chemokine receptor CCR4 and not of the type-1 related receptor CXCR3, we found that DF is able to suppress only IL-5 production (Fig. 3e). IFN- γ secretion was unaltered when nickel-stimulated T-cells were cultured in the presence of DF (Fig. 3f). These results indicate that DF has an immunomodulatory effect on nickel-induced T cell reactivity, by preferential suppression of type-2 cells.

Discussion

Down-regulation of T cell activation by immunosuppressive drugs is a major therapeutically target in the treatment of skin inflammatory diseases, such as psoriasis, AD and ACD [1–7]. The most important mechanism of action is inhibition of IL-2

Suppression of type-1 and type-2 T cells

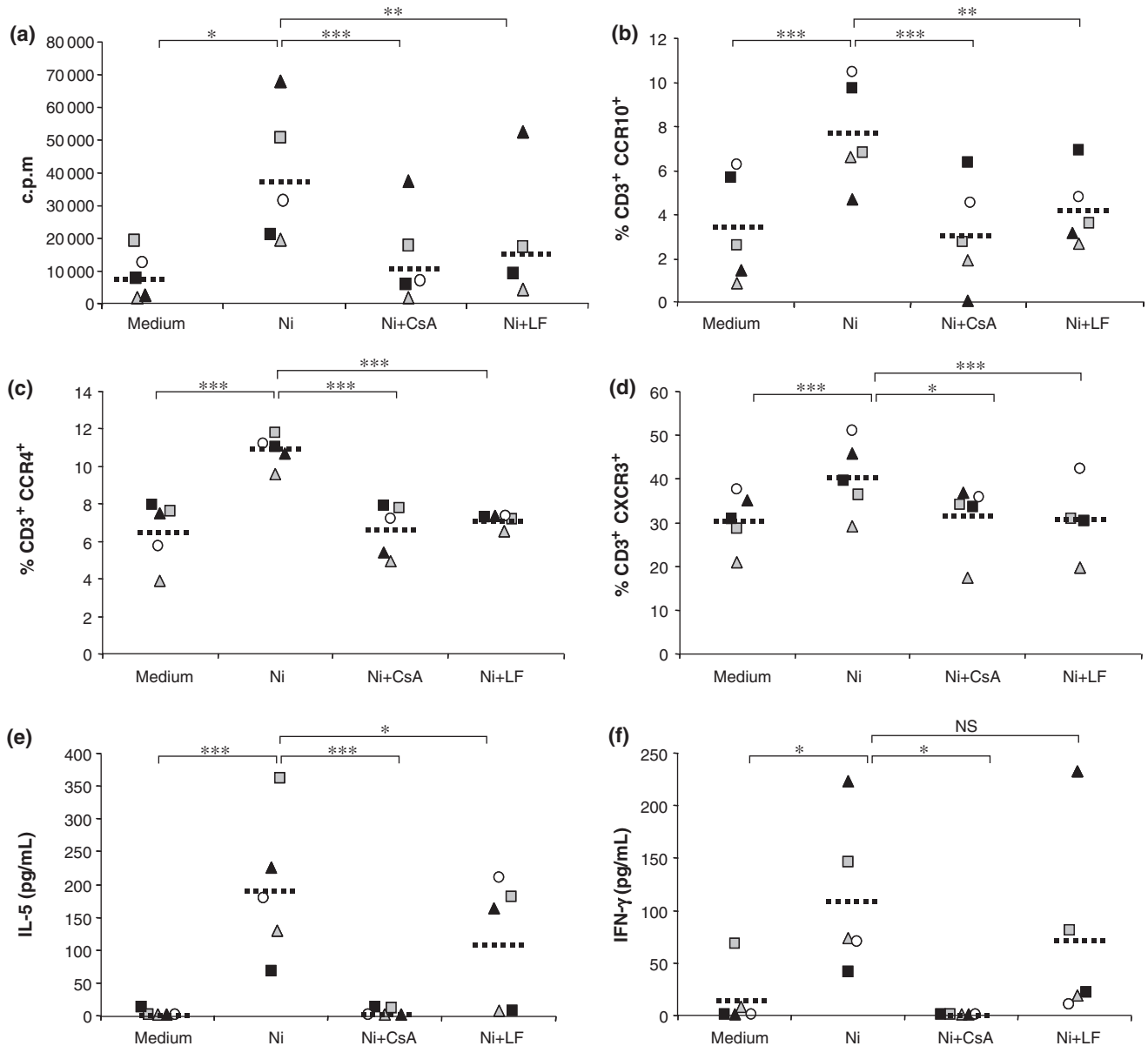


Fig. 1. Immunosuppressive effect of cyclosporin A (CsA) and lactoferrin (LF) on nickel activated T cells. Peripheral blood mononuclear cells of nickel-allergic patients were cultured in the presence or absence of 50 μ M nickel sulphate in combination with increasing concentrations of CsA or LF. After 6 days, proliferation (a), chemokine receptor expression (CCR10: b, CCR4: c, CXCR3: d) and cytokine production (IL-5: e, IFN- γ : f) were measured. Different symbols used in the figures correspond to the data from the different individuals tested, with each individual as a unique symbol. The effect of CsA or LF on proliferation, chemokine receptor expression and cytokine secretion is shown in the concentration which has significant suppressive effect on the proliferative response (10^{-10} M for CsA and 10^{-6} M for LF). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.005$.

production, and thereby blocking proliferative capacity of T cells [3, 5, 11–13]. A more selective strategy to suppress T cell hyper-reactivity is by preferential down-regulation of type-1 cytokines, potentially favourable in diseases such as psoriasis, or suppression of type-2 cytokines that are effective in type-2 related disorders such as AD. Another way of treating inflammatory diseases is to prevent T cells from migrating into the skin, e.g. by suppressing chemokine receptors involved in skin homing. In the present study, we evaluated the suppressive and modulatory effects of various AIDs used in dermatological diseases (i.e. CsA; LF; VD₃; HC; DMF; and DF) *in vitro*. To this end an *in vitro* model was set up with

lymphocytes from nickel-allergic donors that show both type-1 and type-2 T cell responsiveness. Three patterns are revealed: total suppression of T cell activity (mediated by CsA and LF), selective suppression of type-1 response (VD₃, HC, DMF) or type-2 T cells (DF), respectively.

The AID CsA is the most suppressive of all drugs tested. As expected from literature [23], it completely suppressed T cell function already at low concentrations. These *in vitro* test concentrations are within the range of therapeutic concentrations (from 10^{-8} to 10^{-7} M) [24]. CsA is useful for treating not only dermatological disorders, but for all immune system-related diseases where exaggerated T cell responses are

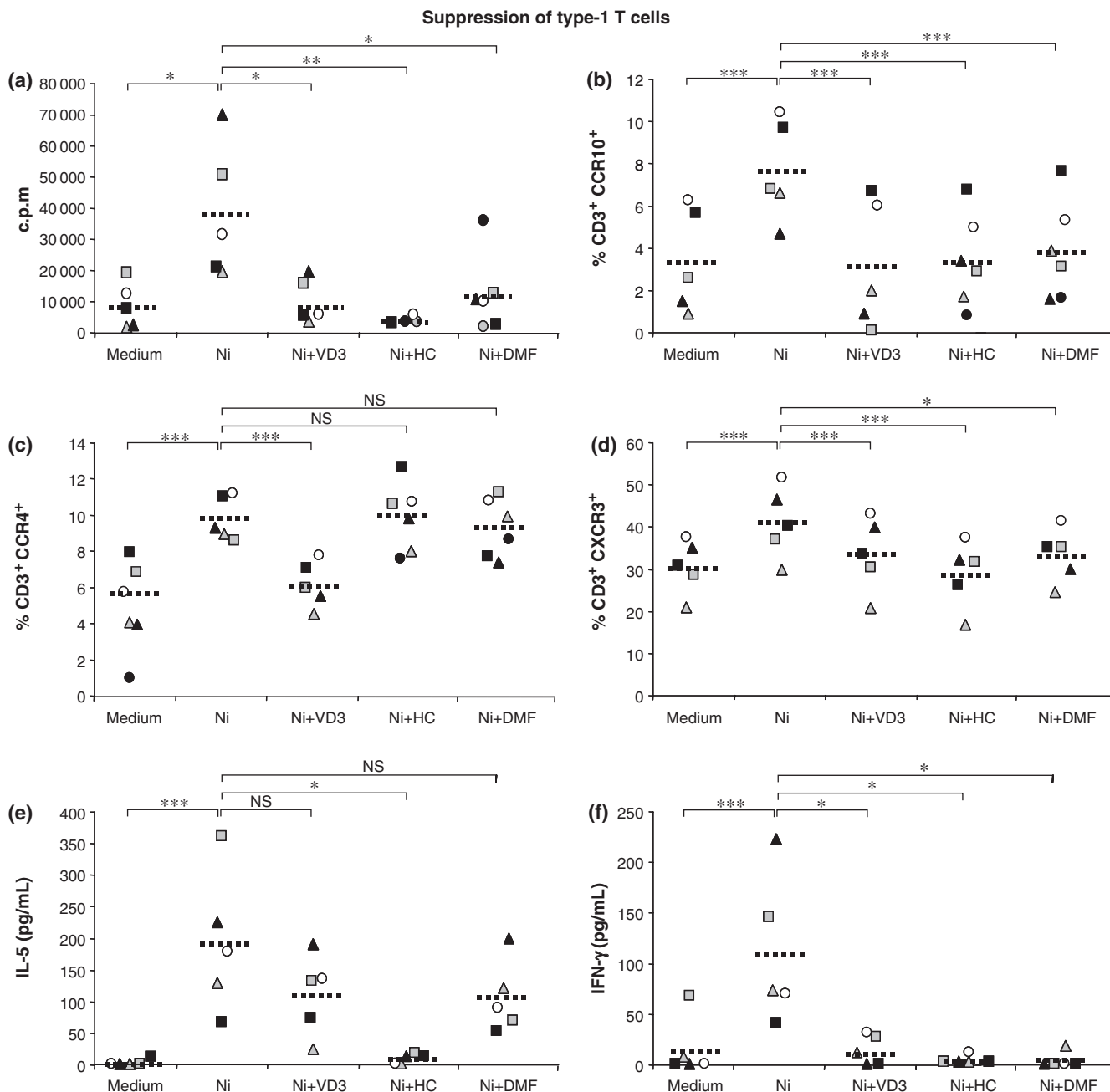


Fig. 2. Immunosuppressive/modulatory effect of 1α,25-dihydroxyvitamin D₃ (VD₃), hydrocortisone (HC) and di-methyl-fumarate (DMF) on nickel-activated T cells. Peripheral blood mononuclear cells of nickel allergic patients were cultured in the presence or absence of 50 μM nickel sulphate in combination with increasing concentrations of VD₃, HC or DMF. After 6 days, proliferation (a), chemokine receptor expression (CCR10: b, CCR4: c, CXCR3: d) and cytokine production (IL-5: e, IFN-γ: f) were measured. Different symbols used in the figures correspond to the data from the different individuals tested, with each individual as a unique symbol. The effect of VD₃, HC or DMF on proliferation, chemokine receptor expression and cytokine secretion is shown in the concentration which has significant suppressive effect on the proliferative response (10⁻⁶ M for VD₃, 10⁻⁴ M for HC and 10⁻⁴ M for DMF). **P* < 0.05; ***P* < 0.01; ****P* < 0.005.

involved [23]. It blocks T cell function by mechanisms that involve the inhibition of the key signalling phosphatase calcineurin [23]. As a consequence, total T cell activity is suppressed, and results in down-regulation of immune reactivity.

Next to the well-studied drug CsA, we investigated the immunosuppressive ability of the protein LF. LF has been shown to be involved in inflammatory, antitumoral and differentiation processes [25, 26]. It can influence and control

local cytokine production in the skin, mainly by local inhibition of TNF-α [27]. Moreover, Griffiths et al. [10] Cumberbatch et al. [28] demonstrated that topical LF administration prior to allergen exposure resulted in a reduction of allergen-induced Langerhans cell migration from the epidermis. This supports the view that LF is capable of influencing cutaneous inflammatory responses. Zimecki et al. [29] described that LF has inhibitory effects on T-helper type 1 cell lines, which can be explained by the observation that LF

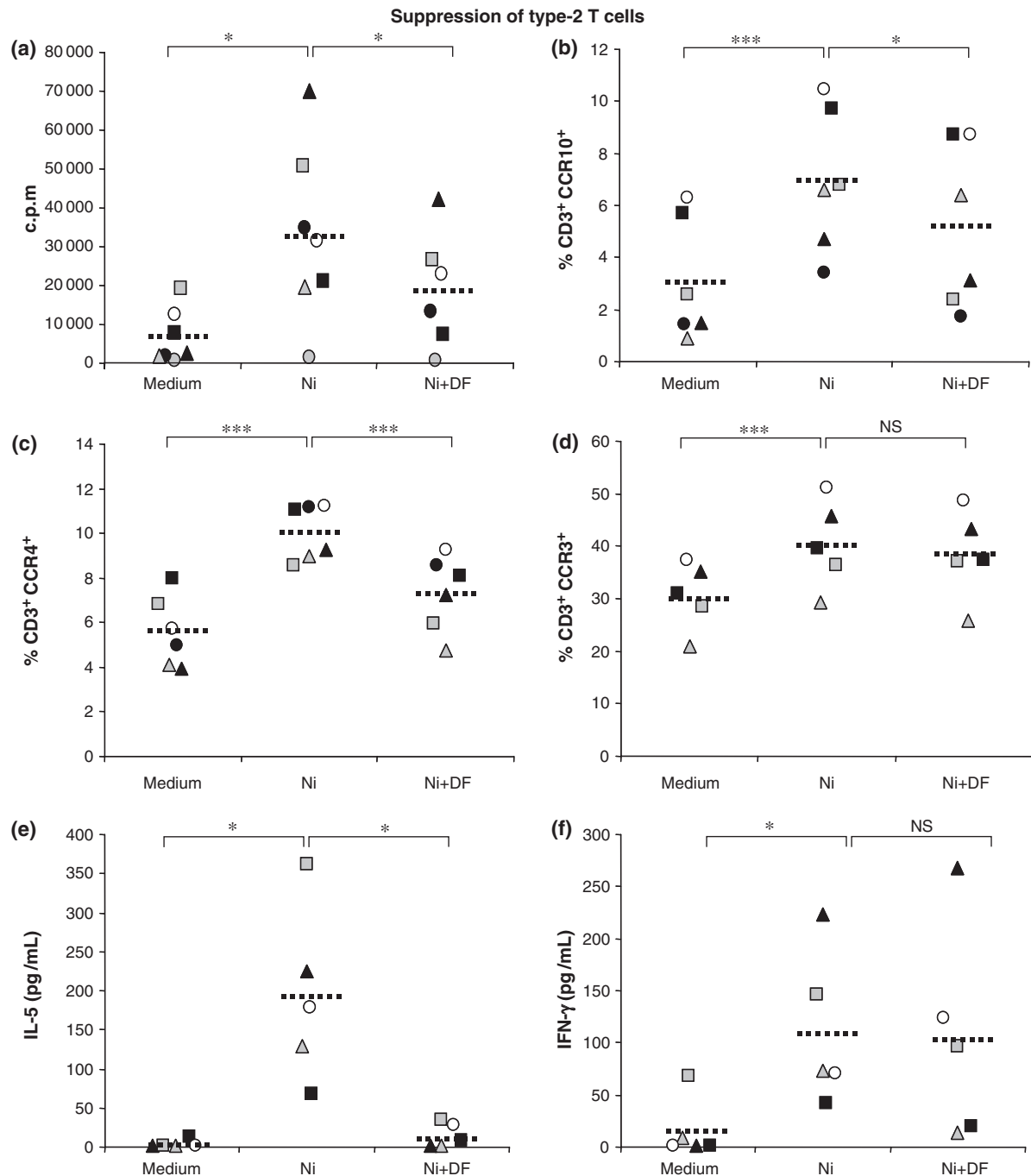


Fig. 3. Immunosuppressive/modulatory effect of diclofenac (DF) on nickel-activated T cells. Peripheral blood mononuclear cells of nickel-allergic patients were cultured in the presence or absence of 50 μ M nickel sulphate in combination with increasing concentrations of DF. After 6 days, proliferation (a), chemokine receptor expression (CCR10: b, CCR4: c, CXCR3: d) and cytokine production (IL-5: e, IFN- γ : f) were measured. Different symbols used in the figures correspond to the data from the different individuals tested, with each individual as a unique symbol. The effect of DF on proliferation, chemokine receptor expression and cytokine secretion is shown in the concentration, which has significant suppressive effect on the proliferative response (10^{-4} M). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.005$.

mediates the down-regulation of IL-2 receptor expression. Moreover, the LF receptor is expressed on activated and not on unstimulated human blood lymphocytes [30], indicating a role for LF in the regulation of T cell responses. Our results showed that LF is capable of suppressing general T cell activity, by down-regulation of proliferative responsiveness as well as chemokine receptor expression and cytokine production, although less effective than CsA. Since LF can be

applied topically onto the skin it is a very promising agent for the treatment of a broad spectrum of inflammatory skin diseases, in the near future.

VD₃ is described as an immunoregulatory steroid hormone involved in differentiation, growth and function of a broad range of cells, including immune cells and keratinocytes [31, 32]. *In vivo*, it has been demonstrated to suppress type-1-mediated immune diseases by increasing IL-4 production and

decreasing IFN- γ levels [31]. Inhibition of type-1 cell immunity is thought to be mediated via the reduced IL-12 production of antigen-presenting cells [33, 34]. Here, we demonstrate that VD₃ affects T cells by down-regulating nickel-induced T cell proliferation and by specifically suppressing nickel-induced IFN- γ but not IL-5 secretion. However, in contrast to cytokine production, both type-1- and type-2-related chemokine receptors are affected by VD₃. The direct effect of VD₃ to down-regulate, especially, type-1 cytokines, in addition to suppression of chemokine receptor expression, may explain its beneficial effect in psoriasis [6], and may also be a good strategy in the treatment of patients with ACD.

Corticosteroids are potent anti-inflammatory agents, widely used in the treatment of skin inflammation [12]. Modulation of cytokine production seems to be the primary mechanism by which T cells are suppressed [11, 13]. However, contradictory results have been published concerning the effect of HC on cytokine production. Some investigators claim that HC treatment results in IL-4 enhancement and IFN- γ inhibition [35, 36], whereas others state that IL-4 and IFN- γ are both down-regulated with more pronounced effects on IL-4 [37–39]. The present results show that allergen-induced IL-5 and IFN- γ secretion by T cells can be completely suppressed by HC. This decrease of cytokine production is accompanied by a similar decrease in proliferative response. However, whereas proliferation and cytokine production are effectively down-regulated by HC, nickel-induced CCR4 expression is unaffected. This suggests that CCR4-expressing cells are still capable to migrate to the site of inflammation. In addition to HC, more potent corticosteroids (i.e. Dexamethason, Triamcinolone) were tested in this study (data not shown). Like in the clinical situation, these agents were able to suppress *in vitro* T cell reactivity more effectively than HC. Concentrations of 10⁻⁸ M (Dexamethason) and 10⁻⁶ M (Triamcinolone) were found to be equally effective as 10⁻⁴ M HC.

Fumaric acid derivates have been shown to be effective in psoriasis [2, 40]. Recent studies showed that in patients treated with fumarate IFN- γ expression was down-regulated, followed by a down-regulation of IL-4 expression [8]. Moreover, DMF was described to modulate T cell response away from type-1 towards type-2 cytokine production [9, 41]. In line with these results, we demonstrate that DMF suppresses the allergen-induced T cell proliferation and modulates the cytokine balance by suppressing only IFN- γ and not IL-5, restoring the type-1/type-2 balance. In parallel with this specific type-1 suppression, we found that only CXCR3 and not CCR4 receptor expression was down-regulated by DMF. These results indicate that DMF which is effective in the type-1 mediated disorder psoriasis, is a promising agent in all diseases where a type-1 cytokine production is involved.

A distinction should be made between fumaric acid, which has no effect on psoriasis, and its systemically active esters, which have anti-psoriatic activity [2]. In this study we tested, next to DMF, the suppressive effect of fumaric acid on T cell reactivity (data not shown). These results demonstrate that only DMF, and not fumaric acid, is able to suppress T cell proliferation and cytokine production. These results show a clear correlation between *in vivo* effects and *ex vivo* results,

underlining the effectiveness of only DMF in suppressing T cell-mediated disease.

In contrast to DMF, DF has been described to modulate type-1/type-2 T cell balance by up-regulating type-1 associated cytokine mRNA and by down-regulating IL-4 mRNA expression [42]. Our findings corroborate these results at the protein level: only nickel induced IL-5 secretion and increased expression of CCR4 could be suppressed by the NSAID DF, whereas IFN- γ and CXCR3 expression stayed unaltered. Taken together, our results with DF suggest that NSAIDs exert their anti-inflammatory action by specifically suppressing type-2 T cells.

In summary, we have demonstrated that immunosuppressive and modulatory drugs are capable to differentially down-regulate contact-allergen-induced T cell proliferative response, chemokine receptor expression and cytokine production in distinct ways. Whereas some drugs suppress total T cell reactivity, others are able to specifically down-modulate type-1 or type-2 cytokine production. Moreover, chemokine receptor expression, particularly expressed on type-1 or type-2 cells, can also be down-regulated to basal levels, preventing cells to home to the site of inflammation. Interestingly, the modulation of CCR10 paralleled the effects observed for type-1 T cell markers. These results suggest that CCR10, CXCR3 and IFN- γ are regulated by similar mechanisms.

These results could be used in all kinds of inflammatory diseases, where an exaggerated T cell response (either over expression of type-1, type-2, or both kinds of cytokine-producing cells) has to be suppressed. Skin penetration studies are currently in progress to investigate the anti-inflammatory ability of topically applied LF in patients suffering from psoriasis. Since LF suppresses, next to IFN- γ and IL-5, also TNF- α [27] this drug is very promising and may have important implications as a local immunosuppressive drug in inflamed psoriatic skin. Local down regulation of TNF- α production is preferred above the currently used anti-psoriatic therapies, such as infliximab (Remicade[®]; Centocor, Malvern, PA, USA) [43] and etanercept (Enbrel[®]; Wyeth Pharmaceuticals, Philadelphia, PA, USA) [44], in which TNF- α blockers are used systemically.

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