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# A local lymph node assay to analyse immunosuppressive effects of topically applied drugs

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**Abstract**

Topical glucocorticosteroids represent the mainstay of antiinflammatory therapy in the treatment of inflammatory skin diseases. Their clinical use, however, is limited by local and systemic side-effects. Thus, in dermatopharmacology there is a large demand for alternative non-steroidal antiinflammatories. Other than transplantation models, most of the frequently used in vivo test systems for assessment of drug-induced immunosuppression measure changes in inflammatory skin responses by means of skin erythema and edema after challenge of sensitized animals. The aim of this study was to develop an alternative mouse model to detect and analyse immunosuppressive effects of topically applied drugs. On the basis of a modified local lymph node assay, we analysed effects of topical hydrocortisone, dexamethasone, mometasone furoate and FK506 (tacrolimus) during the induction phase of contact hypersensitivity. On 4 consecutive days, NMRI mice were treated on the dorsal surfaces of both ears with increasing concentrations of test compound. During the last 3 days, the mice received in addition the contact sensitizer, oxazolone (1%). On day 5, draining auricular lymph nodes were removed in order to assess lymph node cell counts and perform flow cytometric analysis of lymph node cell subpopulations ($CD4^+/CD25^+$, $Ia^+/CD69^+$, $Ia^+/B220^+$). All test compounds proved to exert significant immunosuppressive effects after topical application, but showed differences in their immunomodulatory potential. In conclusion, the local lymph node assay serves as an appropriate model to characterize immunosuppressive effects of topically applied drugs by measuring immunologically relevant end-points. © 1997 Elsevier Science B.V.

*Keywords:* FK506; Glucocorticosteroid; Immunosuppression; Local lymph node assay; Tacrolimus; Topical drug

## 1. Introduction

Topical glucocorticosteroids represent the mainstay of antiinflammatory therapy in the treatment of inflammatory skin diseases. Their clinical use, however, is limited by local and systemic side-effects, such as skin atrophy, teleangiectasia, striae distensae, diabetes and osteoporosis. Thus, in dermatopharmacology there is great demand for alternative non-steroidal antiinflammatories. Currently, the capacity of new chemicals to cause immunosuppression is assessed by means of in vitro lymphocyte activation tests, such as the mixed lymphocyte reaction (Kino et al., 1987; Swada et al., 1987; Morikawa et al., 1994). So far, only few in vivo models exist that detect and analyse drug-induced immunosuppressive effects (Gleichmann et al., 1989; Basketter et al., 1995). Drug-induced systemic immunosuppression is usually assessed by means of transplantation models, such as experimental rat allogenic heart, kidney and skin transplantation (Thomsen, 1990). Most of the frequently used test systems for drug-induced topical immunosuppression measure changes in skin inflammation. This is assessed by skin erythema and edema after challenge of animals sensitized with a model allergen (Meingassner and Stütz, 1992; Duncan, 1994). Although such assays have been, and continue to be, of proven value, they are not without limitations. The time-consuming tests measure 'subjective' and rather unspecific end-points and re-

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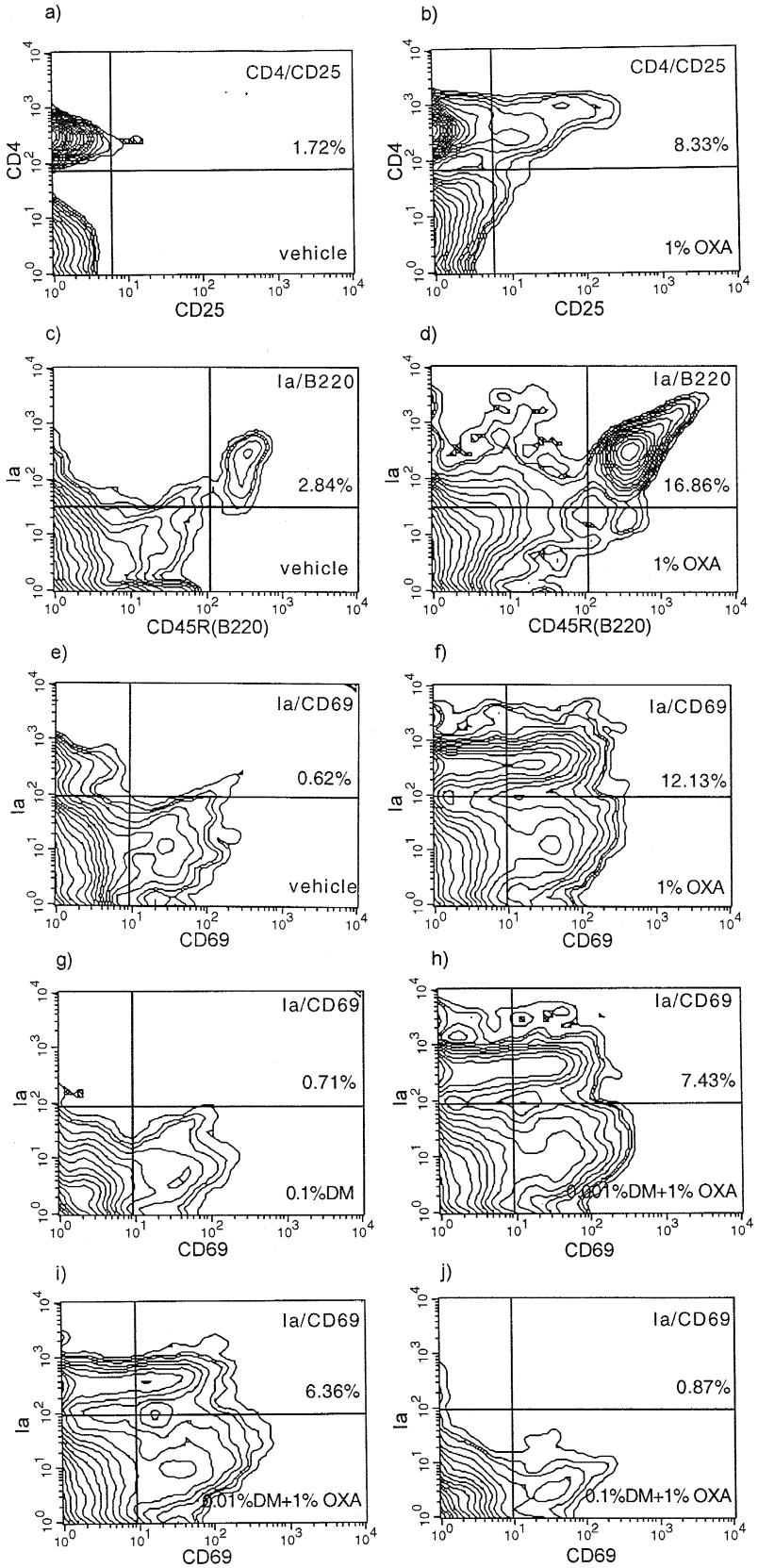
quire large numbers of test animals. Therefore, it is appropriate that attempts are made to design reliable alternative test methods with short-term protocols which measure objective parameters. Contact hypersensitivity has served as a useful model for the primary activation of T cells in skin and skin-associated lymphoid tissue, such as local



draining lymph nodes (Kimber and Weisenberger, 1989; Enk and Katz, 1995). During induction of contact hypersensitivity, antigen-presenting cells of the skin take up antigen and migrate to local lymph nodes where they present relevant antigen determinants together with co-stimulatory molecules to T cells and induce antigen-specific T-cell activation (Baker et al., 1989; Kripke et al., 1990; Enk and Katz, 1995; Kühn et al., 1995). Furthermore, T cells play an important role in inflammatory skin diseases, such as atopic dermatitis and psoriasis (Bos et al., 1994; Cooper, 1994; Bata-Csorgo et al., 1995). With regard to T-cell-mediated skin disorders, immunomodulatory drugs, preferentially macrolide lactones, have attracted special interest in recent years. Lead examples of this class are cyclosporine A, rapamycin and tacrolimus (FK506), which were initially known to act via the inhibition of interleukin-2 gene expression in T cells (Schreiber and Crabtree, 1992). Recently, FK506 has been shown to have therapeutic efficacy in the treatment of psoriasis and atopic dermatitis (Schulz et al., 1993; Nakagawa et al., 1994; Aoyama et al., 1995; Lemster et al., 1995; The European FK506 Multicentre Psoriasis Study Group, 1996). It was the purpose of the present study to examine whether measurement of primary local lymph node activation by means of a modified 'local lymph node assay' would provide a reliable end-point to analyse immunomodulatory effects of topically applied chemicals. Originally, the 'local lymph node assay' was developed by Kimber and Weisenberger (1989) as an alternative method for the identification of contact allergens. We now studied the immunomodulatory effects of topical treatment with the new immunosuppressive compound, FK506, in comparison with the effects of various well established topical glucocorticosteroids on primary immune responses.

## 2. Materials and methods

### 2.1. Chemicals

4-Ethoxymethylen-2-phenyloxazol-5-one (oxazolone) was obtained from Sigma (St. Louis, MO, USA). FK506 was a kind gift of Fujisawa (Osaka, Japan). Hydrocortisone and dexamethasone were obtained from Sigma and mometasone furoate was purchased from Essex Pharma (Munich, Germany). All test compounds were dissolved in 'DAE 433', a mixture of 40% dimethylacetamide (Sigma), 30% acetone and 30% ethanol (Maurer et al., 1980).

### 2.2. Monoclonal antibodies

The following panel of anti-mouse monoclonal antibodies was used: phycoerythrin-conjugated anti-L3T4/CD4 (rat IgG2a) (Boehringer-Mannheim, Mannheim, Germany) which binds specifically to the CD4 antigen; fluorescein isothiocyanate-conjugated anti-CD25 (rat IgG2a) (Boehringer-Mannheim) which reacts with the α-chain of the mouse interleukin-2 receptor; fluorescein isothiocyanate-conjugated anti-CD45R/B220 (rat IgG2a) (Pharmingen, San Diego, CA, USA) which recognizes a form of CD45 molecule expressed on B lymphocytes; phycoerythrin-conjugated anti-Ia (rat IgG2b) (Boehringer-Mannheim) which detects class II major histocompatibility complex molecules; fluorescein isothiocyanate-conjugated anti-CD69 (hamster IgG) (Pharmingen) which recognizes the 'very early activation antigen'. Polyclonal antibodies included fluorescein isothiocyanate-conjugated rat IgG2a, fluorescein isothiocyanate-conjugated hamster IgG, phycoerythrin-conjugated rat IgG2a and phycoerythrin-conjugated rat IgG2b (Pharmingen).

### 2.3. Animals

Female NMRI mice were purchased from Winkelmann (Borken, Germany) and allowed to become accustomed to the new environment for at least 7 days. All animals were maintained on a standard diet and water ad libitum; they were 6–12 weeks old at the start of experiments.

### 2.4. 'Local lymph node assay'

On 4 consecutive days, 5 female NMRI mice per group were topically treated on the dorsal surfaces of both ears with 25 μl of increasing concentrations of chemicals to be tested for their immunosuppressive potential or with vehicle alone. During the last 3 days, the mice received an additional topical treatment with the contact sensitizer, oxazolone (1%), 2 h after application of the test chemical. On day 5, the mice were anesthetized with $CO_2$ and killed by cervical dislocation. The draining lymph nodes of the ears from each animal were removed, pooled and weighed immediately. In addition, single cell suspensions were

Fig. 1. (a–f) Effect of epicutaneous exposure to 1% oxazolone on the expression of lymph node cell surface markers. Five NMRI mice per group were treated topically with 1% oxazolone (OXA) on the dorsum of both ears for 3 consecutive days. On day 4, the auricular lymph nodes were removed and single cell suspensions were prepared. For flow cytometry, $10^6$ pooled lymph node cells per group were double-stained with monoclonal antibodies against the following cell surface markers: (a,b) CD4/CD25; (c,d) Ia/B220; (e,f) Ia/CD69. The contour plots g–j show the effect of 0.001–0.1% dexamethasone (DM) on expansion of $Ia^+/CD69^+$ lymph node cells during oxazolone-induced contact hypersensitivity. Percentages indicate relative number of double-positive lymph node cells. Contour plots show representative data from one of three experiments.

prepared to determine individual cell counts and perform flow cytometry.

### 2.5. Flow cytometric analysis of lymph node cell subpopulations

For double staining of surface antigens, $10^6$ pooled lymph node cells per group ($n = 5$) were incubated with 200 μl of monoclonal antibodies in phosphate buffered saline supplemented with 0.02% sodium azide for 20 min at 4°C, washed twice and analyzed by flow cytometry (FACScan, Becton Dickinson, Mountain View, CA, USA). The following combinations of the above-mentioned monoclonal antibodies were used: anti-CD4/anti-CD25; anti-Ia/anti-CD69 and anti-Ia/anti-CD45R (B220). Rat IgG2a, hamster IgG, and rat IgG2b served as isotype controls.

### 2.6. Statistics

The results are expressed as indices which were defined as the ratio of mean lymph node cell counts from groups treated with test compound to corresponding results of vehicle-treated control groups. All experiments were performed at least three times to ensure consistency of the observations. The statistical significance of differences between two independent groups was analysed on the basis of individual lymph node cell counts by means of the non-parametric Mann-Whitney *U*-test. Values of $P < 0.05$ were considered indicative of significance.

## 3. Results

### 3.1. Effects of different concentrations of oxazolone on local lymph node activation

Skin painting with 25 μl oxazolone on the dorsum of both ears of NMRI mice for 3 consecutive days induced a dose-dependent increase in lymph node weight and cellularity. Lymph node weight and cell count indices were closely correlated to one another. At a concentration of 1% oxazolone significant differences from the vehicle-treated control were observed (Homey et al., 1995a). Therefore this concentration of the contact sensitizer was used in subsequent experiments. Further studies during the induction phase of contact hypersensitivity were focused on flow cytometric analysis of cell surface markers which characterize the activation of antigen-presenting cells ($Ia^+/CD69^+$), T cells ($CD4^+/IL\text{-}2R^+$) and B cells ($Ia^+/B220^+$). Flow cytometry of $10^6$ lymph node cells

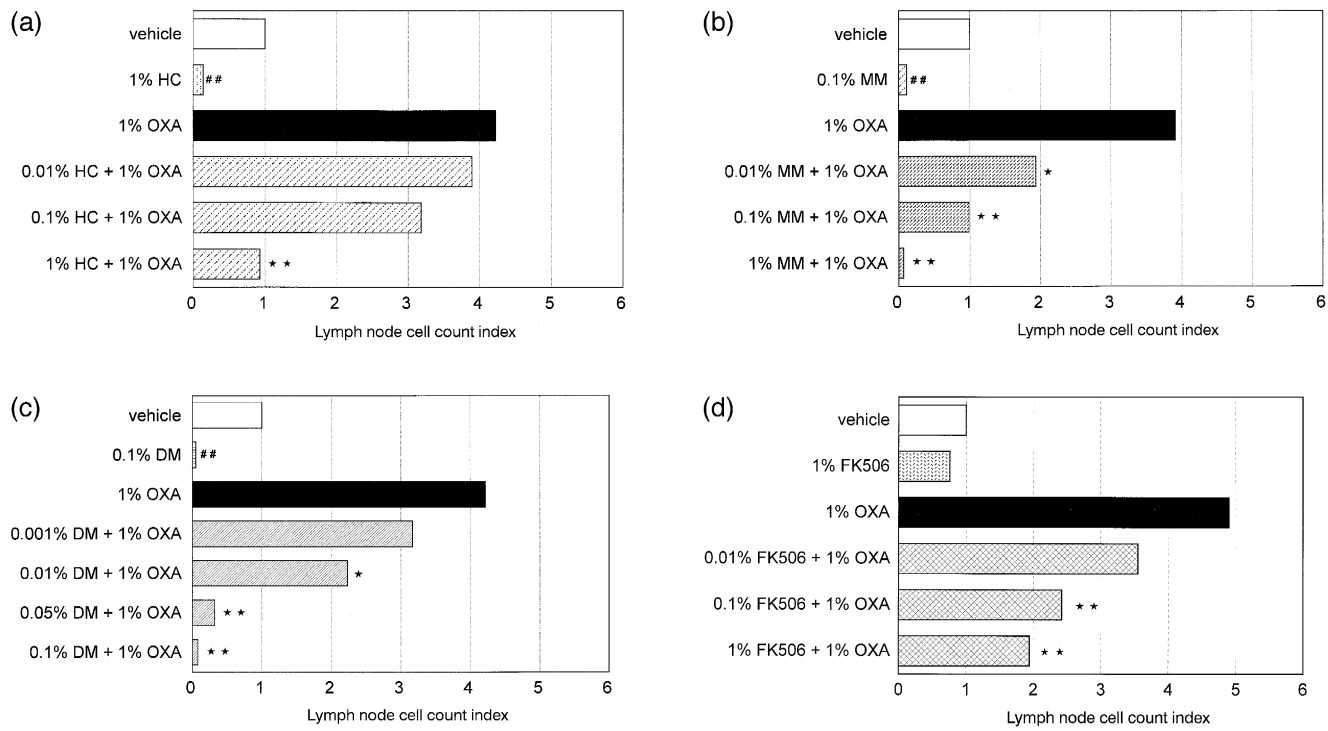



Fig. 2. Effects of topical (a) hydrocortisone (HC), (b) mometasone furoate (MM), (c) dexamethasone (DM), or (d) FK506 on local lymph node proliferation during the induction of contact hypersensitivity. Five NMRI mice per group were topically treated with the indicated concentrations of test compound on the dorsum of both ears for 4 consecutive days. During the last 3 days, the mice received an additional topical treatment with the contact sensitizer, oxazolone (OXA) (1%). On day 5, the auricular lymph nodes were removed and lymph node cell counts were measured. Results are expressed as lymph node cell count indices which were defined as the ratio of mean lymph node cell counts from the test groups to mean lymph node cell counts of the vehicle-treated control group. Statistical significance: $^{*}$ $P < 0.05$, $^{**}$ $P < 0.01$ compared with the oxazolone-treated positive control group; $^{\#}$ $P < 0.05$, $^{\#\#}$ $P < 0.01$ compared with the vehicle-treated control (Mann-Whitney *U*-test).

showed that topical treatment with 1% oxazolone resulted in a marked increase of $Ia^+/CD69^+$, $CD4^+/IL\text{-}2R^+$ and $Ia^+/B220^+$ lymph node cell subpopulations, when compared with lymph node cells of vehicle-treated controls (Fig. 1a–f).

### 3.2. Effects of different glucocorticosteroids on oxazolone-induced contact hypersensitivity

On the basis of the data obtained, a protocol was designed in which NMRI mice were topically treated with various concentrations of glucocorticosteroids or vehicle alone on 4 consecutive days. During the last 3 days, 1% oxazolone was used to induce contact hypersensitivity. On day 5, draining auricular lymph nodes were removed, lymph node cell counts were measured and flow cytometric analysis of cell surface markers was performed. Skin painting with increasing concentrations of hydrocortisone (0.01–1%), mometasone furoate (0.01–1%) or dexamethasone (0.001–0.1%) resulted in a substantial decrease of lymph node activation during oxazolone-induced contact hypersensitivity (Fig. 2a–c and Table 2). Of the 3 glucocorticosteroids tested, 0.1% dexamethasone had the most marked effects (Figs. 1 and 2 and Table 1). Comparable results were obtained with 1% mometasone; both compounds revealed significant immunosuppressive effects even at concentrations as low as 0.01% (Fig. 2b). Dexamethasone (0.1%) and mometasone (1%) both suppressed lymph node cell count indices during oxazolone-induced contact hypersensitivity below the level of those in mice treated with vehicle alone. Topical treatment with 1% hydrocortisone significantly decreased lymph node activation to a level comparable with that of vehicle-treated controls (Fig. 2a). Results obtained for lymph node cell counts were confirmed by flow cytometric analysis of cell surface marker expression. Dexamethasone, mometasone furoate and hydrocortisone markedly suppressed the expansion of $CD4^+/CD25^+$, $Ia^+/CD69^+$ and $Ia^+/B220^+$ lymph node cell subsets (Table 1).

### 3.3. Effects of different glucocorticosteroids on draining auricular lymph nodes

Topical exposure of mice to hydrocortisone (1%), mometasone furoate (0.1%) or dexamethasone (0.1%) alone for 4 consecutive days significantly decreased lymph node

Table 1
Flow cytometric analysis of local lymph node activation: dose dependence of immunomodulatory effects of hydrocortisone, mometasone furoate, dexamethasone and FK506 during oxazolone-induced contact hypersensitivity

| Chemicals | $CD4^+/CD25^+$ (%) | $Ia^+/CD45\ (B220)^+$ (%) | $Ia^+/CD69^+$ (%) |
|---|---|---|---|
| Vehicle | 3.97 | 3.91 | 0.93 |
| 1% hydrocortisone | 3.81 | 3.42 | 1.50 |
| 1% oxazolone | 8.05 | 17.49 | 16.97 |
| 0.01% hydrocortisone + 1% oxazolone | 7.76 | 19.98 | 12.06 |
| 0.1% hydrocortisone + 1% oxazolone | 4.92 | 17.07 | 11.79 |
| 1% hydrocortisone + 1% oxazolone | 3.34 | 6.12 | 4.82 |
| Vehicle | 3.49 | 7.06 | 0.68 |
| 0.1% mometasone | 5.56 | 1.17 | 0.9 |
| 1% oxazolone | 6.12 | 20.57 | 16.31 |
| 0.01% mometasone + 1% oxazolone | 3.63 | 11.7 | 9.21 |
| 0.1% mometasone + 1% oxazolone | 4.79 | 7.0 | 4.35 |
| 1% mometasone + 1% oxazolone | 3.34 | 1.97 | 1.12 |
| Vehicle | 1.96 | 5.33 | 0.62 |
| 0.1% dexamethasone | 2.87 | 1.08 | 0.71 |
| 1% oxazolone | 9.63 | 18.53 | 12.13 |
| 0.001% dexamethasone + 1% oxazolone | 10.63 | 11.04 | 7.43 |
| 0.01% dexamethasone + 1% oxazolone | 7.52 | 11.52 | 6.36 |
| 0.05% dexamethasone + 1% oxazolone | 6.49 | 5.2 | 5.12 |
| 0.1% dexamethasone + 1% oxazolone | 2.74 | 0.91 | 0.87 |
| Vehicle | 3.47 | 5.97 | 0.68 |
| 1% FK506 | 2.64 | 4.70 | 0.37 |
| 1% oxazolone | 6.67 | 24.0 | 16.31 |
| 0.01% FK506 + 1% oxazolone | 9.11 | 18.89 | 12.87 |
| 0.1% FK506 + 1% oxazolone | 3.84 | 16.77 | 4.39 |
| 1% FK506 + 1% oxazolone | 2.26 | 10.71 | 2.87 |

Groups of NMRI mice ($n = 5$) received 25 μl of hydrocortisone, mometasone furoate, dexamethasone, or FK506 in various concentrations on the dorsum of both ears for 4 consecutive days. During the last 3 days, the mice received an additional topical treatment with the contact sensitizer, oxazolone (1%). On day 5, draining auricular lymph nodes were isolated and single cell suspensions were prepared. For flow cytometry, $10^6$ pooled lymph node cells per group were double-stained with anti-CD4/anti-CD25; anti-Ia/anti-CD45R (B220) and anti-Ia/anti-CD69 monoclonal antibodies. Percentages indicate relative number of double-positive lymph node cells. Representative data from one of three experiments are shown.

cellularity when compared with that in vehicle-treated controls (Fig. 2a–c). However, flow cytometric analysis demonstrated only slightly impaired or uninfluenced expression of lymph node cell surface markers (Table 1).

### 3.4. Effects of different concentrations of FK506 on oxazolone-induced contact hypersensitivity

In further experiments, the immunomodulatory potential of topical FK506 was analysed and compared with effects of topical glucocorticosteroids. Skin painting with 1% FK506 alone for 4 consecutive days had almost no effect on lymph node cell counts, but caused impaired expression of cell surface markers when compared with vehicle-treated controls (Fig. 2d and Table 1). First, the dose dependence of FK506 treatment during the induction of contact hypersensitivity was assessed. The administration of 0.01–1% FK506 for 4 consecutive days dose dependently inhibited the lymph node activation induced by oxazolone. Concentrations of 0.1 and 1% FK506 significantly suppressed lymph node cell count indices and correspondingly diminished the population of $CD4^+/CD25^+$ LN cells, when compared to that in the oxazolone-treated positive control (Fig. 2d and Table 1). Similar results were obtained for $Ia^+/CD69^+$ and $Ia^+/B220^+$ lymph node cell subsets (Table 1). Second, effects of 0.1% and 1% FK506 were compared directly with the effects of immunosuppressive concentrations of mometasone furoate (0.1%) and dexamethasone (0.05%) (Table 2). With regard to lymph node cell counts, all compounds significantly suppressed local lymph node activation but the immunosuppressive effect of glucocorticosteroids exceeded that seen in FK506-treated groups (Table 2). Flow cytometry of lymph node cell subsets, however, revealed that topical treatment with 1% FK506 was more effective to prevent a relative increase in $CD4^+/CD25^+$ and $Ia^+/CD69^+$ lymph node cell subpopulations, whereas the effects on $Ia^+/B220^+$ lymph node subsets were comparable with those of topical glucocorticosteroid administration (Table 2).

## 4. Discussion

Our results indicate that a modified ‘local lymph node assay’ may serve as an appropriate tool to detect immunosuppressive effects of topically applied drugs. Topical glucocorticosteroids and the new non-steroidal antiinflammatory, FK506, dose dependently suppressed local lymph node activation during oxazolone-induced contact hypersensitivity, i.e. the expansion of $CD4^+/CD25^+$, $Ia^+/B220^+$ and $Ia^+/CD69^+$ lymph node cell subpopulations. Treatment with FK506 only exerted almost no effect on draining auricular lymph nodes whereas application of dexamethasone (0.1%), mometasone furoate (1%) or hydrocortisone (1%) alone resulted in a marked decrease of lymph node cell counts compared with the vehicle-treated controls. This result with topical glucocorticosteroids might have been due to systemic antiproliferative effects, such as induction of lymphocyte apoptosis, or impaired migration of circulating cells into local draining lymph nodes by modulation of cell surface adhesion molecules, such as intercellular adhesion molecule-1 or vascular adhesion molecule-1 (Cronstein et al., 1992; Krakauer, 1994; Zhao et al., 1995).

Concerning the induction phase of contact hypersensitivity, FK506 suppressed increases in lymph node cell counts less effectively than did glucocorticosteroids, but with regard to cell surface receptor expression, topical FK506 (1%) demonstrated immunosuppressive effects comparable to or stronger than those of 1% hydrocortisone, 0.1% mometasone furoate or 0.05% dexamethasone on activation of T cells ($CD4^+/CD25^+$), B cells ($Ia^+/B220^+$) and antigen-presenting cells ($Ia^+/CD69^+$) (Tables 1 and 2).

The effects of FK506 can be explained by its interactions with cytokine gene transcription. After binding to its cytosolic receptors, FK-binding proteins, the drug-receptor complex inactivates the $Ca^{2+}$-dependent phosphatase, calcineurin, resulting in inhibition of the ‘nuclear factor of activated T cells’ (NF-AT). This transcription factor regu-

Table 2
Flow cytometric analysis of lymph node activation: comparison of immunomodulatory effects of mometasone furoate, dexamethasone and FK506 during oxazolone-induced contact hypersensitivity

| Chemicals | Lymph node cell count index | $CD4^+/CD25^+$ (%) | $Ia^+/B220^+$ (%) | $Ia^+/CD69^+$ (%) |
|---|---|---|---|---|
| Vehicle | 1.0 | 3.77 | 7.21 | 0.72 |
| 1% oxazolone | 3.91 | 6.38 | 20.83 | 16.54 |
| 0.05% dexamethasone + 1% oxazolone | 0.8 [a] | 6.84 | 5.53 | 5.72 |
| 0.1% mometasone + 1% oxazolone | 0.9 [a] | 4.97 | 7.12 | 4.75 |
| 0.1% FK506 + 1% oxazolone | 1.79 [a] | 5.31 | 9.14 | 4.51 |
| 1% FK506 + 1% oxazolone | 1.52 [a] | 3.82 | 5.14 | 2.91 |

Groups of NMRI mice ($n = 5$) were treated as described for Table 1. Lymph node cell proliferation was assessed by measuring individual lymph node cell counts and expressed as lymph node cell count indices. Indices were defined as the ratio of mean lymph node cell counts from groups treated with test compound to corresponding results for a vehicle-treated control group. Afterwards, $10^6$ pooled lymph node cells per group were double-stained with anti-CD4/anti-CD25; anti-Ia/anti-CD45R (B220) and anti-Ia/anti-CD69 monoclonal antibodies. Percentages indicate relative numbers of double-positive lymph node cells. Table shows representative data from one of three experiments. Statistical significance: [a] $P < 0.01$ compared with the oxazolone-treated positive control group (Mann-Whitney $U$-test).

lates the inducible expression of several cytokines, such as interleukin-2, interleukin-3, interleukin-4, granulocyte macrophage-colony-stimulating factor, interferon-γ and tumor necrosis factor-α (Schreiber and Crabtree, 1992; Sigal and Dumont, 1992; Rao, 1994). Thus, FK506 interferes with an early state of cell activation and has the potential to interrupt cytokine-mediated effects, such as induction of cell adhesion molecules, e.g. intercellular adhesion molecule-1, expression of class II major histocompatibility complex molecules and cell activation receptors, e.g. interleukin-2 receptor (CD25) or CD69 which are required for the induction of contact hypersensitivity (Malek and Ashwell, 1985; Wicker et al., 1990; Walliser et al., 1990). With regard to mechanisms of action of glucocorticosteroids, recent results suggest that these compounds suppress the transcription factor, 'nuclear factor-κB' (NF-κB), via induction of the inhibitory factor, IκB (Auphan et al., 1995; Scheinmann et al., 1995).

All chemicals tested in this preliminary validation exercise have been shown to possess immunosuppressive potential on the basis of experimental and clinical studies. We previously reported on the efficacy of topical FK506 to suppress CHS in mice, guinea pigs and humans (Lauerma et al., 1992, 1994; Homey et al., 1995b; Michel et al., 1996). Meingassner and Stütz (1992) compared the immunosuppressive potency of the macrolide lactones, rapamycin, cyclosporine A and FK506, with that of the glucocorticosteroid, clobetasol-17-propionate, in experimental allergic contact dermatitis of farm pigs. Topical application of 0.04–0.4% of FK506 caused a pronounced inhibition of inflammatory skin reactions after challenge with dinitrofluorobenzene. The FK506-related response was similar to the activity of 0.13% of clobetasole-17-propionate, while dexamethasone (1.2%) was less active. Any differences from our results might be due to differences between animal species, different contact sensitizers and the analysis of different end-points. Meingassner and Stütz (1992) evaluated the elicitation phase of contact hypersensitivity visually by subjective and rather unspecific end-points.

So far, activation of local draining lymph node cells has been assessed by measuring lymphocyte proliferation by means of [$^3$H]thymidine incorporation in vivo or ex vivo, flow cytometric analysis of $CD4^+$ lymph node cells expressing the 'proliferating cell nuclear antigen' and interleukin-6 production (Kimber and Weisenberger, 1989; Dearman et al., 1994; Hope et al., 1994; Kühn et al., 1995). In the present study, an alternative method to measure activation of lymph node cells was developed. Flow cytometry was used to detect and differentiate local draining lymph node activation with respect to activation of antigen-presenting cells ($Ia^+/CD69^+$), T cells ($CD4^+/IL\text{-}2R^+$), and B cells ($Ia^+/B220^+$). Regarding T-cell activation, we focussed on activation of $CD4^+$ lymph node cell subpopulations, because these cells have been shown to play a central role in contact hypersensitivity reactions (Hauser, 1990; Kondo et al., 1996). In flow cytometric analysis, the T-cell activation marker, interleukin-2 receptor (CD25), was almost exclusively expressed on $CD4^+$ lymph node cells whereas $CD4^-$ lymph node cells failed to show increased CD25 expression during the induction of contact hypersensitivity to oxazolone (Fig. 1). Furthermore, detailed analysis of activated $CD8^+$ T cells revealed no differences between vehicle controls and oxazolone-treated mice (Homey et al., unpublished results).

During cytokine-mediated B-cell activation, interleukin-4 plays a major role. Incubation with this 'activation factor' led to a striking increase in the density of class II major histocompatibility complex molecules (Paul and Ohara, 1987). Recently, it has been shown that, next to production of T helper type 1 cytokines, such as interleukin-2 and interferon-γ, the production of T helper type 2 cytokines, such as interleukin-4, was also increased in lymph node cells during contact hypersensitivity reactions (Fehr et al., 1994; Homey et al., 1996). Therefore, B-cell activation was analysed by flow cytometry as a function of increased Ia expression on lymph node cells stained with the pan B-cell marker CD45R (B220) (Coffman, 1982; Asensi et al., 1989).

During induction of contact hypersensitivity, antigen-presenting cells can be characterized by their expression of class II major histocompatibility complex molecules (Aiba and Katz, 1990). In order to evaluate antigen-presenting cell activation, we analysed the expression of the 'very early activation antigen', CD69, on the surface of $Ia^+$ lymph node cells (Yokoyama et al., 1988; De Maria et al., 1994).

In conclusion, the modified 'local lymph node assay' represents an appropriate tool to analyse the immunosuppressive potential of chemicals and offers a system for further analysis of mechanisms of action. During the induction phase of contact hypersensitivity, the complex interactions between antigen-presenting cells and T cells, their activation, expression of co-stimulatory molecules and cytokine induction could be characterized not only in local draining lymph nodes but also at the epidermal and dermal level. This screening model for topical immunosuppressants offers important advantages such as speed, relative cost effectiveness, requiring only small amounts of test chemicals and measuring immunologically relevant objective end-points (Basketter et al., 1991; Kimber et al., 1994). Recently, the 'local lymph node assay' was included in the guidelines of immunotoxicological risk assessment of the OECD. Furthermore, a variety of different chemicals with putative immunosuppressive potency are introduced to our environment every day. Next to oral and inhalation exposure, skin contact plays a major role in interactions with chemicals. Thus, in addition to pharmacological drug-development studies, the 'immunosuppression local lymph node assay' might be useful for toxicological screening of chemical-induced immunosuppression.

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