

ORIGINAL PAPER

Misako Tanaka · Setsuya Aiba · Kazuhiro Takahashi
Hachiro Tagami

Reduced proliferative responses of peripheral blood mononuclear cells specifically to *Candida albicans* antigen in patients with atopic dermatitis – comparison with their normal reactivity to bacterial superantigens

Received: 7 August 1995

Abstract Although reduced cutaneous reactivities to *Candida albicans* have been reported in patients with atopic dermatitis (AD), there is still controversy as to whether the in vivo lymphocyte proliferation response is normal or reduced. We have also reported that patients with AD manifest a decreased cutaneous response only to *C. albicans* antigen in scarification patch tests. The purpose of this study was to examine whether patients with AD show normal lymphocyte transformation responses to *C. albicans* antigen. Peripheral blood leucocytes (PBL) from 21 patients with AD and 14 healthy control (HC) subjects were cocultured with optimal concentrations of *C. albicans* antigen and of superantigens (staphylococcal enterotoxin A and B). PBL from the patients with AD showed a significantly lower response to *C. albicans* antigen, but there was no statistically significant difference in PBL responses to superantigens between the patients with AD and the HC subjects. This decreased proliferative response of PBL was particularly noticeable in those whose RAST scores for *C. albicans* antigen were high. These results suggest the development of a specific anergy to *C. albicans* antigen in patients with AD.

Key words Atopic dermatitis · *Candida albicans* · Lymphocyte proliferation test · Skin test · Bacterial superantigens

Introduction

Although the pathogenesis of atopic dermatitis (AD) is still not fully understood, a variety of immunological abnormalities have been observed in patients with AD, in-

cluding elevated serum IgE levels, a transient or persistent reduction in delayed-type hypersensitivity (DTH), and susceptibility to certain cutaneous viral and bacterial infections [1, 2]. We have recently demonstrated using RAST, prick and patch tests that, although generally both IgE-mediated skin hypersensitivity and DTH to various environmental antigens derived from living organisms are pronounced in AD patients [3], these patients show significantly decreased contact sensitivity only to *Candida albicans* antigen. Using intradermal tests, Elliot and Hanifin [4] have also shown that patients with AD show a significantly lower incidence of positive reactions to *C. albicans* antigen than healthy control (HC) subjects. In contrast, however, there is still controversy about the in vitro proliferative responses of peripheral blood leucocytes (PBL) from patients with AD to *C. albicans* antigen [4, 5]. The patients studied by Elliot and Hanifin [4] showed normal proliferative responses of PBL to *C. albicans*. On the other hand, McGeady and Buckley [5] reported that AD patients showed a significantly lower proliferative responses of PBL to *C. albicans* than HC subjects.

To determine whether patients with AD show normal proliferative responses of PBL to *C. albicans* antigen or not, we examined in vivo and in vitro reactivities of 21 patients with AD and 14 HC subjects to *C. albicans* antigen using RAST, prick tests, scarification patch tests and PBL proliferation tests.

Methods

Patients and control subjects

A group of 21 Japanese patients with AD (6 female and 15 male, between 9 and 40 years of age, mean 21 years) were evaluated at the Department of Dermatology, Tohoku University School of Medicine. All patients fulfilled the criteria of Hanifin and Rajka [6] for the diagnosis of AD. They participated in the study during remission of their skin lesions after appropriate topical and systemic treatments, and had clinically lesion-free areas on the flexor surface of the forearms. No patients had been receiving systemic immunotherapy. Seven patients had a past or present history of atopic respiratory disease (ARD), and remaining 14 patients had no personal history or complications of ARD (i.e. they had pure AD). We

M. Tanaka (✉) · S. Aiba · K. Takahashi · H. Tagami
Department of Dermatology,
Tohoku University School of Medicine, 1-1 Seiryō-machi,
Aoba-ku Sendai 980-77, Japan
Tel. 81-22-717-7271; Fax 81-22-717-7275

estimated the severity of the AD according to the criteria of Rajka and Langeland [7]. Three patients had mild, nine moderate and nine severe AD. They showed no signs of cutaneous candidial infection.

A group of 14 healthy volunteers without a personal or family history of AD or ARD (6 male and 8 female, between 24 and 42 years of age, mean 31 years) served as the HC group. All the subjects gave informed consent before the examinations.

Detection of allergen-specific and nonspecific IgE antibodies

Serum IgE levels of the subjects were measured using a fluoroenzyme immunoassay (FEIA; CAP system, Pharmacia, Uppsala, Sweden), and allergen-specific IgE antibodies were measured using a RAST FEIA (CAP system).

Antigen and superantigens

C. albicans antigen in the form of a crude extract of *C. albicans* containing cell wall mannans and cytoplasmic proteins (commercially available from Torii Co, Tokyo, Japan) was used for prick and patch test. It was prepared by the Torii Co. as follows: center mould medium inoculated with *C. albicans* was incubated at 27°C for 30 days and, after separation of *C. albicans* by filtration, was then condensed, dialyzed against distilled water, freeze dried and diluted in saline. For prick tests, the extract was used at a concentration of 1:100 in saline containing 50% glycerin (PNU 6860). For patch tests, the extract was diluted with an equal volume of control solution (Torii Co.) to which was added phenol to obtain a final concentration of 0.5%.

Staphylococcal enterotoxin A (SEA) and B (SEB) were purchased from Sera (Heidelberg, Germany) and Sigma (St. Louis, Mo.), respectively.

Skin tests

All the subjects were prohibited from taking antihistamines and systemic steroids and from applying topical steroids to the volar aspects of the forearms for 1 week before skin testing. Modified prick tests were done according to the method of Pepys [8], described in detail elsewhere [3]. The wheal response was evaluated after 15 min. A wheal with a long diameter of over 5 mm was regarded as positive. If a patient showed a reaction to the control vehicle, reactions to the allergens were recorded as positive when their diameter was twice that of the control reaction [9].

Chamber scarification patch tests were carried out as described by Frosch and Kligman [10], and described in detail elsewhere [3]. In brief, the test area was lightly abraded four times with a 27-gauge needle in a criss-cross fashion. The test solution (20 µl) was applied in a Finn chamber to the volar aspect of the subject's forearm for 48 h. A control solution containing 0.5% phenol was applied in the same way. The patch test reactions were read 72 h and

1 week after application of the patches and the appearance of erythema with papules at 72 h and remaining papules with pigmentation at 1 week was regarded as a positive reaction according to the ICDRG (International Contact Dermatitis Research Group) criteria for patch tests [11]. The skin tests were approved by the ethics committee of the Tohoku University School of Medicine.

Cell proliferation assay

RPMI-1640 medium supplemented with penicillin/streptomycin/Fungizone, 5×10^{-5} M 2-mercaptoethanol, 2 mM glutamine, 10 mM HEPES (Wako, Osaka, Japan) and 10% heat-inactivated AB serum (Sigma, St. Louis, Mo.) was used as the culture medium. PBL were isolated from the AD patients and the HC subjects using Ficoll-Hypaque density gradient centrifugation and washed with culture medium three times, and 1×10^5 cells/well were cultured with the test agents. The cells were cultured for 72 h with SEA or SEB, or for 120 h with *C. albicans* antigen. To each well was added 16 h before harvesting the cells 1 µCi [³H]-thymidine (New England Nuclear, Boston, Mass.). The cells were harvested onto glass filter strips using a semiautomatic cell harvester, and the amount of incorporated [³H]-thymidine was assessed using a liquid scintillation counter.

The results are presented as the means of triplicate cultures $\pm$ SD. The difference between the incorporation of [³H]-thymidine by PBL cultured alone and by those cultured with the antigen and superantigens (Δ cpm) was calculated. For comparison of [³H]-thymidine incorporation in PBL between the HC subjects and the AD patients, the following concentrations of antigen and superantigens were used: 100 µg/ml *C. albicans* extract, 0.25 µg/ml SEA, 4 µg/ml SEB. We selected suboptimal concentrations from dose-response curves for each antigen and superantigen (data not shown).

Statistical analysis

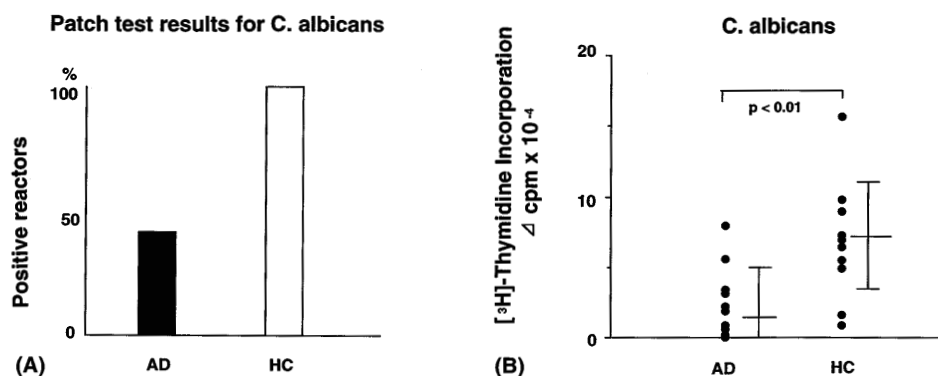
The Mann-Whitney test and Pearson's correlation coefficient were used as appropriate.

Results

Responses of the PBL from the AD patients and from the HC subjects for *C. albicans* antigen and for staphylococcal superantigens

As shown in Fig. 1A, the AD patients showed a much lower incidence of positive scarification patch test reactions to *C. albicans* antigen. Moreover, the cultures stimulated with *C. albicans*, PBL from the AD patients incor-

Fig. 1 **A** An incidence of positive *C. albicans* scarification patch test reactions in AD patients and HC subjects; **B** responses of PBL from AD patients and HC subjects to *C. albicans* antigen (Longer horizontal bars mean, shorter horizontal bars standard deviation)



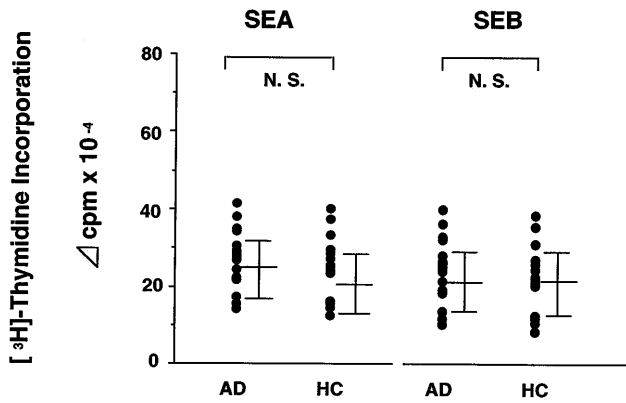


Fig. 2 Responses of PBL from AD patients and HC subjects to SEA and SEB (N.S. not significant)

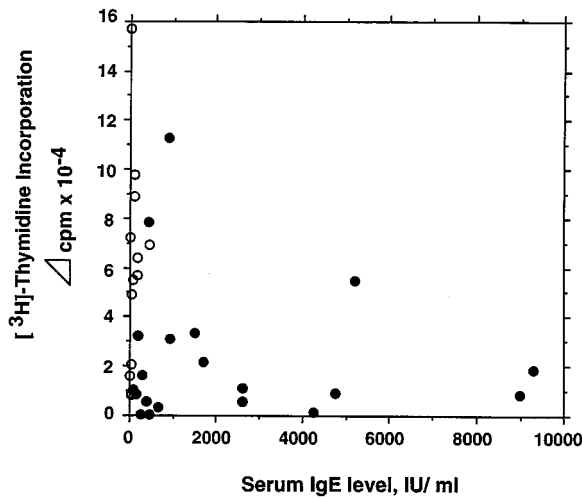


Fig. 3 Correlation between responses of PBL to *C. albicans* antigen and serum IgE levels in AD patients and HC subjects

porated much less [^3H]-thymidine than those from the HC subjects ($P < 0.01$, Mann-Whitney test; Fig. 1B). [^3H]-thymidine incorporation by PBL stimulated with SEA and SEB did not show any differences between the AD patients and the HC subjects (Fig. 2). The basal proliferation responses of the PBL were not different between the AD patients and HC subjects, and neither age nor sex influenced the PBL proliferative responses in either group.

Correlation between the in vitro responses of PBL and the serum IgE levels in the AD patients and the HC subjects

The AD patients who showed higher serum IgE levels tended to display lower lymphocyte proliferative responses than those who showed lower serum IgE levels, although the correlation was not statistically significant (Pearson's correlation coefficient, Fig. 3). Most HC subjects showed low serum IgE levels and high lymphocyte proliferative responses. There was no special correlation between them.

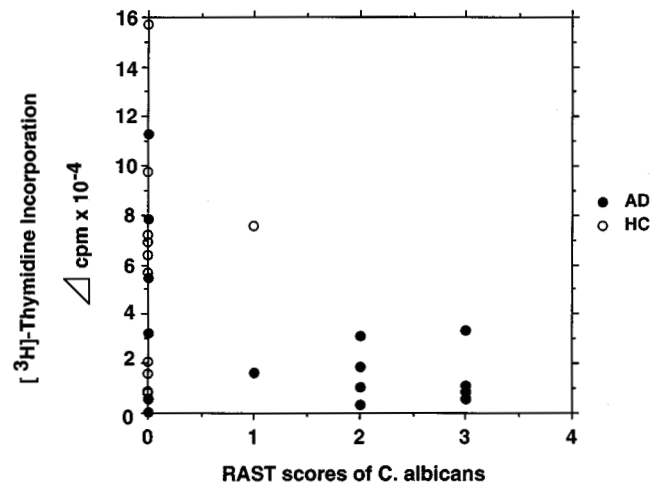


Fig. 4 Correlation between responses of PBL to *C. albicans* antigen and RAST scores in AD patients and HC subjects

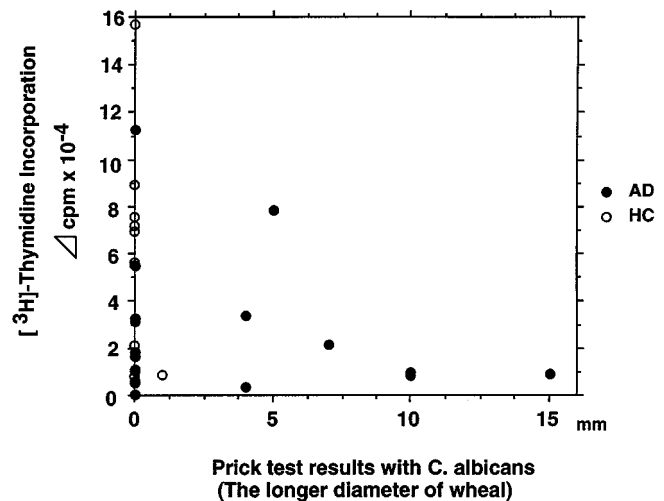


Fig. 5 Correlation between responses of PBL to *C. albicans* antigen and *C. albicans* antigen prick test results in AD patients and HC subjects

Correlation between in vitro responses of PBL and the RAST scores in the AD patients and the HC subjects

The patients with AD who showed higher RAST scores tended to display lower lymphocyte proliferative responses than those who showed lower RAST scores, although the correlation was not statistically significant (Pearson's correlation coefficient, Fig. 4). Most HC subjects showed low RAST scores and high lymphocyte proliferative responses, but there was no significant correlation between them.

Correlation between in vitro responses of PBL and the result of prick tests in the AD patients and the HC subjects

The AD patients who showed larger prick test responses tended to display lower lymphocyte proliferative responses than those who showed smaller prick test responses, although the correlation was not statistically significant (Pearson's correlation coefficient, Fig. 5). Most HC subjects showed no positive prick test response but high lymphocyte proliferative responses, but there was no significant correlation between them.

Discussion

In our previous in vivo study, we demonstrated that patients with AD show a low incidence of positive reactions for *C. albicans* antigen in scarification patch tests compared with HC subjects, in contrast to the finding that AD patients show a higher incidence of positive patch test reactions to other environmental allergens than HC subjects [3]. These results suggest that DTH in AD patients is not generally impaired, although it is decreased for certain antigens, possibly those derived from the body microflora. In the present study we succeeded in demonstrating in vitro that AD patients specifically show a decreased proliferative response of PBL for *C. albicans* antigen in comparison with a nonspecific proliferative response of PBL against other superantigens, in this case SEA and SEB. Our results differ from those of Elliot and Hanifin [4] but are similar to those of McGeady and Buckley [5]. The in vitro reactivity of PBL for *C. albicans* seems reasonable because it corresponds well with the results of in vivo scarification patch tests. In our previous study we also found that there was a negative correlation between the RAST scores and the results of scarification patch tests to *C. albicans* antigen [3]. Likewise, in the present study, the patients with AD were found to show a rather decreased PBL proliferative response when their RAST scores were high against *C. albicans*.

In contrast to these reaction patterns to *C. albicans* antigen, we have found a positive correlation between the RAST scores and the results of scarification patch tests to housedust mite antigen in patients with AD [3]. Rawle et al. [12] also reported that the proliferative response of PBL from allergic patients to the major antigen of housedust mite correlates positively with its serum IgE antibody. Thus, the observed immunological reactivities to *C. albicans* antigen in AD patients, who tended to show negative patch test reactions and decreased proliferative responses of PBL together with rather high RAST scores to *C. albicans* antigen, seem to be unique as compared with those against other environmental allergens. We have found by scarification patch testing that neonates are all nonreactive to *C. albicans* antigen; they seem to acquire contact sensitivity of the antigen probably as a result of developing cutaneous candidiasis early in life in the form of diaper dermatitis or by mucosal exposure to it, because

the contact sensitivity is demonstrable in almost all healthy individuals [13]. In experimentally induced dermatophytosis, Jones et al. [14] observed that DTH to fungal antigens is a predominant immunological response. However, they found that atopic individuals show a unique sequence of events: an initially delayed sensitivity is later replaced by immediate-type hypersensitivity. In a similar fashion, it is possible that, although AD patients may at first demonstrate DTH to *C. albicans*, they gradually lose it by developing prominent IgE-mediated hypersensitivity from repeated exposure to this common microorganism of the body during their life.

Recent studies using mouse helper T-cell clones suggest that there are two types of T-cell clones that are clearly separable on the basis of the panel of cytokines they produce [15]; Th1 clones secrete IL-2 and IFN- γ and preferentially induce DTH [16], while Th2 clones produce IL-4, IL-5 [15] and IL-10 [17] and provide superior help for IgE production [18, 19]. Wierenga et al. [20] have demonstrated the presence of Th2 type T cells in AD patients. The demonstration that healthy subjects with a normal immune response show high PBL proliferative responses as well as positive scarification patch tests to *C. albicans* antigen [3], suggests the dominant presence of Th1 type T cells specific to *C. albicans* antigen. In contrast, in the present study the AD patients had high RAST scores to *C. albicans*, which suggests the presence of Th2 type T cells in patients with AD. Therefore, it is conceivable that Th1 type T cells to *C. albicans* antigen are replaced by Th2 type T cells in AD.

Several researchers have demonstrated that allergic subjects carrying *C. albicans* in the nasopharynx and anus show high RAST scores but reduced skin DTH for *C. albicans* [21–23]. It has been reported that the incidence of faecal *C. albicans* is higher in patients with AD than in control subjects [24]. Our finding that, in addition to a lower incidence of positive patch test reactions, PBL from AD patients show a markedly reduced response to *C. albicans* antigen seems to indicate that the Th lymphocytes themselves are in the process of acquiring or have acquired a state of anergy due to repeated exposure to excessive *C. albicans* antigen.

Recently, Picker et al. [25] have reported the presence of skin-homing T lymphocytes. These T cells have a unique homing receptor, called cutaneous lymphocyte antigen (CLA), which can interact with ELAM-1 [26]. Furthermore, there are several reports [27, 28] that Th2 T cell clones specific to housedust mite antigen derived from epicutaneously challenged skin express CLA, while T cell clones derived from peripheral blood do not. Our present results seem to rule out the possibility that T cells reactive to *C. albicans* express CLA, unlike housedust mite antigen-specific T cells that can express it, because we found not only reduced patch test reactions but also decreased PBL responses to *C. albicans* antigen in AD patients.

References

- Clark RAF (1989) Cell-mediated and IgE-mediated immune responses in atopic dermatitis. *Arch Dermatol* 125:413–416
- Brown MAJ, Hanifin M (1990) Atopic dermatitis. *Curr Opin Immunol* 2:531–534
- Tanaka M, Aiba S, Matsumura N, Aoyama H, Tabata N, Sekita Y, Tagami H (1994) IgE-mediated hypersensitivity and contact sensitivity to multiple environmental allergens in adult patients with atopic dermatitis: compiled results of RAST, prick and patch testing. *Arch Dermatol* 130:1393–1401
- Elliott ST, Hanifin JM (1979) Delayed cutaneous hypersensitivity and lymphocyte transformation. *Arch Dermatol* 115:36–39
- McGeedy SJ, Buckley RH (1975) Depression of cell-mediated immunity in atopic eczema. *J Allergy Clin Immunol* 56:393–406
- Hanifin JM, Rajka G (1980) Diagnostic features of atopic dermatitis. *Acta Derm Venerol Suppl* 92:44–47
- Rajka G, Langeland T (1989) Grading of the severity of atopic dermatitis. *Acta Derm Venereol Suppl* 144:13–14
- Pepys J (1975) Skin testing. *Br J Hosp Med* 14:412–417
- Ito K, Nakagawa T, Morita Y, Okudaira H, Yamashita T (1989) Immunologic analysis of steroid-dependent asthma. *Ann Allergy* 62:15–20
- Frosch PJ, Kligman AM (1976) The chamber-scarification test for irritancy. *Contact Dermatitis* 2:314–324
- Fisher AA (1986) The role of patch testing. In: Fisher AA (ed) *Contact dermatitis*. Lea & Febiger, Philadelphia, pp 9–29
- Rawle FC, Mitchel EB, Platts-Mills TAE (1984) T cell responses to the major allergen from the house dust mite *Dermatophagoides pteronyssinus*, antigen P₁: comparison of patients with asthma, atopic dermatitis, and perennial rhinitis. *J Immunol* 133:195–201
- Tagami H, Urano-Suehisa S, Hatchome N (1985) Contact sensitivity to *Candida albicans*: comparative studies in man and animal (guinea-pig). *Br J Dermatol* 113:415–424
- Jones HE, Reinhardt JH, Rinaldi MG (1974) Immunologic susceptibility to chronic dermatophytosis. *Arch Dermatol* 110:213–220
- Mosmann TR, Cherwinski H, Bond MW, Giedlin MA, Coffman RL (1986) Two types of murine helper T cell clone I. Definition according to profiles of lymphokine activities and secreted proteins. *J Immunol* 136:2348–2357
- Stout RD, Bottomly K (1989) Antigen-specific activation of effector macrophages by IFN- γ (Th1) T cell clones. Failure of IL-4-producing (Th2) T cell clones to activate effector function in macrophages. *J Immunol* 142:760–765
- Fiorentino DF, Bond MW, Mosmann TR (1989) Two types of mouse T helper cell IV. Th2 clones secrete a factor that inhibits cytokine production by Th1 clones. *J Exp Med* 170:2081–2095
- Boom WH, Liano D, Abbas AK (1988) Heterogeneity of helper/inducer T lymphocytes II. Effects of interleukin 4- and Interleukin-2-producing T cell clones on resting B lymphocytes. *J Exp Med* 167:1350–1363
- Killar L, MacDonald G, West J, Woods A, Bottomly K (1987) Cloned, I-A restricted T cells that do not produce interleukin 4 (IL-4)/B cell stimulatory factor 1 (BSF-1) fail to help antigen-specific B cells. *J Immunol* 138:1674–1679
- Wierenga EA, Snoek M, Groot C de, Chretien I, Bos JD, Jansen HM, Kapsenberg ML (1990) Evidence for compartmentalization of functional subsets of CD4⁺ T lymphocytes in atopic dermatitis. *J Immunol* 144:4651–4656
- Koivikko A, Kalimo K, Nieminen E, Viander M (1988) Relationship of immediate and delayed hypersensitivity to nasopharyngeal and intestinal growth of *Candida albicans* in allergic subjects. *Allergy* 43:201–205
- Savolainen J, Koivikko A, Kalimo K, Nieminen E, Viander M (1990) IgE, IgA and IgG antibodies and delayed skin response towards *Candida albicans* antigens in atopics with and without saprophytic growth. *Clin Exp Allergy* 20:549–554
- Salvolainen J, Lammintausta K, Kalimo K, Viander M (1993) *Candida albicans* and atopic dermatitis. *Clin Exp Allergy* 23:332–339
- Buslau M, Menzel I, Holzman H (1990) Fungal flora of human faeces in psoriasis and atopic dermatitis. *Mycoses* 33 (2):90–94
- Pickler LJ, Treer JR, Ferguson-Darnell B, Collins PA, Buck D, Terstappen LWMM (1993) Control of lymphocyte recirculation in man I. Differential regulation of the peripheral lymph node homing receptor L-section in T cells during the virgin to memory cell transition. *J Immunol* 150:1105–1136
- Pickler LJ, Kishimoto TK, Smith CW, Warnock RA, Butcher EC (1991) ELAM-1 is an adhesion molecule for skin-homing T cells. *Nature* 349:796–799
- Rossiter H, Reijssen F von, Mudde GC, Kalthoff F, Bruijnzeel-Koomen CAFM, Pickler LJ, Kupper TS (1994) Skin disease-related T cells bind to endothelial selections: expression of cutaneous lymphocyte antigen (CLA) predicts E-selectin but not P-selectin binding. *Eur J Immunol* 24:205–210
- Babi LFS, Pickler LJ, Soler MTP, Drzimalla K, Flohr P, Blaser K, Hauser C (1995) Circulating allergen-reactive T cells from patients with atopic dermatitis and allergic contact dermatitis express the skin-selective homing receptor, the cutaneous lymphocyte-associated antigen. *J Invest Dermatol* 181:1935–1940