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Adhesion molecules and IL-1 costimulate T lymphocytes in the autologous MECLR in psoriasis

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Abstract **Membrane molecules such as CD36 (OKM5), intercellular adhesion molecule-1 (ICAM-1, CD54), gamma interferon-induced protein 10 (γ-IP10) and IL-1 are induced and/or upregulated in psoriatic epidermis. These molecules have important accessory, trafficking or signalling functions in the immune system and also play a role in the pathophysiology of psoriasis. The relevance of adhesion molecules, CD36 and epidermal IL-1 in psoriasis was studied in vitro in the autologous mixed epidermal cell – T lymphocyte reaction (MECLR). Their level of expression was quantitated in epidermal cell suspensions (ECS) from patients with psoriasis and their function was assessed by blocking with specific mAbs and antisera or by depleting $CD36^+$ cells from the ECS prior to the MECLR. ECS from psoriatic lesions contained increased numbers of $CD36^+$ (23 ± 12%), $ICAM\text{-}1^+$ (31 ± 14%) and $IL\text{-}1^+$ (57 ± 21%) cells. The autologous MECLR was inhibited in samples from all patients by mAb to CD2 (LFA-2), CD11a (LFA-1α), CD18 (LFA-1β), ICAM-1, CD58 (LFA-3) and an antiserum to IL-1β. Thus, adhesion molecules facilitate inflammation in psoriasis not only via adhesion and recruitment of T lymphocyte in psoriatic lesions, but also via activation of T cells. Furthermore CD36 molecules on psoriatic epidermal cells do not costimulate autologous T lymphocytes in psoriasis. The observed costimulatory function of IL-1β in the MECLR emphasizes its relevance in psoriasis.**

Key words Adhesion molecules · IL-1 · T cells · Autologous MECLR · Psoriasis

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Introduction

The autologous mixed epidermal cell-T lymphocyte reaction (MECLR) is elevated in psoriasis patients [21]. This is mainly due to the spontaneous stimulation of autologous T lymphocytes by $HLA\text{-}DR^+/CD1a^-$ epidermal antigen-presenting cells (APC) in the absence of exogenously added antigen [19]. Cytokines produced by activated T lymphocytes may induce the 'psoriatic phenotype' and upregulate the expression of gamma interferon-induced protein 10 (γ-IP10), HLA-DR, ICAM-1 (CD54) and CD36 (OKM5, thrombospondin receptor) on keratinocytes [1, 15]. Epidermal cells (EC) from psoriatic lesions do indeed express increased levels of CD36, ICAM-1 and γ-IP10 [2, 3, 10, 11].

In psoriatic lesions, CD36 is intensely expressed on keratinocytes, sporadically on intraepidermal dendritic cells and consistently on dermal dendritic and endothelial cells [3]. Peripheral blood $CD36^+/CD11b^-$ monocytes are potent stimulators of autologous T lymphocytes in the autologous mixed lymphocyte reaction (MLR) [22]. Epidermal $CD36^+/CD11b^-$ APC have also been shown to be efficient stimulators of autologous T lymphocytes [1]. However, the proportion of such APC is low and is increased in healthy human skin only after irradiation with 3 to 5 MED UVB [1]. Based on the crucial role of $CD36^+$ APC in the MLR, it is considered possbile that $CD36^+$ EC from psoriatic lesions could serve as accessory cells in the autologous MECLR [1, 6, 26].

The integrins CD11a (LFA-1α), CD18 (LFA-1β) and their ligands have been shown to be crucial for successful (allo)antigen and mitogen-induced T-cell activation and proliferation [13]. Costimulation via adhesion molecules on epidermal APC is essential for effective antigen-specific T-cell proliferation [16, 20]. The LFA-1/ICAM-1 adhesion pathway may be involved in the pathogenesis of psoriasis by facilitating lymphocyte adhesion, trafficking, activation and stimulation [15]. The expression of IL-1 is abnormal in psoriatic lesions [8, 18]. IL-1 is known to costimulate T lymphocytes during antigen presentation

[14]. In the study reported here, the expression of the above-mentioned molecules was quantified and their function in psoriasis was investigated.

Materials and methods

Patients and controls

Patients with stable untreated, plaque-type psoriasis underwent shave biopsies and venipuncture after informed consent had been obtained. The autologous MLR and the MECLR were performed with samples from all patients. The number of patients samples of which were tested in each experiment is shown in Tables 2 and 3 and Figs. 1 and 2. EC and peripheral blood mononuclear cells (PBMC) were immunophenotyped. EC isolated from uninvolved skin of nickel dermatitis patients and from patients undergoing abdominal or breast plastic surgery served as controls. Isolated PBMC from these patients and other healthy individuals served also as controls.

Preparation of epidermal cell suspensions

Split-skin specimens from involved skin of psoriasis patients and from excised skin of controls were obtained using a dermatome. In nickel dermatitis patients, blister roofs were obtained using the suction blister technique [20]. Single cell suspensions of EC were prepared using standard methods [19]. Briefly, split-skin specimens were rinsed with phosphate-buffered saline (PBS), cut into pieces of ± 0.5 cm^2 and floated in Ca^{2+}- and Mg^{2+}-free PBS containing 0.0625% trypsin (ICN, Cleveland, Ohio, USA) and 0.1% EDTA for 45 min at 37°C in an atmosphere containing 5% CO_2, and in the presence of 0.025% deoxyribonuclease solution (DNase; Sigma, St. Louis, Mo., USA) during the last 15 min of the incubation. Epidermal sheets were separated from the dermis using fine forceps, cut with scissors and the EC were filtered through sterile 100-μm and 30-μm mesh nylon gauzes and resuspended in RPMI-1640 supplemented with antibiotics, fresh L-glutamine, 20 m*M* Hepes (RPMI complete medium), 20% heat-inactivated human AB serum and 0.01% DNase. Cells were counted using a haemocytometer and their viability was determined by trypan blue exclusion.

Removal of $CD36^+$ cells from epidermal cell suspensions

$CD36^+$ cells were removed from the epidermal cell suspensions (ECS) using anti-CD36 mAb (Table 1) and antimouse Ig-conjugated paramagnetic beads (Dynal, Oslo, Norway) [17]. Briefly, ECS were incubated for 30 min at 4°C with primary mAb, rinsed three times, incubated with the conjugated paramagnetic beads and centrifuged. After 30 min the pellet was resuspended and the rosettes counted. Rosetted cells were attracted to one side of the tube by a strong magnet and the non-rosetted cells were collected. The number of rosetted cells was compared with the number of $CD36^+$ EC before depletion and the efficiency of the depletion was checked again later by immunofluorescent staining. In pilot experiments IgG1 isotype control mAb was used as the first step followed by the normal immunomagnetic separation procedure.

Isolation of peripheral blood mononuclear cells and purification of T lymphocytes.

PBMC were isolated using density gradient centrifugation on Lymphoprep (density 1.077; Nyegaard, Oslo, Norway). All rinsing and isolating of other cells was done in RPMI complete medium containing 1% human AB serum. FCS was not used in this study.

T lymphocytes were isolated with a rosetting technique using 2-aminoethylisothiouronium bromide (AET)-treated sheep red blood cells. These purified cells consisted of approximately 95% $CD3^+$, 99% CD2 $(OKT11)^+$, 0–0.5% $CD14^+$ and 0–0.5% $CD20^+$ cells [17, 18]. The accessory capacity of this fraction was negligible because this population could not be stimulated by antigen alone [2, 13].

Monoclonal antibodies and immunophenotyping of cell suspensions

In addition to the mAbs in Table 1, the following mAbs were used for immunophenotyping: CD3 (Leu-4), CD4 (Leu-3), CD5 (Leu-1), CD8 (Leu-2), CD16 (Leu-11b), CD25 (IL-2 receptor α chain; Becton Dickinson), CD20 (B1; Coulter, Hialeah, Fl., USA), CD11b (CR3, OKM1), CD36 (OKM5; Ortho Diagnostic), CD71 (66IG10; Dr. M. van der Rijn, Amsterdam), CD14 (My4; Dr. J. D. Griffin, Boston, Mass., USA), CD15 (VIM-D5; Dr. W. Knapp, Vienna), CD29 (VLA-β1, 4B4; Coulter), CD45 (HLe-1, FITC-conjugated; Becton Dickinson), CD45RA (2H4; Coulter), IL-1β (Cistron, Pine Brook, N.J., USA) and TcR-γδ (11F2; Dr. J. Borst, Amsterdam). Immunophenotyping of EC, PBMC or whole blood samples was performed using direct, indirect and double-labelling (SimulSET) immunofluorescence techniques and flow cytometry as described by Van Dongen et al. [28]. CD36/CD45 double-labelling of ECS was carried out by the addition of rhodamine-labelled goat-anti-mouse IgG to detect the primary OKM5 mAb, and incubation with 10% normal mouse serum followed by HLe1-FITC mAb. Double-labelling of PBMC was also performed to detect CD3/CD4, CD3/CD8, CD3/CD25 and CD3/HLA-DR double-positive cells according to standard procedures (SimulSET). The FACscan data were analysed using the SimulSET and Lysis programs (Becton Dickinson). The results were expressed as the mean percentage ± SD.

Autologous MECLR and MLR

Mixed cultures of unpurified EC and PBMC or purified T lymphocytes were set-up in quadruplicate in round-bottomed mi-

Table 1 Source and specificity of antibodies used in depletion and blocking experiments. Mouse IgG1 (10 μg/ml) and IgG2a (1 μg/ml) isotype control mAbs, and normal goat and normal rabbit sera at a dilution of 1/100 were used as controls

mAb	Source	Specificity	Isotype	Final concentration
CLB-LFA1/2	Central Blood Transfusion Laboratory	LFA-1α/CD11a	IgG1	10 μg/ml
CLB LFA1/1	Central Blood Transfusion Laboratory	LFA-1β/CD18	IgG1	10 μg/ml
OKT11	Ortho Co.	LFA-2/CD2	IgG2a	1 μg/ml
TS 2/9	T.S. Springer	LFA-3/CD58	IgG1	1 μg/ml
RR1/1.1.1	R.R. Rothlein	ICAM-1/CD54	IgG1	1 μg/ml
HLA-DR	Becton Dickinson	–	IgG2a	0.25 μg/ml
OKM5	Ortho Co.	CD36	IgG1	1 μg/ml
Polyclonal antibodies				
α-IL-1β	Van Damme et al. [27]	Human IL-1β	–	1/1000
α-IL-6	Helle et al. [12]	Human IL-6	–	1/1000

crotitre plates (Costar, Cambridge, Mass., USA). To each well, 4×10^4 EC and $1–2 \times 10^5$ PBMC were added to a final volume of 0.2 ml complete RPMI medium supplemented with 15% human AB serum. The autologous MLR comprised the spontaneous proliferation of separately plated fractions of PBMC. Only non-irradiated EC were used because we had previously shown that autologous MECLR responses with non-irradiated EC did not differ significantly from those with irradiated EC [19]. The cultures were pulsed with 18.5 kBq tritiated thymidine ([^{3}H]TdR, 185 GBq/mmol; Amersham, UK) on days 6, 7 and 8 and harvested 8 h later onto glass fibre filters. The amount of incorporated [^{3}H]TdR was determined by liquid scintillation counting (β-plate; Pharmacia/LKB, Uppsala, Sweden). The results were expressed as mean ± SEM counts per min (cpm) of quadruplicate cultures.

Blocking of the autologous MECLR with antibodies

The mAbs used in the blocking experiments are shown in Table 1. Sodium azide (NaN_3) was removed from all mAbs by overnight dialysis at 4° C. Anti-HLA-DR mAb was used as a positive control, and a mAb to an irrelevant epitope (anti-CD14, My4) as well as IgG1 and IgG2a mouse isotype control mAbs (Becton Dickinson) were used as negative controls in the blocking experiments. The IL-1 antiserum (2580) was raised in a rabbit by immunization with highly purified natural human IL-1β. It contained no preservatives and has been shown to neutralize natural and human recombinant IL-1β (Dr. J. Van Damme, Leuven) [27]. At our laboratory this antiserum (dilution 1/16 000) completely neutralized 2.5 IU/ml rIL-1β in the D10N bioassay. The antiserum to IL-6 was raised in a goat by immunization with human recombinant IL-6 and has been shown to neutralize natural and recombinant human IL-6 (Dr. M. Helle and Prof. L. Aarden, CLB, Amsterdam) [12]. This antiserum (dilution 1/16 000) completely neutralized 5 IU rIL-6/ml in the B9 bioassay. The EC were preincubated with the antibodies prior to adding PBMC.

Blocking with mAb of in vitro T-lymphocyte proliferation to control antigens

Pure tetanus toxin (from *Clostridium tetani*), without preservatives or aluminium hydroxide; Calbiochem, San Diego, Calif., USA) was used at a final concentration of 3 μg/ml. Concanavalin A (Con-A) type IV-S (Sigma Chemical Co.) was used at a final concentration of 10 μg/ml in the cultures. Nickel sulphate ($NiSO_4 \cdot 6H_2O$, analytic grade; Merck, Darmstadt) was used at final concentrations of 5, 7.5 and 10 μg/ml [20]. The culture and labelling conditions and other procedures were similar to those used in the autologous MECLR.

Statistical analysis

The results were analysed with STATA (computer program for statistical analysis; CRC, Los Angeles, Calif., USA). The tests used to determine statistical significance were Wilcoxon test (WT) and Wilcoxon's signed rank sum test (WSRT).

Results

Immunophenotyping of peripheral blood cells

The absolute number of peripheral blood leucocytes, lymphocytes and monocytes fell within the normal range in all psoriasis patients. The absolute number (mean ± SD) of activated peripheral blood T cells was increased in the majority of the patients: 199 ± 62 μl $CD3^+/CD25^+$ cells and 173 ± 76 μl $CD3^+$/HLA-DR^+ cells, as compared with an average of 100/μl in healthy individuals ($P < 0.01$, WT, $n = 12$). The number of TcRγδ$^+$ T lymphocytes, $CD5^+$ B cells and LFA-1α$^+$, LFA-1β$^+$, ICAM-1^+, LFA-3^+, $OKM1^+$, $CD36^+$ and IL-1β$^+$ leucocytes did not differ significantly from those of controls.

Immunophenotyping of ECS

In the ECS of psoriatic lesions, 3.2 ± 0.5% (mean ± SD) $CD45^+$ cells were observed, but no TcRγδ$^+$ cells. In ECS of psoriatic skin, the numbers of $CD36^+$, ICAM-1^+ and IL-1β$^+$ cells were clearly increased as compared with ECS of uninvolved psoriatic and healthy control skin. Immunofluorescence microscopy showed that ECS of lesional psoriatic skin contained 23 ± 12% $CD36^+$, 31 ± 14% ICAM-1^+ and 57 ± 21% IL-1β$^+$ cells (Table 2; $P < 0.02$, WT, when compared with ECS of normal control skin). In all experiments the proportions of psoriatic EC that were positive for both CD45 and CD36 after double staining were less than 1%. ECS of uninvolved skin from psoriasis patients contained 0–2% $CD36^+$ and ICAM-1^+ cells, but 12 ± 3% IL-1β$^+$ cells ($P < 0.05$, WT, when compared with ECS of normal control skin). ECS control skin contained a mean of 0.5 ± 0.1% (range 0–2%) $CD36^+$ and ICAM-1^+ cells, and 4 ± 0.5% IL-1β$^+$ cells (Table 2).

Table 2 CD36 $(OKM5)^+$, CD54 $(ICAM\text{-}1)^+$ and IL-1β$^+$ EC in lesional, uninvolved psoriatic skin and in healthy control skin. Values are mean percentages ± SD

	Lesional psoriatic skin ($n = 15$)	Uninvolved psoriatic skin ($n = 8$)	Healthy control skin ($n = 10$)
$CD36^+$	23 ± 12%	0.3 ± 0.1%	0.5 ± 0.1%
ICAM-1^+	31 ± 14%	0.6 ± 0.1%	0.5 ± 0.1%
IL-1β$^+$	57 ± 21%	12 ± 3%	4 ± 0.5%

Blocking of the autologous MECLR with mAb to adhesion molecules

The functional role of adhesion molecules was investigated by adding antibodies to the autologous MECLR. The proliferative responses obtained were compared with the proliferative responses of the 'untreated' MECLR. The results obtained using mAbs specific for the adhesion molecules CD2 (LFA-2/T11), CD11a (LFA-1α), CD18 (LFA-1β), CD54 (ICAM-1) and CD58 (LFA-3) are shown in Fig. 1. These mAbs clearly and consistently inhibited the autologous MECLR ($P < 0.0025$, WSRT). The isotype control mAb had no effect in this in vitro system. The mean ± SEM [^{3}H]TdR incorporation expressed as cpm in the autologous MECLR in the presence of IgG1 and IgG2a control mAbs were 21 277 ± 3095 and 20 831 ± 3217 ($n = 10$), respectively, which did not differ significantly from the untreated MECLR.

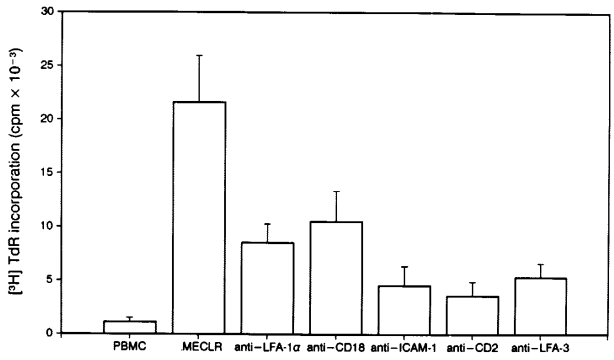

Fig. 1 The effect of blocking with anit-LFA mAb on the autologous MECLR. Each bar represents the mean ± SEM [^{3}H]TdR incorporated in the autologous MLR, MECLR and blocked MECLR with samples from 12 patients. All mAbs significantly inhibited the autologous MECLR ($P < 0.0025$, WSRT). The mean ± SEM [^{3}H]TdR incorporation in the autologous MECLR in the presence of IgG1 and IgG2a isotype control mAbs was 21277 ± 3095 and 20831 ± 3217 ($n = 10$), respectively, which did not differ significantly from the values from the untreated MECLR

Blocking of the autologous MECLR with an IL-1β-specific antiserum

The IL-1β-specific antiserum resulted in a consistent inhibition (mean 35 ± 14%, $P < 0.002$, WSRT) of the autologous MECLR (Fig. 2). The controls comprised EC preincubated with IgG2a isotype control mAb, 1% normal rabbit serum, a goat IL-6-specific antiserum and anti-HLA-DR mAb prior to the autologous MECLR. Anit-IL-6 antiserum had no effect on the autologous MECLR, whereas anti-HLA-DR mAb drastically inhibited the reaction (91 ± 8%, $P < 0.002$, WSRT; Fig. 2). The mean ± SEM [^{3}H]TdR incorporation in the autologous MECLR in the presence of IgG2a isotype control mAb and 1% normal rabbit serum were 20831 ± 3217 and 23682 ± 2644 ($n = 10$), respectively. These values did not differ significantly from the results from the untreated MECLR. When the same antibodies and highly purified AET-SRBC-T lymphocytes from three psoriatic patients were used in the autologous MECLR, identical results were obtained.

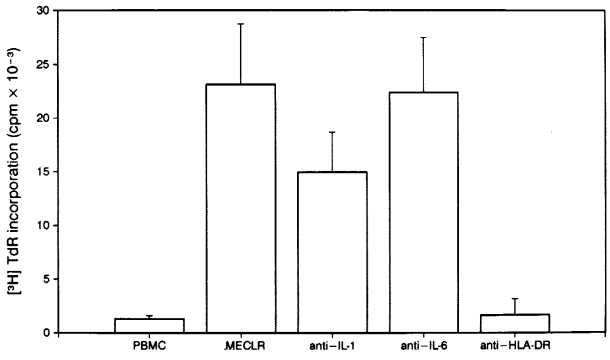

Fig. 2 The effect of blocking with anti-IL-1β, anti-IL-6 and anti-HLA-DR antibodies on the autologous MECLR. Each bar represents the mean ± SEM [^{3}H]TdR incorporated in the autologous MLR, MECLR and blocked MECLR with samples from 15 patients. The anti-HLA-DR mAb and anti-IL-1β polyclonal antiserum significantly inhibited the autologous MECLR ($P < 0.002$, WSRT). The mean ± SEM [^{3}H]TdR incorporation in the autologous MECLR in the presence of IgG2a isotype control mAb and 1% normal rabbit serum were 20831 ± 3217 and 23682 ± 2644 ($n = 10$), respectively. These values did not differ significantly from the values from the untreated MECLR

Furthermore, as a control, autologous EC preincubated with anti-IL-1β antiserum were used to present nickel to highly purified peripheral blood T lymphocytes from eight different patients with proven contact dermatitis to nickel. In these experiments the anti-IL-1β antiserum caused varying effects. A mean inhibition of 9% in three cases and a slight increase in the proliferative response of 16% in five cases were observed. The results in these cases were in clear contrast to the consistent inhibitory effects obtained in the autologous MECLR with psoriatic samples.

Depletion of CD36$^+$ cells from psoriatic ECS

Depletion of CD36$^+$ cells using an immunomagnetic rosetting technique was reproducible with an almost complete removal of CD36$^+$ cells from the ECS. After depletion, ECS of psoriatic lesions, contained 0–0.5% CD36$^+$ cells as determined by immunofluorescence microscopy. As shown in Table 3, the removal of CD36$^+$ cells did not significantly affect the autologous MECLR. In three cases a slight decrease and in six cases varying increases in the proliferative responses were observed. The results of controls using IgG1 mAb are shown in Table 3.

Table 3 The effect of depletion of EC positive for CD36 (OKM5) on the autologous MECLR with samples from psoriatic patients. Values represent the mean ± SEM [^{3}H]TdR incorporation of quadruplicate cultures. The source and concentration of the anti-CD36 and control mAb used are shown in Table 1 (*NT* not tested)

Patient no.	Proliferation (cpm)			
	Non-depleted EC		CD36-depleted EC	
	PBMC	MECLR	MECLR	IgG1 control
1	944 ± 175	24636 ± 2536	27351 ± 1605	NT
2	484 ± 180	16196 ± 1628	19029 ± 1254	14384 ± 1945
3	1773 ± 862	15369 ± 4759	21843 ± 6830	20707 ± 3208
4	613 ± 206	20940 ± 2132	24911 ± 2037	18653 ± 2911
5	1093 ± 223	16428 ± 1452	14052 ± 1175	19479 ± 1613
6	951 ± 317	15324 ± 1596	18773 ± 2567	NT
7	2537 ± 763	37442 ± 7059	39190 ± 4872	43810 ± 6459
8	1319 ± 341	26635 ± 1755	24860 ± 2199	NT
9	1161 ± 128	15496 ± 1235	12864 ± 1307	NT

Discussion

The increased expression of CD36 and ICAM-1 observed in this study in psoriatic lesions has also been observed in previous studies on psoriasis by others. Increased CD36

expression has also been described in other inflammatory dermatoses, suggesting upregulation of these molecules by proinflammatory cytokines [2, 3, 11]. The microscopic appearance of $CD36^+$ EC in ECS was that of medium to large-sized keratinocytes. The low number of $CD45^+$/$CD36^+$ EC as determined by double-labelling, and their expression in the upper epidermal layers indicate that the majority of the $CD36^+$ cells in lesional skin are keratinocytes. Although, intense expression of CD36 was observed on a considerable proportion of psoriatic keratinocytes, the results of the depletion experiments suggest that $CD36^+$ keratinocytes do not serve as accessory cells in the autologous MECLR. These results and those of a previous study on the autologous MECLR in psoriasis suggest that keratinocytes do not serve as accessory cells in this system. This may be explained by the fact that a limited number of keratinocytes in psoriatic lesions express MHC class II antigens which seem necessary for such an accessory function [26]. Consistent with this is the profound inhibitory effect of anti-HLA-DR mAb in the autologous MECLR (Fig. 2).

The results of the blocking studies using mAbs to the integrin family of adhesion molecules and their ligand(s) indicate a clear costimulatory role of these molecules in the autologous MECLR in psoriasis. This finding is consistent with the reported costimulatory function of these adhesion molecules in comparable in vitro systems using skin APC [25]. In accordance with the decreased stimulation of T cells after addition of anti-LFA mAb, in this study a reduced cell–cell aggregation (less clustered cells) was observed in the mixed cultures upon light-microscopic examination. Further illustrations of the importance of adhesion molecules in psoriasis are the LFA-mediated adherence of peripheral blood lymphocytes to cryostat sections of psoriatic lesions and the activation of keratinocytes by this adherence [7, 24]. Taken together, the results of our investigations indicate that in psoriatic patients, LFA-3 and ICAM-1 membrane molecules on $HLA\text{-}DR^+/CD1a^-$ epidermal APC deliver costimulatory signals to T cells in the autologous MECLR [19]. In the same system, the role of $ICAM\text{-}1^+$ keratinocytes seems restricted to co-immobilization of T lymphocytes resulting in cluster formation, and consequent T cell activation by epidermal APC. However, under certain in vitro conditions, keratinocytes may serve as accessory cells in T-cell-mediated responses [16, 23]. IFN-γ-treated cultured keratinocytes serve as accessory cells in an autologous mAb T-cell-mediated response to bacterial superantigen, PHA and immobilized anti-CD3 mAb, but not in an allogeneic T-cell-mediated response [16]. In this type of stimulation of autologous T cells, costimulatory signals are delivered via the LFA-1/ICAM-1 pair of adhesion molecules. It would be useful to investigate the effects of nominal or autologous antigen in this system to further delineate its in vivo relevance. MHC class I- and class II-independent, LFA-1/ICAM-1-mediated stimulation of allogeneic and autologous PBMC by PMA-treated cultured keratinocytes, but abrogation of their stimulatory capacity by IFN-γ pretreatment, has also been reported [23].

The observed inhibition by the antibody to IL-1β indicates that $IL\text{-}1\beta^+$ psoriatic EC deliver costimulatory signals to autologous T lymphocytes in the MECLR. The cellular source of the costimulatory IL-1 in the autologous MECLR remains unclear, because both keratinocytes and dendritic epidermal APC are known to produce this cytokine [4, 9]. Stimulation via peripheral blood monocyte-derived IL-1 appeared to be negligible in our system because of (1) the consistent, identical results when highly purified T cells were used and (2) the diverse effects of the same antibody to IL-1β in tetanus toxin and nickel-stimulated T cells using EC as APC. The functional relevance of IL-1 has been well documented in several in vitro systems, but its role in chronic inflammatory skin disease is not yet fully understood. Indirect indications of its importance are the findings that dendritic cell-derived IL-1β is an essential molecule required for the initiation of primary immune responses in skin and that skin-derived IL-1 is a potent inflammatory agent in human skin in vivo [5, 9]. The accessory function of IL-1 in the autologous MECLR and its elevated expression on EC from uninvolved and lesional skin observed in this study emphasizes the relevance of the reported IL-1 dysregulation in psoriasis [8].

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