The Effect of In Vivo Interferon-Gamma on the Distribution of LFA-1 and ICAM-1 in Normal Human Skin

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Lymphocyte function associated antigen 1 (LFA-1) and its ligand intercellular adhesion molecule 1 (ICAM-1) are cell surface adhesion molecules important in many lymphocyte-mediated responses. Recent in vitro studies have demonstrated that the cytokine interferon-gamma (IFN-γ) can induce ICAM-1 expression by keratinocytes, and that lymphocytes adhere to IFN-γ treated keratinocytes. In view of the importance of keratinocyte/lymphocyte interactions in the pathogenesis of cutaneous disease, we have examined the effects of in vivo IFN-γ on cutaneous expression of LFA-1 and ICAM-1. Fourteen volunteers received intradermal IFN-γ (dose:1 or 10 μg) daily for 3 d. Biopsy was obtained on day 6. Cryostat sections were stained by the peroxidase anti-peroxidase technique employing murine monoclonal antibodies to CD11, CD18, and ICAM-1. IFN-γ intensified ICAM-1 expression by dermal endothelial cells and induced keratinocyte expression of ICAM-1. Furthermore, after administration of 10 μg of IFN-γ LFA-1 positive (LFA + ve) lymphocytes were observed along the basement membrane zone closely related to ICAM-1 + ve basal keratinocytes and also surrounding dermal endothelium. Exposure to IFN-γ induced expression of both CD11a and CD18 antigens on epidermal Langerhans cells. These studies suggest that the distribution of adherence molecules expression within cutaneous tissue in vivo is modulated by IFN-γ, and that these alterations may be important in interactions involving cutaneous immunocompetent cells. *J Invest Dermatol 93:439–442, 1989*

The leukocyte integrins comprise a family of three heterodimer glycoproteins termed lymphocyte function associated antigen-1 (LFA-1), Mac 1, and p 150,95. Each is composed of a common chain (CD18) and a distinct chain (CD11a, CD11b, and CD11c, respectively) [1]. LFA-1, Mac 1, and p 150,95 are cell surface glycoproteins involved in intercellular adhesion reactions [2] unlike the other members of the integrin family, which act as receptors for extracellular matrix components such as fibronectin [3]. While expression of Mac 1 and p 150,95 is limited to cells of the monocyte/macrophage lineage and neutrophils, LFA-1 is expressed by all leukocytes, including T and B lymphocytes and null cells [4]. Antigen-independent adhesion via LFA-1 appears to be essential in the development of most lymphocyte mediated responses, including helper T lymphocyte responses, cytotoxic T cell responses, and adhesion of lymphocytes to endothelial cells and fibroblasts [5].

More recently, the ligand for LFA-1, termed intercellular adhesion molecule-1 (ICAM-1), has been identified [6,7]. It is a single chain glycoprotein of molecular weight 90 kd showing significant sequence homology with molecules belonging to the so-called immunoglobulin supergene family [8]. It has a similar tissue distribution to HLA-DR, being expressed on the surface of endothelial cells, fibroblasts, dendritic cells, and certain epithelial cells; for example, thymic epithelium [9]. In addition, cytokines such as interferon-gamma (IFN-γ), interleukin-1 [9], and tumour necrosis factor [10] regulate ICAM-1 expression by certain cell types, including vascular endothelial cells. This suggests that cytokine controlled ICAM-1/LFA-1 interactions may be involved in the sequestration of lymphocytes (LFA-1 + ve) into sites of local inflammation, because adhesion of lymphocytes to endothelial cells appears to be an important step in this process.

In normal human epidermis keratinocytes do not express ICAM-1 [9]. However, in vitro studies have demonstrated that recombinant IFN-γ treated human keratinocytes express ICAM-1 [11], and that lymphocytes adhere to keratinocytes incubated with IFN-γ [12], a reaction that can be blocked by monoclonal antibodies to either ICAM-1 or LFA-1. Furthermore, adherence via ICAM-1/LFA-1 is the only known adhesion pathway between keratinocytes and lymphocytes [11].

Whether the in vitro effects of IFN-γ on human keratinocytes occur in vivo is not known. In view of the potentially important role of keratinocyte-lymphocyte interactions in the pathogenesis of inflammatory cutaneous disease [13], we have assessed the effects of intradermally administered recombinant human IFN-γ on the distribution of these adherence molecules in normal human skin, in vivo.

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Abbreviations:
APAAP: alkaline phosphatase anti alkaline phosphatase
ICAM-1: intercellular adhesion molecule-1
IFN-γ: interferon-gamma
LFA-1: lymphocyte function associated antigen-1
μg: microgram
PAP: peroxidase anti-peroxidase

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MATERIALS AND METHODS

Subjects Fourteen adult male volunteers (age range 18–44) were recruited for the study. All received a general medical examination, including blood pressure and temperature measurement, and were screened for haematologic and biochemical abnormalities.

Interferon-Gamma Recombinant human interferon-gamma (specific activity = 2×10^7 U per mg) was kindly donated by Biogen Incorporated. Each volunteer received an intradermal injection of recombinant IFN-γ diluted in 0.1 ml sterile water, to one precise area on the left buttock daily for three consecutive days. Nine volunteers received a daily dose of 10 μg IFN-γ, and four received 1μg daily. A similar area on the right buttock received 0.1 ml of diluent only for three consecutive days, as control.

Biopsy Biopsy was obtained 5 d after the initial injection (i.e., study day 6) from injected sites on both buttocks by 6 mm punch biopsy under local anaesthetic. Each specimen was bisected with half snap frozen prior to storage in liquid nitrogen and the second half fixed and processed for staining with haematoxylin and eosin. 5-μm cryostat sections were then cut from each frozen biopsy, air dried, and fixed in acetone for 10 min prior to immunohistochemical analysis.

Immunohistochemistry The three-step peroxidase anti-peroxidase (PAP) immunohistochemical assay was used, as described elsewhere [14], employing diaminobenzidine as substrate. This provides more sensitive results than standard indirect immunoperoxidase reactions, without loss of specificity. The primary murine monoclonal antibodies used are listed in Table I. Positive staining was identified as a brown reaction product under the light microscope.

To determine the phenotype of epidermal cells expressing LFA-1 (see later), a double immunoenzymatic technique as described by Allen et al [15] was employed, using the antibodies OKT6 (CD1) and MHM24 (CD18). OKT6 was linked to an alkaline phosphatase anti-alkaline phosphatase catalysed reaction (APAAP), and MHM24 was linked to a PAP reaction. In this way CD1 molecules were identified as a blue reaction product and CD18 as a brown product. Double labeled cells appeared black and could easily be differentiated from singly labeled cells.

RESULTS

ICAM-1 In the control biopsy specimens (no IFN-γ administered) positive staining for ICAM-1 was limited to dermal endothelial cells (Fig 1). ICAM-1 expression by Langerhans cells, keratinocytes, and cells of the acrosyringium was uniformly absent. One microgram of recombinant human IFN-γ injected daily for three consecutive days induced cell surface expression of ICAM-1 by basal keratinocytes, while suprabasal keratinocyte and Langerhans cell staining was absent (Fig 2). Within the dermis a sparse infiltrate was observed of similar degree to that observed within the control biopsies. Ten micrograms of recombinant human IFN-γ induced intense ICAM-1 expression on the surface of most keratinocytes; staining was maximal along the basal layer (Fig 3). A few scattered cells throughout a moderate dermal inflammatory infiltrate also expressed ICAM-1. The intensity of staining for ICAM-1 by dermal endothelial cells was observed to be greater after administration of IFN-γ (both 1 μg and 10 μg) (Fig 4).

Table I. Table of the Primary Murine Monoclonal Antibodies (McAb) Used in this Study

McAb	Specificity	Dilution	Source
RR1/1	ICAM-1	1:5000	T. Springer (6)
MHM23	CD11a	1:160	Dako
MHM24	CD18	1:160	Dako
Leu1	CD3	1:20	Becton-Dickinson
OKT6	CD1	1:20	Ortho Diagnostics

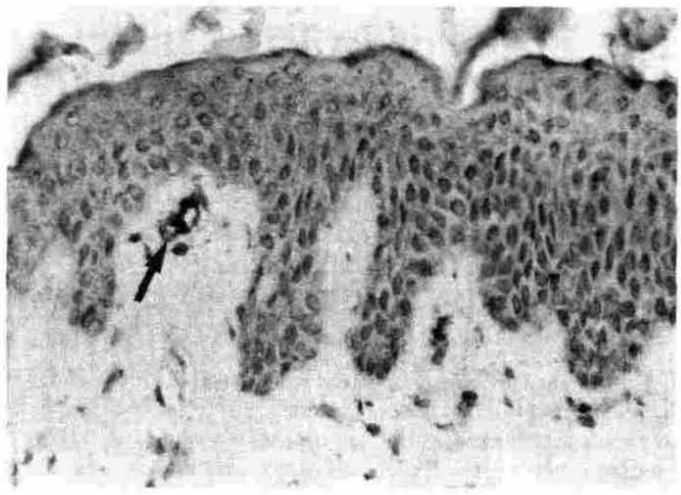

Figure 1. Photomicrograph of normal skin that received diluent only stained with antibody RR1/1. Only dermal endothelial cells *(arrow)* express ICAM-1 (×150).

LFA-1 In normal human skin not exposed to IFN-γ, cells expressing both CD11a and CD18 antigens, presumably leukocytes, were observed scattered throughout the sparse dermal infiltrate. These were situated mainly around dermal vessels. No epidermal positivity was noted for either antigen; in particular, dendritic cell expression of CD18 was not observed. No change was noted after administration of 1 μg of recombinant human IFN-γ. When 10 μg of recombinant human IFN-γ was administered intradermally a moderate dermal infiltrate was produced. Of these infiltrating cells, nearly all expressed both CD11a and CD18 antigens. The majority were closely arranged around dermal vessels (ICAM-1 + ve), but a significant proportion were also observed along the basement membrane zone, closely applied to basal keratinocytes (ICAM-1 + ve) (Fig 5). Immunoperoxidase labeling of serial 5 μm sections with Leu 1 antibody, a pan T cell marker, demonstrated that the majority of these cells were T lymphocytes.

As previously mentioned, expression of LFA-1 by epidermal Langerhans cells in control biopsy specimens was not observed. Ten micrograms of IFN-γ, however, induced expression of both CD11a and CD18 antigens on dendritic cells throughout the epidermis, although CD18 expression was more intense (Fig 3). Double label-

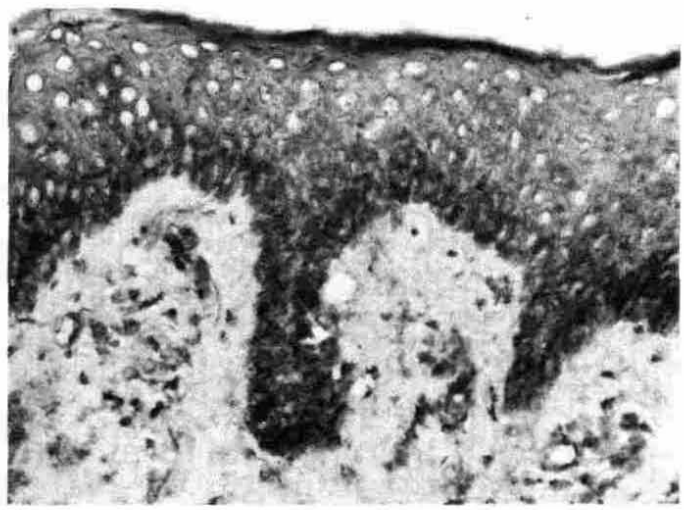

Figure 2. Photomicrograph of normal skin injected with 1 μg recombinant IFN-γ (×3) stained for ICAM-1. Basal keratinocytes express ICAM-1, but suprabasal staining is absent (×150).

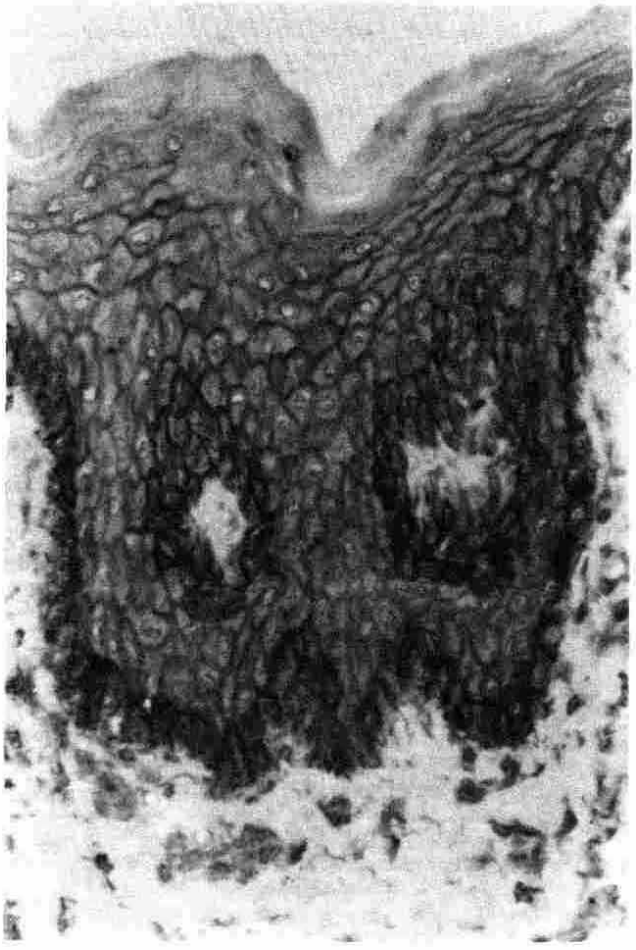

Figure 3. Photomicrograph of normal skin injected with 10 μg recombinant IFN-γ (×3) stained for ICAM-1. Keratinocytes display strong cell surface expression of ICAM-1, which is maximal in the basal layer (×200).

ing experiments revealed that 60% of epidermal CD18 positive cells co-localized with OKT6 antibody.

Histologic examination of fixed sections stained with haematoxylin and eosin revealed no evidence of keratinocyte damage. In particular, no dyskeratotic cells nor hydropic change was observed.

DISCUSSION

This study has demonstrated that, in vivo, *IFN-γ induced keratinocyte* ICAM-1 expression, the intensity of which was dependent on the concentration of IFN-γ administered. Furthermore, LFA-1 + ve cells were observed along the basement membrane zone, consistent with adhesion of lymphocytes to ICAM-1 + ve basal keratinocytes. In the epidermis, Langerhans cells expressed both CD11a and CD18 antigens after exposure to IFN-γ. Within the dermis, endothelial cell ICAM-1 expression was intensified, and LFA + ve cells were observed in close proximity to these cells.

In vitro studies have demonstrated that lymphocytes adhere to keratinocytes via LFA-1, a lymphocyte surface glycoprotein, and its ligand ICAM-1 is expressed by keratinocytes [11,12]. This interaction, however, is dependent on prior exposure of keratinocytes to IFN-γ, because unstimulated keratinocytes do not possess ICAM-1. The importance of these molecules lies in the fact that adhesion of lymphocytes to target cells via this pathway is an obligate step in the induction and maintenance of T-cell responses such as T helper cell and cytotoxic T-cell activity. Alterations in keratinocyte-lymphocyte interactions are thought to play a major role in the pathogenesis of many inflammatory cutaneous diseases [13], such as discoid lupus erythematosus, lichen planus, and cutaneous graft versus host disease, where lymphocytes are in close apposition to keratinocytes. Each of these diseases is further characterized by an infiltrate rich in activated T lymphocytes [16] (the presumed source of IFN-γ) and by keratinocyte expression of HLA-DR [17], suggesting that IFN-γ has potent epidermal effects in vivo. Our study demonstrates that IFN-γ induces similar changes in vivo to those observed in vitro, suggesting that it may play a central role in regulating keratinocyte/lymphocyte interactions in vivo.

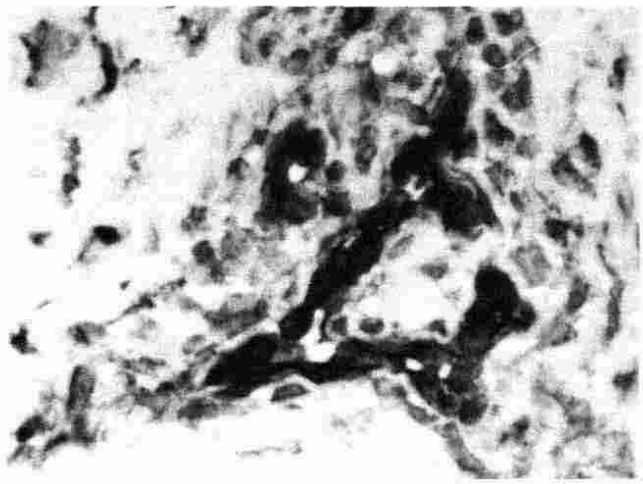

Figure 4. Photomicrograph of normal skin injected with 10 μg recombinant IFN-γ (×3) stained for ICAM-1. Intense expression of ICAM-1 by dermal endothelial cells is observed (×250).

Although the immunoperoxidase assay used in this study is not quantitative, it is our impression that intradermal IFN-γ increased the expression of ICAM-1 on dermal endothelial cells, consistent with the results of in vitro studies [9]. The consequences of increased expression may be to facilitate the sequestration of LFA-1 + ve cells into the dermis, thereby helping to promote an inflammatory infiltrate. This is further suggested by the observed close apposition of LFA-1 + ve cells to ICAM-1 + ve endothelial cells and by other group's findings that lymphocytes adhere to dermal endothelial cells, in vitro [18]. It is interesting to speculate what the initial

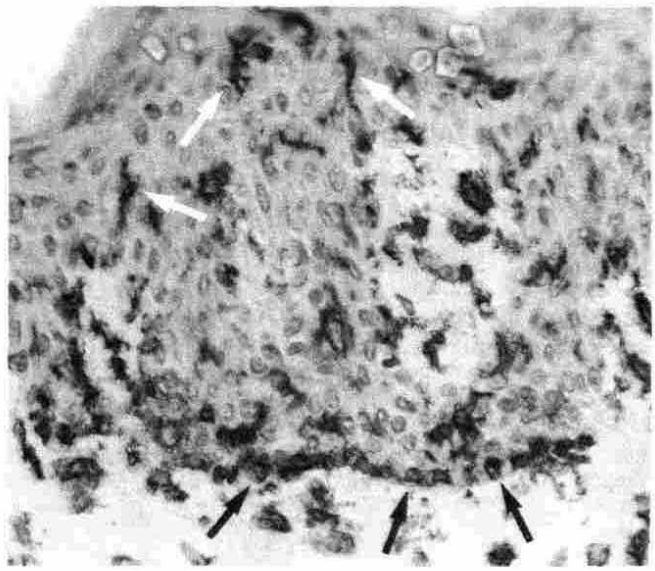

Figure 5. Photomicrograph of normal skin injected with 10 μg recombinant IFN-γ (×3) stained with antibody to CD18. Small round infiltrating cells *(black arrows)* are seen along the basement membrane zone. Scattered dendritic figures *(white arrows)* are seen throughout the epidermis (×250).

trigger for this may be. Cytokine production by antigen-stimulated keratinocytes may be responsible for the enhanced expression of ICAM-1 by endothelium. One potentially responsible peptide is IL-1, a known product of keratinocytes [19], which is capable of inducing ICAM-1 expression by endothelial cells, but not keratinocytes [11], in vitro.

It is of interest that dendritic cells within the epidermis expressed both CD11a and CD18 antigens after exposure to IFN-γ. Double labeling experiments with antibodies OKT6 and MHM24 demonstrated that most dendritic epidermal cells expressing CD18 molecules were also CD1 positive. Although we did not identify positive staining of Langerhans cells for either antigen in the control biopsies, other investigators have observed expression of CD18, but not CD11a antigens, on Langerhans cells, implying that they may be immature leukocytes [20]. The inducible changes in Langerhans cell LFA-1 expression suggest that IFN-γ may be involved in the regulation of Langerhans cell function. We cannot discount the possibility, however, that the observed changes in Langerhans cell surface antigen expression are secondary effects of mediators such as other cytokines, released by cells within the IFN-γ induced inflammatory infiltrate. Nevertheless, this seems unlikely, because epidermal dendritic cell expression of LFA-1 was observed in sites distant from the perivascular infiltrate.

The alterations in distribution of adhesion molecule expression induced in normal skin by IFN-γ strongly suggest that it is important in regulation of dermal endothelium/lymphocyte, keratinocyte/lymphocyte, and, perhaps, keratinocyte/Langerhans cell interactions. Furthermore, the inducible expression of ICAM-1 on keratinocytes provides further evidence of an important role for keratinocytes in skin immune responses.

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REFERENCES

1. Sanchez-Madrid F, Krensky AM, Ware CF, Robbins E, Strominger JL, Burakoff SJ, Springer TA: A human leukocyte differentiation antigen family with distinct subunits and a common subunit: the lymphocyte function-associated antigen (LFA-1), the C3bi complement receptor (OKM1/Mac-1) and the p150,95 molecule. J Exp Med 158:1785–1803, 1983
2. Anderson DC, Springer TA: Leukocyte adhesion deficiency: an inherited defect in the Mac-1, LFA-1, and p150,95 glycoproteins. Ann Rev Med 38:175, 1987
3. Hynes RO: Integrins: a family of cell surface receptors. Cell 48:549–554, 1987
4. Krensky AM, Sanchez-Madrid F, Robbins E, Nagy JA, Springer TA, Burakoff SJ: The functional significance, distribution, and structure of LFA-1, LFA-2, LFA-3: cell surface antigens associated with CTL-target interactions. J Immunol 131:611–616, 1983
5. Springer TA, Dustin AI, Kishimoto TK, Marlin SD: The lymphocyte function-associated LFA-1, CD2, and LFA-3 molecules: cell adhesion receptors of the immune system. Ann Rev Immunol 5:223, 1987
6. Rothlein R, Dustin ML, Marlin SD, Springer TA: A human intercellular adhesion molecule (ICAM-1) distinct from LFA-1. J Immunol 137:1270–1274, 1986
7. Marlin SD, Springer TA: Purified intercellular adhesion molecule-1 (ICAM-1) is a ligand for lymphocyte function-associated antigen 1 (LFA-1). Cell 51:813–819, 1987
8. Staunton DE, Marlin SD, Stratowa C, Dustin ML, Springer TA: Primary structure of ICAM-1 demonstrates interaction between members of the immunoglobulin and integrin supergene families. Cell 52:925–933, 1988
9. Dustin ML, Rothlein R, Bhan AK, Dinarello CA, Springer TA: Induction by IL-1 and interferon, tissue distribution, biochemistry and function of a natural adherence molecule (IACM-1). J Immunol 137:245–254, 1986
10. Pober JA, Gimbrone MA, Jr., Lapierre LA, Mendrick DL, Fiers W, Rothlein R, Springer TA: Overlapping patterns of activation of human endothelial cells by interleukin 1, tumor necrosis factor and immune interferon. J Immunol 137:1893, 1986
11. Dustin ML, Singer KH, Tuck DT, Springer TA: Adhesion of T lymphoblasts to epidermal keratinocytes is regulated by interferon gamma and is mediated by intercellular adhesion molecule-1 (ICAM-1). J Exp Med 167:1323–1340, 1988
12. Nickoloff BJ, Lewinsohn DM, Butcher EC, Krensky AM, Clayberger: Recombinant gamma interferon increases the binding of peripheral blood mononuclear leukocytes and a Leu-3+ T lymphocyte clone to cultured keratinocytes and to a malignant cutaneous squamous carcinoma cell line that is blocked by antibody against the LFA-1 molecule. J Invest Dermatol 90:17–22, 1988
13. Breathnach SM, Katz SI: Cell mediated immunity in cutaneous disease. Hum Pathol 17:161–167, 1986
14. Holden CA, MacDonald DM: Immunoperoxidase techniques in dermatopathology. Clin Exp Dermatol 8:443–457, 1983
15. Allen MH, Markey AC, MacDonald DM: Development of a reproducible immunocytochemical technique for demonstrating co-localisation of cutaneous antigens (unpublished)
16. Barker JNWN, Navsaria HA, Leigh IM, MacDonald DM: Gamma-interferon induced human keratinocyte HLA-DR synthesis: the role of dermal activated T lymphocytes. Br J Dermatol 119:567–572, 1988
17. Aubock J, Romani N, Grubauer G, Fritsch P: HLA-DR expression by keratincoytes is a common feature of diseased skin. Br J Dermatol 114:467–472, 1986
18. Haskard D, Cavender D, Beatty P, Springer T, Ziff M: T lymphocyte adhesion to endothelial cells: mechanisms demonstrated by anti-LFA-1 monoclonal antibodies. J Immunol 137–2901, 1986
19. Luger TA, Stadler BM, Katz SI, Oppenheim JJ: Epidermal cell (keratinocyte) derived thymocyte activating factor (ETAF). J Immunol 127:1493, 1981
20. Gothelf Y, Hanau D, Schmitt DA, Cazenave J-P, Gazit E: Expression of the lymphocyte function-associated antigen 1 (LFA-1) glycoprotein family on human epidermal Langerhans cells. J Invest Dermatol (abstr) (in press).