The Chemokine Receptor CCR6 Identifies Interferon-γ Expressing T Cells and is Decreased in Atopic Dermatitis as Compared with Psoriasis

To the Editor:

Atopic dermatitis (AD) is associated with an increased production of T helper (Th) 2 cytokines such as interleukin (IL)-4 and IL-13 and a decrease in the production of the Th1 cytokine, interferon (IFN)-γ, in the peripheral blood and acute skin lesions (Jujo *et al*, 1992; Hamid *et al*, 1994). Increased Th2 cytokines leads to the synthesis of IgE and induction of adhesion molecules that are involved in the migration of allergic inflammatory cells such as eosinophils into the skin lesions (Akdis *et al*, 1999; Bochner, 2000). On the other hand, a decrease in IFN-γ contributes to the production of Th2 cytokines and IgE synthesis as this cytokine downregulates Th2 responses (Leung, 2000).

Chemokines are a group of polypeptides that recruit leukocytes to the site of tissue inflammation (Lukacs *et al*, 2001). CCR6 is a chemokine receptor that has been shown to be highly expressed in skin-homing CLA^+ (cutaneous lymphoid antigen) memory T cells (Homey *et al*, 2000). The chemokine, CCL20 (previously known as macrophage inflammatory protein-3α, MIP-3α) has been identified as the natural ligand for CCR6 (Rossi *et al*, 1997; Greaves *et al*, 1997). Expression of CCR6 has been shown to be upregulated in psoriasis (Homey *et al*, 2000); however, the cytokine expression of $CCR6^+$ T cells in psoriasis were not determined. Therefore, we were interested in determining whether $CCR6^+$ T cells are decreased in AD and whether they are associated with increased IFN-γ expression.

Six patients with moderate to severe chronic AD of more than 1 y (skin involvement of 15–80%) and two control groups were studied: (i) six normal subjects with no history of AD, allergic rhinitis, or asthma, and (ii) six patients with psoriasis (skin involvement of 10–70%). Flow cytometry was used to examine the frequency of blood $CCR6^+$ T cells. Expression of IFN-γ and IL-4 was studied using intracellular cytokine staining.

Table I summarizes the mean percentages of $CD3^+/CCR6^+$, $CD4^+/CCR6^+$, and $CD8^+/CCR6^+$ T cells in the study groups. The percentage of $CD3^+/CCR6^+$ T cells was significantly lower in AD patients compared with that of psoriasis patients ($p<0.05$). Comparison of the expression of CCR6 in the $CD4^+$ and $CD8^+$ T cell populations in the three study groups showed similar results with AD patients having significantly lower percentages of $CD4^+/CCR6^+$ and $CD8^+/CCR6^+$ blood T cells compared with that of psoriasis patients ($p<0.05$). This table also shows that within the T cell compartment, CCR6 is mainly expressed by the $CD4^+$ cells in all three study groups.

As CCR6 is mainly expressed by $CD4^+$ cells within the T cell compartment, we also compared the expression of CCR6 in $CD4^+$ T cells, which express the skin-homing receptor, CLA, in the three study groups. Our data show that both AD patients and normal subjects have a significantly lower percentage of $CD4^+/CLA^+/CCR6^+$ T cells than that of psoriasis patients ($p<0.05$). There was no significant difference between the percentage of $CD4^+/CLA^+/CCR6^+$ T cells between AD patients and normal subjects.

Table II shows the expression of IFN-γ and IL-4 by $CCR6^+$ blood T cells in the study groups. IFN-γ was expressed by $46.3\pm8.1\%$ of $CD4^+/CCR6^+$ T cells in AD patients, $53.0\pm2.8\%$ in psoriasis patients, and $62.8\pm9.2\%$ in normal subjects, whereas only $9.4\pm8.1\%$ of the $CD4^+/CCR6^-$ T cells in AD, $14.1\pm2.8\%$ in psoriasis and $9.6\pm9.2\%$ in normal subjects express IFN-γ ($p<0.05$). On the other hand, the percentage of $CD4^+/CCR6^+$ blood T cells expressing IL-4 was relatively low in all three study groups, with values of $0.8\pm0.6\%$ in AD patients, $1.1\pm0.5\%$ in psoriasis patients, and $0.3\pm0.2\%$ in normal subjects. The expression of IL-4 in the $CD4^+/CCR6^-$ T cell population was significantly higher than $CD4^+/CCR6^+$ T cells for all three study groups ($p<0.05$). Therefore, $CD4^+/CCR6^+$ blood T cells are associated with increased IFN-γ, expression compared with $CD4^+/CCR6^-$ blood T cells.

This study demonstrates that the frequency of $CCR6^+$ blood T cells are decreased in AD compared with that of another inflammatory skin disease, i.e., psoriasis. This is of interest as psoriasis has been shown to be primarily a Th1-mediated skin disease associated with increased CCR6 cells in the CLA^+ T cell subset (Schlaak *et al*, 1994). This latter study did not examine cytokine expression of CCR6 cells. Our results therefore extend this finding in that we have found $CCR6^+$ cells in the $CD4^+$ T cell subset, of all three groups of study subjects, to be associated with increased IFN-γ expression compared with $CD4^+/CCR6^-$ T cells.

This decrease in $CD4^+/CLA^+/CCR6^+$ T cells may play an important part in the increased Th2 cytokine profile seen in acute AD skin lesions as compared with psoriasis skin lesions (Hamid

Table I. Expression of CCR6 on $CD3^+$, $CD4^+$, and $CD8^+$ T cells[a]

	CCR6 expression on:		
	CD3	CD4	CD8
AD (n = 6)	$4.4\pm1.1\%^b$	$5.8\pm1.5\%^b$	$1.8\pm1.1\%^a$
Normal (n = 6)	$6.7\pm2.9\%$	$7.5\pm2.2\%$	$3.1\pm1.2\%$
Psoriasis (n = 6)	$9.3\pm3.3\%$	$12.2\pm4.8\%$	$4.1\pm1.3\%$

[a]In these experiments, peripheral blood mononuclear cells from the study subjects were stained with phycoerythrin-conjugated anti-CCR6 or an isotype-matched antibody (R&D Systems, Inc., Minneapolis, MN), and Peridinin Chlorophyll protein-conjugated anti-CD3, and antigen-presenting cell-conjugated anti-CD4 or anti-CD8 for 30 min. Analysis was performed using a FACScalibur flow cytometer (Becton Dickinson, San Jose, CA). An isotype-matched antibody control was used to verify the staining specificity of CCR6. [b]$p<0.05$, as compared with psoriasis.

Manuscript received May 8, 2002; revised August 29, 2002; accepted for publication August 31, 2002

Reprint requests to: Donald Y.M. Leung, MD, PhD, National Jewish Medical and Research Center, Department of Pediatrics, Room K926, 1400 Jackson Street, Denver, CO 80206, U.S.A. Email: leungd@njc.org

Table II. IFN-γ and IL-4 expression in CD4/CCR6$^+$ and CD4/CCR6$^-$ T cells[a]

	%IFN-γ in:		%IL-4 in:	
	CD4/CCR6$^+$	CD4/CCR6$^-$	CD4/CCR6$^+$	CD4/CCR6$^-$
AD (n = 6)	46.3 ± 8.1%[b]	9.4 ± 8.1%	0.8 ± 0.6%[b]	3.1 ± 0.6%
Normal (n = 6)	62.8 ± 9.2%[b]	9.6 ± 9.2%	0.3 ± 0.2%[b]	1.7 ± 0.2%
Psoriasis (n = 6)	53.0 ± 2.8%[b]	14.1 ± 2.8%	1.1 ± 0.5%[b]	3.5 ± 0.5%

[a] 2×10^6 peripheral blood mononuclear cells per ml were stimulated with 50 ng per ml phorbol myristate acetate and 1 μg per ml ionomycin in the presence of 10 μg per ml brefeldin A (Sigma, St. Louis, MO) in RPMI 1640 with 10% fetal bovine serum for 4 h at 37°C. The cells were then spun down and resuspended in staining solution at 2×10^7 per ml and incubated in a 96-well microtiter plate with phycoerythrin-conjugated anti-CCR6 or an isotype-matched antibody and Peridinin Chlorophyll protein-conjugated anti-CD4 for 30 min at 4°C. The cells were spun down and incubated for 10 min with fluorescence-activated cell sorter-lysing solution and permealizing solution (B-D Pharmigen, San Diego, CA) at room temperature. The cells were then washed once with wash buffer and resuspended at 2×10^7 per ml in staining solution and incubated with fluorescein isothiocyanate-conjugated anti-IFN-γ and antigen-presenting cell-conjugated anti-IL-4 (Becton-Dickinson) for 30 min at room temperature. The cells were then washed two times and fixed in 1% paraformaldehyde solution for four-color flow cytometric analyses. [b] $p < 0.05$ as compared with CD4/CCR6$^-$ cells.

et al, 1996). In mouse models of AD, it has been found that IFN-γ deficiency is associated with increased systemic and skin directed Th2 responses (Habu *et al*, 2001). Interestingly, it has been shown that chronic AD skin lesions have a higher level of CCL20 and CCR6 compared with normal skin (Nakayama *et al*, 2001). Based on our observation that CCR6 is a marker for IFN-γ expressing T cells, an increased infiltration of CD4$^+$/CLA$^+$/CCR6$^+$ T cells to chronic AD skin lesions may account for the increased IFN-γ expression in these lesions as compared with normal skin (Grewe *et al*, 1994).

The increased Th2 cytokine profile in AD skin lesions likely occurs as the result of T cells expressing other chemokine receptors that contribute to polarization of the Th2 immune response. A recent study has shown that the frequency of blood CD4$^+$/CCR4$^+$ T cells, which are associated with increased IL-4 expression, is increased in AD patients (Nakatani *et al*, 2001). Therefore, the combination of both a decrease in CD4$^+$/CCR6$^+$ T cells and an increase in CD4$^+$/CCR4$^+$ T cells may further increase the predominance of Th2 cytokines in AD.

This work was supported in part by NIH grants HL36577, AR41256, HL37260, General Clinical Research Center grant MO1 RR00051 from the Division of Research Resources, and the University of Colorado Cancer Center. Dr Ong was the recipient of a NIH National Research Service Award (T32 AI 07365) and an AAAAI Fujisawa Health Care Allergic Skin Diseases Award.

Peck Y. Ong* and Donald Y. M. Leung*†
*Division of Allergy and Immunology, Department of Pediatrics, The National Jewish Medical and Research Center, Denver, CO 80206, U.S.A.; †Department of Pediatrics, University of Colorado Health Sciences Center, Denver, CO 80262, U.S.A.

REFERENCES

Akdis CA, Akdis M, Simon D, *et al*: T cells and T cell-derived cytokines as pathogenic factors in the nonallergic form of atopic dermatitis. *J Invest Dermatol* 113:628–634, 1999

Bochner BS: Road signs guiding leukocytes along the inflammation superhighway. *J Allergy Clin Immunol* 106:817–828, 2000

Greaves DR, Wang W, Dairaghi DJ, *et al*: CCR6, a CC chemokine receptor that interacts with macrophage inflammatory protein 3alpha and is highly expressed in human dendritic cells. *J Exp Med* 186:837–844, 1997

Grewe M, Gyufko K, Schopf E, Krutmann J: Lesional expression of interferon-gamma in atopic eczema. *Lancet* 343:25–26, 1994

Habu Y, Seki S, Takayama E, *et al*: The mechanism of a defective IFN-gamma response to bacterial toxins in an atopic dermatitis model, NC/Nga mice, and the therapeutic effect of IFN-gamma, IL-12, or IL-18 on dermatitis. *J Immunol* 166:5439–5447, 2001

Hamid Q, Boguniewicz M, Leung DY: Differential *in situ* cytokine gene expression in acute versus chronic atopic dermatitis. *J Clin Invest* 94:870–876, 1994

Hamid Q, Naseer T, Minshall EM, Song YL, Boguniewicz M, Leung DY: *In vivo* expression of IL-12 and IL-13 in atopic dermatitis. *J Allergy Clin Immunol* 98:225–231, 1996

Homey B, Dieu-Nosjean MC, Wiesenborn A, *et al*: Up-regulation of macrophage inflammatory protein-3 alpha/CCL20 and CC chemokine receptor 6 in psoriasis. *J Immunol* 164:6621–6632, 2000

Jujo K, Renz H, Abe J, Gelfand EW, Leung DY: Decreased interferon gamma and increased interleukin-4 production in atopic dermatitis promotes IgE synthesis. *J Allergy Clin Immunol* 90:323–331, 1992

Leung DY: Atopic dermatitis new insights and opportunities for therapeutic intervention. *J Allergy Clin Immunol* 105:860–876, 2000

Lukacs NW, Prosser DM, Wiekowski M, Lira SA, Cook DN: Requirement for the chemokine receptor CCR6 in allergic pulmonary inflammation. *J Exp Med* 194:551–555, 2001

Nakatani T, Kaburagi Y, Shimada Y, Inaoki M, Takehara K, Mukaida N, Sato S: CCR4 memory CD4$^+$ T lymphocytes are increased in peripheral blood and lesional skin from patients with atopic dermatitis. *J Allergy Clin Immunol* 107:353–358, 2001

Nakayama T, Fujisawa R, Yamada H, *et al*: Inducible expression of a CC chemokine liver- and activation-regulated chemokine (LARC)/macrophage inflammatory protein (MIP)-3 alpha/CCL20 by epidermal keratinocytes and its role in atopic dermatitis. *Int Immunol* 13:95–103, 2001

Rossi DL, Vicari AP, Franz-Bacon K, McClanahan TK, Zlotnik A: Identification through bioinformatics of two new macrophage proinflammatory human chemokines: MIP-3alpha and MIP-3beta. *J Immunol* 158:1033–1036, 1997

Schlaak JF, Buslau M, Jochum W, *et al*: T cells involved in psoriasis vulgaris belong to the Th1 subset. *J Invest Dermatol* 102:145–149, 1994