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Lymphocyte and Monocyte Localization of Altered Adrenergic Receptors, cAMP Responses, and cAMP Phosphodiesterase in Atopic Dermatitis

A Possible Mechanism for Abnormal Radiosensitive Helper T Cells in Atopic Dermatitis

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Atopic dermatitis (AD) is a chronic pruritic inflammatory skin disease demonstrating a wide spectrum of clinical manifestations (1). The varied inflammatory processes are suggestive of multiple immune abnormalities which may encompass forms of IgE-mediated immediate hypersensitivity as well as delayed cell-mediated reactive processes. This paper addresses relationships between the cells involved in the immune abnormalities and the disordered regulation of cyclic nucleotides in AD.

What are the cells which express immune abnormalities in AD? There is ample clinical evidence for T-cell dysfunction in AD as reflected by the increased susceptibility to cutaneous fungal and viral infections, reduced incidence of allergic contact dermatitis, and reduced sensitization to poison oak and dinitrochlorobenzene (DNCB) (1). In vitro studies of T-cell parameters demonstrate depressed mitogen and antigen responsiveness in severe cases of AD and reduced numbers of T cells (2, 3); specifically, those T cells bearing suppressor/cytotoxic phenotypic features, such as FcIgG receptors (4, 5), OKT8 antigen (6), and histamine H_2 receptors (7). Although depressed cytotoxic T-lymphocyte activity against class I alloantigen-bearing targets has been demonstrated (8), evidence for a nonspecific suppressor T-cell defect has been controversial (5, 9). It often has been proposed that an isotype-specific, suppressor T-cell defect in regulation of IgE production will be found in AD, since these patients demonstrate elevated serum IgE levels (10) and their peripheral blood B cells synthesize markedly elevated quantities of IgE in vitro (11–13). The clear demonstration of an IgE-specific suppressor T-cell defect in B-cell regulation remains to be demonstrated (13, 14); rather, B-cell IgE synthesis is stimulated by T-cell factors (15, 16). T-cell dependent IgG synthesis by B-cells is reduced in AD, and although a helper T-cell defect in the atopic patients was observed, an intrinsically reduced ability to produce IgG could not be excluded (5). Thus, it is possible that an abnormality of B-cells, as well as T cells, is present in AD.

Large granular lymphocytes (LGL) comprise a small, FcIgG receptor bearing subpopulation of blood mononuclear leukocytes (MNL) and are particularly potent in natural killer activity against certain target cells. MNL from patients with AD have reduced natural killer activity (17, 18).

Immunologic functions of monocytes have been much less studied than those of T cells in AD but reports similarly suggest altered immune function of these cells. Monocytes and macrophages from atopic patients demonstrate reduced chemotaxis (19), decreased phagocytosis (20), decreased antibody dependent cellular cytotoxicity (ADCC) (21), depressed prostaglandin E (PGE) synthesis after stimulation with histamine-induced suppressor factor (22), and increased expression of FcIgE receptors (23).

Studies have suggested that elevated histamine release by IgE bearing basophils from patients with AD (24) may account for the elevated skin and blood histamine levels sometimes seen in these patients (25). Brief exposure of normal mononuclear leukocytes

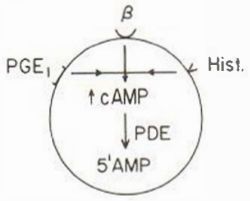
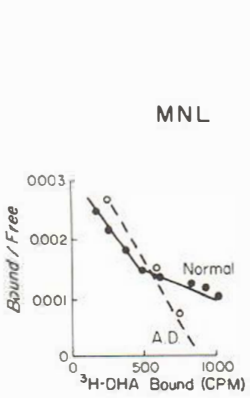


Fig. 1



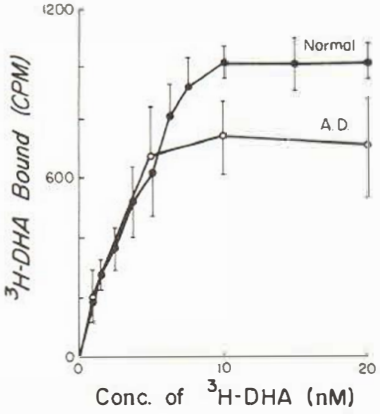


Fig. 2

(MNL) to histamine (at concentrations similar to those found in tissues of patients with AD) results in alterations of beta adrenergic surface receptors (26), reduced cAMP responsiveness to all cAMP elevating agonists (heterologous desensitization) (27), and elevated cAMP phosphodiesterase (28). These changes are identical to those seen in MNL from AD patients (26–28).

How might this disordered cellular regulation of cyclic nucleotide metabolism affect immun cellular functions? Most immune reactions are accomplished through a complex series of interactions, some requiring cell-cell contact and others mediated through factor production. Many regulatory signals to immune cells are mediated by factors which act by binding cell surface receptors linked to adenylate cyclase. Activation of adenylate cyclase results in "second messenger" cAMP formation, which in turn modulates cell functions often through protein kinase activation. However, cells from patients with AD have altered surface receptors, adenylate cyclase and cAMP phosphodiesterase (PDE). Thus, even if receptor binding occurs and cAMP is raised, the elevated PDE so quickly catabolizes the newly formed cAMP that appropriate modulation of cell function may not occur (Fig. 1).

Immunoregulatory signals such as histamine, prostaglandins, neuroendocrine adrenergic effects, and T-cell-inducing thymic hormones (29) all act by raising cAMP. Chemoattractants may act by raising cAMP through PDE inhibition (30); if PDE is elevated, chemotactic migration may not occur.

Clearly AD is a condition with immunologic and cyclic nucleotide regulatory abnormalities of MNL. We sought to dissect which particular subsets of MNL display disordered regulation of cyclic nucleotides in order to determine whether all or just certain cells have altered responses to immunomodulatory signals mediated through cAMP.

To study the problem we prepared MNL subpopulations from peripheral blood according to two purification schemes. MNL were allowed to settle onto plastic tissue culture flasks for 45 min. Nonadherent lymphocytes and adherent monocytes were harvested separately from the flasks. Nonadherent lymphocytes were mixed with sheep erythrocytes to form E rosettes which were passed twice through Ficoll Hypaque to enrich E-rosette-forming T-cells in the pellet and B-enriched cells at the interface. Alternatively, MNL were layered on a discontinuous Percoll density gradient, spun, and low density monocyte-enriched cells harvested separately from high-density, T-enriched cells sedimenting lower in the gradient. Cyclic AMP responses were determined in cells from non-atopic

control subjects and from AD patients after a 15 minute incubation with 10^{-4} M isoproterenol. Reduced cAMP responses were seen in both AD T-cells and monocytes purified from patients with AD, whereas there was no difference between the low cAMP responses of normal and atopic B cells (Table I) (31).

Were reduced cAMP responses due to altered surface receptors? The numbers and affinities of adrenergic surface receptors on MNL subpopulations were measured by the binding of propranolol-displaceable ^{3H}-dihydroalprenolol (^{3}H-DHA) to cell surfaces. Unfractionated atopic MNL showed reduced numbers of receptors per cell as compared to normals, demonstrating reduced specific binding at saturating concentrations of ligand. We also observed absence of a normal, lower affinity subpopulation of high affinity beta-adrenergic receptors, resulting in a linear Scatchard plot of beta-adrenergic binding to MNL from atopic patients, instead of the biphasic plot seen in normal control cells (Fig. 2).

These alterations of surface receptors for cAMP-elevating ligands were localized to T cells and monocytes of patients with atopic dermatitis. In contrast, atopic B-cell receptor numbers and affinities were identical to those of normal B cells (26).

We next compared intracellular cAMP-PDE activity in MNL subsets from normal and AD subjects (Table I). Monocytes from patients with AD demonstrated a markedly higher PDE activity. High density Percoll enriched T-cells (containing less than 5% B cells), also demonstrated higher PDE activity in cells of patients with AD as compared to controls. In another study, Percoll-separated lymphocytes, which contained more cells from lower density bands (including some B cells), did not show statistically different PDE activity between normal lymphocytes and lymphocytes from patients with AD (32). This is probably due to the higher level of PDE activity in B cells (Table I) masking the difference between the T-cells of AD and normal.

Thus, the disordered cellular regulation of cyclic nuclotides in AD seem particularly localized to monocytes and to certain T-cell subsets. Since both monocytes and T cells demonstrated multiple abnormalities on a biochemical basis, one could easily postulate abnormal immune interactions between these cells.

The interaction of Ia^{+} antigen-presenting monocytic cells with T cells is critical and central to immunologic functions (33). There is substantial evidence for defects in monocyte immune function in AD. Depressed chemotaxis may be related to altered PDE, as discussed previously. Decreased monocyte ADCC, phagocytosis, and prostaglandin synthesis all support the concept of generalized monocyte/macrophage dysfunction (14–22).

A lymphocyte subset which is central to many immune functions and is triggered by self

Table I. *Correlation of cAMP-phosphodiesterase activity with isoproterenol stimulated cAMP in leukocyte subpopulations from normal and atopic dermatitis donors*

	Normal		Atopic dermatitis	
	% increase[a]	PDE[b]	% increase[a]	PDE[b]
MNL	298	3.5	21	10.2
T-cells	142	3.2	0	7.3
B-cells	0	200.0	0	228.0
Monocytes	326	22.0	18	56.0

[a]Percentage increase above basal level of cAMP due to stimulation by 10^{-4} M isoproterenol for 15 minutes. Data extracted from reference (32).
[b]PDE—pmol cAMP hydrolysed/min/10^{8} cells.

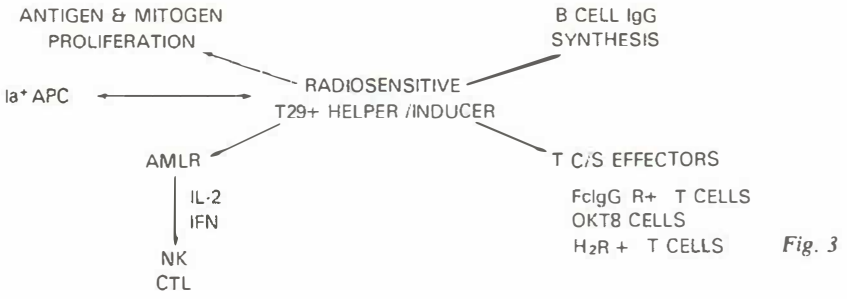


Fig. 3

Ia-bearing antigen presenting monocytic cells, has been identified as a radiosensitive (functionally dependent upon a proliferative step), $OKT4^+$, $T29^+$ helper/inducer T-cell (34–38). A defect in the generation or function of these radiosensitive T-helper/inducer cells would then result in abnormalities of immune function (dependent upon the proliferation and function of these cells). Indeed, many immune functional abnormalities in AD related to macrophage—T helper cell interactions; it is therefore of interest to examine the evidence for altered radiosensitive T cells.

Pokeweed mitogen (PWM) stimulates a pool of blood B cells which have recently been activated by antigen in vitro to produce large quantities of IgG, IgM, and IgA in vitro over a 7-day culture period. Purified B cells plus PWM in the absence of helper T cells do not produce any immunoglobulin. Induction of PWM-stimulated immunoglobulin synthesis requires the presence of either radioresistant helper T cells or radiosensitive helper T cells (34–37). When we used irradiated T cells to provide help for PWM-stimulated B cells, as measured by the level of IgG produced in vitro, there was no difference between the helper function of irradiated T cells from normal controls and AD patients; that is, radioresistant T cell help was normal in AD. In contrast, when unirradiated T cells were used to provide help for PWM-stimulated B cells, T cells from normal controls provided by T cells in patients with AD generated normal levels of B cell imunoglobulin production (6). In functional studies of the radiosensitive helper T-cell population, T cells from normal controls generated significantly higher levels of B-cell immunoglobulin production than did T cells from patients with AD. Thus, we concluded that these patients had a defect in the radiosensitive helper T-cell population responsible for PWM B-cell responsiveness (6).

Subsequently, Leung et al. (38) have provided direct evidence for a defect in the development of the helper/inducer T cell population. The monoclonal antibody-defined $T29^+$-cell is a subset of $OKT4^+$ helper/inducer T cells which proliferates in response to Ia^+ antigen-presenting monocytic cells in the presumed absence of antigen (38). This reaction is a reflection of self-recognition and generation of T cells with immunoregulatory function for a variety of processes and is termed the autologous mixed lymphocyte reaction (AMLR). When T cells from patients with AD were mixed with their own (autologous) antigen presenting cells, they demonstrated significantly less proliferation than did T cells from normal controls tested in the AMLR (39). Further confirmatory evidence was provided when these authors showed that the number of $T29^+$ cells responsible for proliferation in the autologous mixed lymphocyte reaction were reduced in the blood of patients with AD as compared to controls (38).

An abnormality in the function or numbers of the radiosensitive, $T29^+$, helper/inducer T cells generated by the interaction with autologous Ia^+ antigen presenting monocytic cells may explain other immune abnormalities in AD. Soluble mitogen-stimulated proliferation is critically dependent on successful monocyte/T-cell interaction, and can be reduced in patients with severe AD (2, 39) (Fig. 3, upper left).

The development of the pool of blood PWM-recruitable B cells for in vitro Ig production requires induction by a radiosensitive T-cell inducer (34, 36, 40). In addition to the T-cell defects in patients with AD noted above, B cells from patients with AD also demonstrate decreased PWM-stimulated Ig secretion, even when corrected for number or when normal T cells are used to provide helper function (6). One likely interpretation was that the pool of PWM-recruitable B cells was reduced in AD. The generation of PWM recruitable B cells is normally dependent on radiosensitive T helper cells (34–37) (Fig. 3, upper right).

The development of mature suppressor and cytotoxic effector T cells also requires the help of such an inducer T cell (41–43) (Fig. 3, lower right). Numerous authors have looked at the numbers of blood T cells associated with these funcions. Indeed, T cells with FcIgG receptors, OKT8 cells, and histamine H_2-receptor-bearing T cells often show significantly reduced values in patients with AD (4–8, 44).

Similarly, cytotoxic T cell function against class I alloantigen-bearing targets is reduced in patients with AD as compared to controls and this is proportional to the reduced number of OKT8 cells (8). Development of cytotoxic T lymphocytes is critically dependent upon Ia^+ monocyte stimulation of inducer T cell factors such as Interleukin-2 (41) (Fig. 3, lower left).

Natural killer cell growth and function by peripheral blood lymphocytes is enhanced by products of proliferating T cells such as IL-2 and interferon (41). Reduction in the production of these factors by abnormal helper/inducer T cells or an altered ability to respond to these signals in AD may be etiological factors in the reduced natural killer activity of lymphocytes from patients with AD (17, 18) (Fig. 3, lower left).

Disordered cellular regulation of cyclic nucleotide metabolism in monocytes and T cells may play a role in the central Ia^+ antigen-presenting monocytic cell/T cell interaction proposed in Fig. 2. Alternatively, each of the immune abnormalities listed could be due to altered immune signal processing by more distal effector cells with their own abnormal cAMP intracellular messenger systems. An interesting example has been raised by the work of Rocklin et al. on H_2-receptor bearing T cells ($H2R^+$ T cells) (22, 45). These cells, which overlap considerably with T and OKT8 cells, normally generate suppressor factors in response to histamine or prostaglandin E_2. As would be predicted by our data on reduced atopic T cell responses to histamine, atopic $H2R^+$ T cells demonstrate a reduced ability to produce these suppressive factors in response to histamine.

These authors proposed that acute inflammation (i.e., Type I), once provoked in patients with AD, would not be subject to the normal down regulatory influences of the T cell derived suppressive factors, and could eventuate into histamine H_1-receptor bearing T cell mediated chronic inflammation.

The concept of elevated cAMP PDE as a contributing factor in immune dysfunction in AD raises new therapeutic possibilities. Ro20–1724 is a PDE inhibitor that inhibits the cAMP PDE isozyme which is elevated in AD leukocytes (46). In vitro exposure to Ro20–1724 normalizes histamine release by leukocytes from patients with AD (24). Similarly, incubation of blood MNL with Ro20–1724 resulted in significantly reduced spontaneous IgE synthesis by cells from patients with AD (47). Topically applied Ro20–1724 or other PDE inhibitors may be of benefit in AD (48).

Integration of our understanding of disordered cyclic nucleotide metabolism and immune dysfunction in AD may thus lead to new rational therapeutic interventions in this disease.

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REFERENCES

1. Hanifin JM. Atopic dermatitis. J Allergy Clin Immunol 1984; 73: 211–222.
2. Lobitz WC, Honeyman JF, Winkler MW. Suppressed cell mediated immunity in two adults with atopic dermatitis. Br J Dermatol 1972; 86: 317–328.
3. McGeady SJ, Buckley RH. Depression of cell-mediated immunity in atopic eczema. J Allergy Clin Immunol 1975; 56: 393–406.
4. Schuster DL, Bongiovanni BA, Pierson DL, Barbaro JF, Wong DT, Levinson Al. Selective deficiency of a T cell subpopulation in active atopic dermatitis. J Immunol 1980; 124: 1662–1667.
5. Cooper KD, Kazmierowski JA, Wuepper KD, Hanifin JM. Immunoregulation in atopic dermatitis: Functional analysis of T-B cell interactions and the enumeration of Fc receptor bearing T cells. J Invest Dermatol 1983; 80: 139–145.
6. Leung DYM, Rhodes AR, Geha RS. Enumeration of T cell subsets in atopic dermatitis using monoclonal antibodies. J Allergy Immunol 1981; 67: 450–455.
7. Beer DJ, Osband ME, McCaffrey RP et al. Abnormal histamine induced suppressor cell function in atopic individuals. N Engl J Med 1982; 306: 454–458.
8. Leung DY, Wood N, Dubey D, Rhodes AR, Geha RS. Cellular basis of defective cell mediated lympholysis in atopic dermatitis. J Immunol 1983; 130: 1678—1682.
9. Schuster DL, Pierson D, Bongiovanni B, Levinson AF. Suppressor cell function in atopic dermatitis associated with elevated immunoglobulin E. J Allergy Clin Immunol 1979; 64: 139–143.
10. Juhlin L, Johansson SGO, Bennich H, Hogman C, Thyresson N. Immunoglobulin E in dermatoses. Arch Dermatol 1969; 100: 12–16.
11. Buckley RH, Becker WG. Abnormalities in the regulation of human IgE synthesis. Immunol Rev 1978; 41: 288–314.
12. Tjio AH, Hull WM, Gleich GJ. Production of human immunoglobulin E antibody in vitro. J Immunol 1979; 122: 2131–2133.
13. Saxon A, Morrow C, Stevens RH. Subpopulations of circulating B cells and regulatory T cells involved in in vitro IgE production in atopic patients with elevated serum IgE. J Clin Invest 1980; 65: 1457–1468.
14. Sampson H, Buckley R. Human IgE synthesis in vitro: a reassessment. J Immunol 1981; 127: 829–834.
15. Saryan J, Leung D, Geha R. Induction of human IgE synthesis by a factor derived from T cells of patients with hyper-IgE states. J Immunol 1983; 130: 242–247.
16. Romagnani S, Maggi E, Del Prete G, Ricci M. IgE synthesis in vitro induced by T cell factors from patients with elevated serum IgE levels. Clin Exp Immunol 1983; 52: 85–88.
17. Jensen JR, Sard TT, Jorgensen AS, Thestrup-Pedersen K. Modulation of natural killer cell activity in patients with atopic dermatitis. J Invest Dermatol 1984; 82: 30–34.
18. Kuscimi NT, Trentin JJ. Natural cell mediated cytotoxic activity in the peripheral blood of patients with atopic dermatitis. Arch Dermatol 1982; 118: 568–571.
19. Rogge JL, Hanifin JM. Immunodeficiencies in severe atopic dermatitis. Arch Dermatol 1976; 112: 1391–1396.
20. Godard P, Chaintrevel J, Damon M, Coupe M, Flandre O, Crastes de Paulet A, Michel F. Functional assessment of alveolar macrophages: Comparison of cells from asthmatics and normal subjects. J Allergy Clin Immunol 1982; 70: 88.
21. Kragballe K, Herlin T. Antibody dependent monocyte mediated cytotoxicity in atopic dermatitis. Allergy 1981; 36: 27–32.
22. Beer DJ, Rocklin RE. Histamine-induced suppressor cell activity. J Allergy Clin Immun 1984; 73: 439–452.
23. McLewicz F, Zeiger R, Mellon M, O'Conner R, Spiegelberg H. Increased peripheral blood monocytes with Fc receptors for IgE in patients with severe allergic disorders. J Immunol 1981; 126: 1592–1595.
24. Butler JM, Chan SC, Stevens SR, Hanifin JM. Increased leukocyte histamine release with elevated cAMP phosphodiesterase activity in AD. J Allergy Clin Immunol 1983; 71: 490–497.
25. Juhlin L. Localization and content of histamine in normal and diseased skin. Acta Dermatovener 1967; 42: 218–219.
26. Cooper KD, Chan SC, Hanifin JM. Absence of low affinity beta adrenergic receptors in atopic dermatitis and histamine desensitized normal leukocytes. Clin Res 1982; 30: 27A.
27. Safko MJ, Chan SC, Cooper KD, Hanifin JM. Heterologous desensitization of leukocytes: A possible mechanism of beta adrenergic blockade in atopic dermatitis. J Allergy Clin Immunol 1981; 68: 218–255.
28. Grewe SR, Chan SC, Hanifin JM. Elevated leukocyte cyclic AMP phosphodiesterase in atopic

disease: A possible mechanism for cAMP hyporesponsiveness. J Allergy Clin Immunol 1982; 70:452–457.
29. Goldstein G, Sheid IM, Boyse A, Brand A, Gilmour D. Thymopoietin and Bursopoietin: Induction signals regulating early lymphocyte differentiation. Cold Spring Harbor Symposium on Quantitative Biology 1977; 41: 5–9.
30. Verghese M, McPhail L, Fox K, Snyderman R. Inhibition of phosphodiesterase by chemoattractants in human neutrophils: A potential new mechanism for receptor mediated enhancement of cAMP. Clin Res 1984; 32:471A.
31. Cooper KD, Chan SC, Hanifin JM. Differential T and B cell beta adrenergic responsiveness implies alternative regulatory mechanisms in atopy. Clin Res 1981; 29: 281A.
32. Holden CA, Austen DR, Chan SC, Hanifin JM. Defective cyclic nucleotide metabolism in atopic monocytes. Clin Res 1984; 32: 591A.
33. Shevach EM,: Macrophages and other accessory cells. In: Paul W, ed. Fundamental immunology. New York: Raven Press. 1984; 99–1–2.
34. Rheinherz EL, Kung P, Goldstein G, Schlossman S. Further characterization of helper/inducer T-cell subsets defined by monoclonal antibody. J Immunol 1979; 123: 2894–2896.
35. Thomas Y, Sosman J, Robozinski L, Irigoyen O, Kung P, Goldstein G, Chess L. Regulation of helper factor production by T-cell subsets. J Immunol 1981; 126: 1948–1951.
36. Rheinherz EL, Morimoto C, Penta JA, Schlossman S. Subpopulations of the T4 positive inducer cell subset in man. J Immunol 1981; 126: 67–80.
37. Uchiyama T, Nelson D, Fleisher T, Waldmann T. A monoclonal antibody (Anti-T) reactive with activated and functionally mature human T cells. II. Expression of Tac antigen on activated cytoxic killer T-cells. J Immunol 1981; 126: 1398–1403.
38. Leung DYM, Saryan JA, Stobo JB, Geha RS. Cellular basis of the impaired autologous mixed lymphocyte reaction in atopic dermatitis. Clin Res 1983; 31: 164A.
39. Elliott ST, Hanifin JM. Lymphocyte response to phytohemagglutinin in atopic dermatitis. Arch Dermatol 1979; 115: 1424–1426.
40. Stevens R, Macy E, Morrow C, Saxon A. Characterization of a circulating subpopulation of spontaneous anti-tetanus toxid antibody production. J Immunol 1980; 122: 2498–2503.
41. Henney CS, Gillis S. Cell mediated cytotoxicity. In Paul W, ed. Fundamental immunology. New York: Raven Press, 1984: 677–679.
42. Rheinherz E, Schlossman S: Regulation of the immune response-inducer and suppressor T-lymphocyte subsets in human beings. N Engl J Med 1980; 303: 370–373.
43. Broder S, Uchiyama TT, Muul L, Goldman C, Sharrow S, Poplack D, Waldman T. Activation of leukemic pro-suppressor cells to become suppressor effector cells. N Engl J Med 1981; 304: 1382–1387.
44. Kang K, Cooper KD, Vanderbark A, Strannegard IL, Strannegard O, Hanifin JM. Immunoregulation in atopic dermatitis: T-lymphocyte subsets defined by monoclonal antibodies. Semin Dermatol 1983; 2 (1): 59–80.
45. Rocklin RE, Beer DJ: Histamine and immune modulation. Adv Intern Med 1983; 28: 225–251.
46. Giustina TA, Chan SC, Baker JW, Hanifin JM. Increased leukocyte sensitivity to PDE inhibitors in atopic dermatitis: Tachyphylaxis after theophylline therapy. J Allergy Clin Immunol 1984 (in press).
47. Cooper KD, Kang K, Chan SC, Hanifin JM. Phosphodiesterase inhibition by Ro20–1724 reduces Hyper IgE production by atopic dermatitis cells in vitro. J Invest Dermatol 1984 (submitted for publication).
48. Kaplan RJ, Daman L, Rosenberg EW, Feigenbaum S. Topical use of caffeine with hydrocortisone in the treatment of atopic dermatitis. Arch Dermatol 1978; 114: 60–62.

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