Impairment of the Autologous Mixed Lymphocyte Reaction in Atopic Dermatitis

DONALD Y. M. LEUNG, JOHN A. SARYAN, RENE FRANKEL, MARIANNE LAREAU, and RAIF S. GEHA, *Divisions of Allergy and Immunology, Children's Hospital Medical Center and the Department of Pediatrics, Harvard Medical School, Boston, Massachusetts 02115*

ABSTRACT The T cell proliferative response to autologous non-T cells is termed the autologous mixed lymphocyte reaction (AMLR). Recent studies have suggested that the AMLR represents an inducer circuit for the activation of T8+ suppressor/cytotoxic effector cells. Since atopic dermatitis (AD) patients are deficient in T8+ cytolytic T cell function, we investigated the AMLR in AD. When sheep erythrocytes were used to separate T cells from non-T cells, the AMLR was found to be significantly decreased ($P < 0.001$) in AD patients ($n = 11$; Δcpm = 1,550±393) when compared with normal control subjects ($n = 13$; Δcpm = 25,819±4,609). To exclude the possibility that these results were an artifact of the sheep erythrocyte separation, T cells were also separated on a fluorescence-activated cell sorter after treatment of peripheral blood lymphocytes with the OKT3 monoclonal antibody. AD T cells separated by the latter method were also found to have a significantly reduced AMLR response when compared with similarly treated normal T cells. Co-culture studies using cells from AD patients and their HLA identical siblings indicated that the defect resided at the responder T cell level rather than at the stimulator non-T cell level. Co-culture studies revealed no evidence for excessive suppressor cell activity resulting in the decreased AMLR. However, enumeration of T cells reactive with the monoclonal antibody T29, which recognizes a subset of T cells proliferating in the AMLR, demonstrated that AD patients ($n = 8$; % T29 = 2.5±0.7) had a significantly decreased ($P < 0.001$) number of circulating T29+ T cells when compared with normal controls ($n = 8$; % T29 = 10.4±0.8). These studies suggest that a deficiency of T4+ T29+ cells contributes to the deficient AMLR in AD and possibly underlies the abnormalities of T8+ effector cells present in this disease.

INTRODUCTION

Defective cell-mediated immunity has been reported in up to 80% of patients with atopic dermatitis (AD)[1] (1–5). Clinically, these patients are susceptible to develop disseminated viral infections with herpes simplex and vaccinia viruses (5). This in vivo observation correlates with in vitro experiments showing that the majority of AD patients are unable to generate normal T8+ cytotoxic T cell activity after allogeneic stimulation (6). Furthermore, the latter study also demonstrated that the defect in generation of cytotoxic T cells resided in both the helper/inducer T4+ cells and the effector T8+ cells. Since recent studies suggest that the autologous mixed lymphocyte reaction (AMLR) represents an inducer circuit for the activation of T8+ effector cells (7), the current investigation was carried out to determine whether AD patients had a defective AMLR and, if so, to determine the cellular basis of this defect.

METHODS

Subjects. The study population consisted of 16 patients with chronic AD, nine males and seven females, 4–40 yr of age. They all satisfied the criteria suggested by Hanifin and Lobitz (5) for the diagnosis of AD, and had moderate to severe chronic AD defined by multiple sites of skin involvement for at least 3 mo, as well as a personal and/or family

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[1] *Abbreviations used in this paper:* AD, atopic dermatitis; AMLR, autologous MLR; E, sheep erythrocyte; MLR, mixed lymphocyte reaction; PBMC, peripheral blood mononuclear cells.

history of allergic diseases. Two age-matched control populations were studied: the first consisted of 15 healthy subjects, nine males and six females, 6–36 yr of age, with no skin disease; the second control population consisted of five patients with nonatopic skin disease (Netherton's disease, psoriasis, and pityriasis rosea: one each, and inflammatory acne: two patients) aged 5–25 yr. Consent was obtained either from subjects or from their parents before obtaining blood. None of the individuals studied had previously received oral steroids, and topical corticosteroids were held for at least 1 wk before the study.

Isolation of peripheral blood mononuclear cell (PBMC). PBMC were isolated from heparinized venous blood by means of Ficoll-Hypaque gradient density centrifugation and incubated on plastic petri dishes for 30 min at 37°C to remove excess macrophages, which have been shown to suppress the AMLR (7). The nonadherent cells were rosetted with sheep erythrocytes (E) and fractionated into T cells (E+) and non-T cells (E−) as previously described (8).

AMLR and allogeneic mixed lymphocyte reaction (MLR). AMLR cultures contained 1×10^5 responder E+ cells and 1×10^5 irradiated (5,000 R) stimulator E− cells in 200 μl of complete culture medium (RPMI 1640 with 10% AB+, 2 mM L-glutamine, 10^{-6} M indomethacin, 50 μg penicillin, and 50 μg streptomycin/ml). Cultures were performed for 6 d at 37°C in humidified 5% CO_2. 18 h before termination of the cultures, 1 μCi [^{3}H]thymidine (New England Nuclear, Boston, MA) was added to each culture. Results were expressed as mean counts per minute±SEM of triplicate cultures minus background counts per minute (cultures of autologous T cells and irradiated autologous T cells), i.e., Δcpm. Allogeneic MLR were performed in an identical manner, except that 1×10^5 irradiated allogeneic non-T cells were used as stimulators. To determine whether T cells suppressive of the AMLR were present, 5×10^4 cells from the patient were added to the AMLR of a healthy HLA-identical sibling.

In selected experiments, T cells were separated from non-T cells on the basis of reactivity with the OKT3 (Ortho Pharmaceutical, Raritan, NJ) monoclonal antibody. Nonadherent PBMC were incubated with OKT3 antibody (10×10^6 cells/μg antibody in 0.2 ml RPMI with 1% human serum albumin) for 45 min on ice, washed twice, and incubated at 4°C for 30 min with 1:40 dilution of fluorescein-conjugated goat anti-mouse IgG (Cappel Laboratories, Cochranville, PA). After washing the cells three times, T3-positive (T3+) T cells were separated from T3-negative (T3−) non-T cells on a fluorescence-activated cell sorter (FACS)-IV cell sorter (Becton, Dickinson & Co., Mountain View, CA). At the end of the cell-sort, T3+ cells and T3− cells were washed once, resuspended at 1×10^6 cells/ml complete culture medium, and AMLR cultures were set up as described above.

Analysis of peripheral blood T lymphocytes with T29 monoclonal antibody. The T29 monoclonal antibody was previously shown to react with ~10% of peripheral blood T cells and to define a subpopulation of T cells that proliferate during the AMLR (9). 1 million T lymphocytes were suspended in 0.1 ml of T29 monoclonal antibody (generous gift of Dr. J. Stobo, San Francisco, CA) or with 0.1 ml of 1:10 dilution of mouse serum at 4°C for 40 min, washed once, and incubated at 4°C for 30 min with 0.1 ml of a 1:20 dilution of fluorescein-conjugated goat anti-mouse IgG. After washing the cells three times, cytofluorographic analysis was performed on a FACS-IV. Uniformly, <2% of the cells exhibited fluorescent staining with the reagent control.

RESULTS

Impaired autologous MLR in AD. Results of the AMLR after separation of T cells by E in AD patients are shown in Fig. 1. The mean AMLR of 13 normal age-matched controls was 25,819±4,609 cpm above background. The AMLR of 11 AD patients averaged only 1,550±392 cpm and was significantly depressed ($P < 0.01$) compared with normal subjects and controls ($P < 0.001$). In contrast, the AMLR in five subjects with miscellaneous skin diseases was not significantly different from the normals ($P > 0.1$). There was no significant difference between background cpm in AD subjects and control subjects. Although the results obtained for each patient are represented only once in Fig. 1, several patients and normal controls were tested on multiple occasions (up to five times) over a 1-yr period and the results were unchanged. Furthermore, in two AD patients studied on multiple occasions, the AMLR remained abnormally low regardless of whether they were in short term remission (<6 mo) or had active skin disease.

The AMLR response of three AD patients and four normal controls were also determined after separation of T3+ T cells from T3− non-T cells in a FACS-IV cell sorter. As shown in Table I, separation of T cells on the basis of reactivity with the OKT3 monoclonal antibody resulted in similar results as those obtained by separating T cells using E, i.e., the mean AMLR response by AD T cells (Δcpm = 4,169±205) was sig-

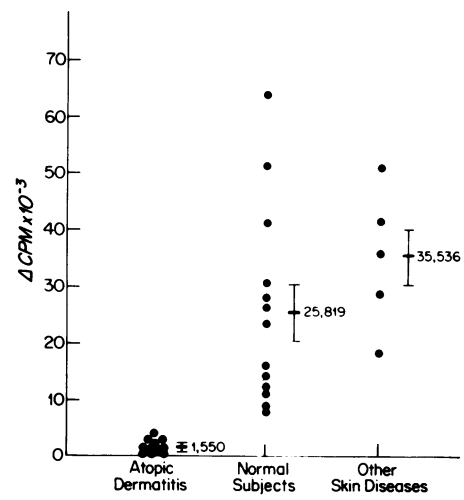

FIGURE 1 AMLR in 11 AD patients, 13 normal subjects, and five patients with nonatopic skin diseases. Results of the AMLR are expressed as the mean±SEM of triplicate cultures. The AMLR of AD patients was significantly below ($P < 0.001$) that of normal controls.

TABLE I
AMLR after Cell Sort with OKT3 Monoclonal Antibody

Donor no.	AMLR (Δcpm) Normal	AD
1	26,350±4,512	4,448±187
2	42,060±5,415	3,667±345
3	13,726±578	4,381±913
4	16,605±1,997	
Mean AMLR	24,685±5,534	4,169±205

The AMLR response of four normal donors and three AD patients were determined after T cells were separated from non-T cells by cell sorting PBMC for T3+ cells on a FACS-IV cell sorter. Results are expressed as mean±SEM of triplicate cultures. The AMLR of AD patients was significantly below ($P < 0.02$) that of normal controls.

nificantly lower than that of normal controls (Δcpm = 24,685±5,534; $P < 0.02$). These results suggest that the basis for the decreased AMLR in AD was not due to an abnormal proliferative response to E antigens.

In contrast to the impairment of the AMLR, allogeneic MLR in patients with AD was normal. Allogeneic MLR determined in eight out of the 11 patients with AD (mean±SEM of Δcpm = 54,524±10,047) was not significantly different ($P > 0.3$) from the allogeneic MLR determined in eight control subjects (mean±SEM of Δcpm = 60,107±7,902).

Cellular basis of the impaired AMLR in AD. To determine whether the impaired AMLR was due to a defect of responder T cells, stimulator cells, or both, two AD patients and their healthy HLA-identical siblings were studied. In these experiments, T cells from a patient, from an HLA-A-, B-, and DR-identical sibling and from an unrelated normal subject were cultured with non-T cells from each of these three donors. The results in Table II show that T cells from the healthy HLA-identical siblings were stimulated equally well by autologous non-T cells and by the patient's non-T cells (Table II, lines 2 and 5, columns 3 and 4). In contrast, T cells from the patients did not proliferate in response to either autologous non-T cells or HLA-identical normal non-T cells (Table II, lines 1 and 4, columns 3 and 4). These results suggested that the defect in AD patients resided at the level of the responder T-cell, rather than at the level of the stimulator non-T cells. The deficiency in T cell response was not generalized because the patients' T cells response to allogeneic non-T cells was equivalent to that of T cells from their HLA-identical siblings (Table II, column 5).

The possibility that suppressor T cells were responsible for the decreased AMLR in AD was tested by studying the effect of the addition of T cells from two AD patients to the AMLR of their healthy HLA-identical siblings. As shown in Fig. 2, T cells from AD patients did not possess excessive suppressor cell activity for the AMLR. These was no significant difference ($P > 0.1$) in proliferation when normal T cells were stimulated with autologous non-T cells in the presence of absence of HLA-identical AD T cells.

Enumeration of T29 cells in AD. The possibility that the decreased AMLR in AD was accompanied by a numerical reduction of autoreactive T cells was considered. To test this possibility, T cells from normal controls and AD patients were enumerated for reactivity with the T29 monoclonal antibody, previously shown to react with a subpopulation of T cells that proliferate in the AMLR. As shown in Fig. 3, the T29 antigen was present on 10.4±0.8% of normal T cells

TABLE II
Defective T Cell Response in the AMLR of Patients with AD

Experiment	Responder T cells	[^{3}H]thymidine incorporated in the presence of stimulator non-T cells: Patient	HLA-identical sibling	Unrelated donor
			cpm	
A	Patient 1	965±143	2,499±80	148,028±1,900
	HLA-identical sibling	68,475±7,783	63,651±1,553	146,598±13,400
	Unrelated donor	149,326±13,476	163,940±10,541	
B	Patient 2	120±27	226±80	58,983±10,930
	HLA-identical sibling	27,247±252	28,568±2,552	34,176±6,038
	Unrelated donor	51,276±2,815	66,110±3,304	

Results reported as the mean±SEM of triplicate cultures.

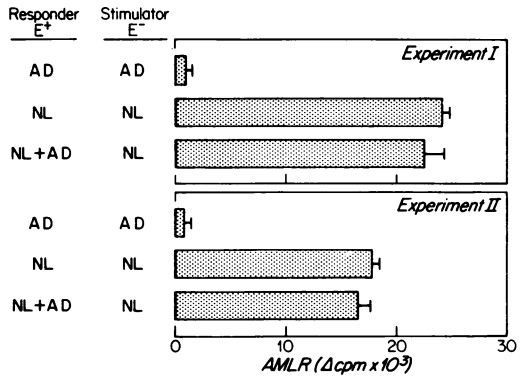

FIGURE 2 Effect of HLA-identical AD T cells on the normal AMLR response. To determine whether the defective AMLR in AD is due to the presence of excessive suppressor T cell activity, AMLR cultures of normal HLA-identical siblings were carried out in the presence or absence of AD T cells. Results shown in this table are derived from two AD patients and demonstrate that AD T cells do not possess excessive suppressor activity for the AMLR. *NL*, normal leukocyte.

(n = 8). In contrast, patients with AD (n = 8) had significantly decreased percentage of circulating T29 positive cells (2.5±0.7%, $P < 0.001$). Since patients with AD have normal or decreased numbers of circulating T cells (10, 11), these results suggest that the absolute number of circulating T29+ cells is also decreased in AD.

DISCUSSION

Our data indicate that the T cells of patients with AD respond poorly to autologous non-T cells. Similar results were obtained whether T cells were separated on the basis of rosetting with E or cell sorting after treatment with the OKT3 monoclonal antibody. The defective AMLR in AD was not simply secondary to the skin inflammation, because other patients with skin disease had normal AMLR (Fig. 1) and because, in two patients with AD who were studied on multiple occasions, impaired AMLR was present even during short term remissions (<6 mo), i.e., when they had no evidence of skin disease.

Reciprocal mixing experiments using cells from the patients and from their healthy HLA-identical siblings indicated that a defect in responder T cells rather than in stimulator non-T cells was the basis of the defective AMLR in AD. Indeed, in each of two cases studied, T cells from the healthy HLA-identical sibling mounted normal proliferative responses to both autologous and AD non-T cells. Patients' T cells, however, failed to respond to either autologous or normal sibling's non-T cells. Similar experimental approaches by other investigators have demonstrated that the decreased AMLR in Hodgkins' Disease (12) and systemic lupus erythematosus (13) may be due to defective responder T cells, whereas a defect of autologous stimulator cells has been described in patients with mononucleosis (14) and chronic lymphocytic leukemia (15).

The T cell defect in AMLR was neither generalized nor appeared to be the result of excess suppressor cells. Indeed, T cells from patients with AD proliferated normally to allogeneic cells. Suppression of the AMLR by non-T cells was not a factor, since non-T cells from the patients were excellent stimulators of HLA-identical normal T cells (Table II). Suppression by T cells was tested for and ruled out in co-culture experiments in which T cells from AD patients were added to the AMLR of their normal HLA-identical siblings (Fig. 2).

The defective AMLR in AD could have resulted from a functional and/or numerical impairment of T cells committed to the AMLR. Two subpopulations of T cells are known to paticipate in the AMLR. One population bears the surface antigen recognized by the T29 monoclonal antibody and proliferates in response to adherent monocytes. A second population lacks the T29 surface antigen and responds to nonadherent E− cells, i.e., B cells and null cells (7). Our studies show a deficiency in the T29+ population in AD (Fig. 2). The major portion of the proliferative AMLR response is due to T29− cells (7, 9). Because the mean AMLR of AD patients was <10% of the normal AMLR, it is

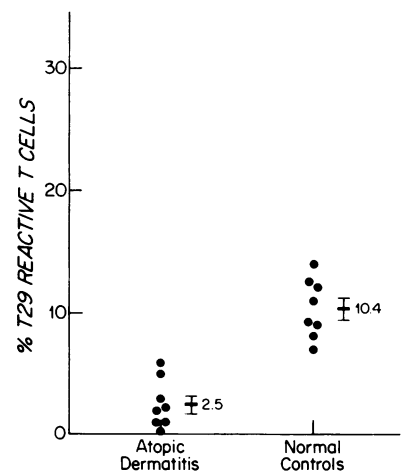

FIGURE 3 Enumeration of T29+ cells in AD. T lymphocytes from eight AD patients and eight healthy controls were analyzed for reactivity with the T29 monoclonal antibody previously shown to recognize a subset of T cells that proliferate during the AMLR. AD patients had a significantly lower percentage of T29+ cells ($P < 0.001$) than normals. The mean±SEM of each group is shown.

likely that the T29− population as well as the T29+ cells were deficient (functionally and/or numerically).

The deficient AMLR in patients with AD has to be viewed in the context of other immunoregulatory abnormalities present in this disease and in the light of the cellular events that occur in the AMLR. The subset of AMLR reactive T cells includes T cells that proliferate in response to soluble antigens (9). In this regard, lymphocytes from AD patients proliferate poorly in response to candida and tuberculin antigens (16). Isolated T4+ cells proliferate in the AMLR whereas isolated T8+ cells do not (17, 18). However, in the presence of T4+ cells, T8+ cells will proliferate; this suggests that the AMLR can function as an inducer circuit for the activation of T8+ effector cells (17). A variety of effector functions have been attributed to AMLR-activated T cells. They include the induction of cytotoxic T cells (19, 20), of helper T cells (21, 22), and of suppressor T cells (12, 23). In this regard the majority of patients with AD are deficient in T8+ cytolytic T cell function (6). This deficiency has been shown to be due in part to the failure of their T4+ helper cells to induce normal T8+ cytotoxic T cell function (6). Further studies are required to delineate the relationship between the deficient AMLR and impaired induction of cytolytic T cells in patients with AD.

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REFERENCES

1. Juhlin, L., S. G. O. Johansson, H. Bennich, C. Hogman, and N. Thyresson. 1969. Immunoglobulin E in dermatoses: levels in atopic dermatitis and urticaria. *Arch. Dermatol.* 100:12–16.
2. Byrom, N. A., and D. M. Timlin. 1979. Immune status in atopic eczema: a survey. *Br. J. Dermatol.* 100:491–498.
3. McGeady, S. J., and R. H. Buckley. 1975. Depression of cell-mediated immunity in atopic dermatitis. *J. Allergy Clin. Immunol.* 56:393–406.
4. Lobitz, W. C., Jr., J. F. Honeyman, and N. W. Winkler. 1972. Suppressed cell mediated immunity in two adults with atopic dermatitis. *Br. J. Dermatol.* 86:317–328.
5. Hanifin, J. M., and W. C. Lobitz. 1972. Newer concepts of atopic dermatitis. *Arch. Dermatol.* 113:663–670.
6. Leung, D. Y. M., N. Wood, D. Dubey, and R. S. Geha. 1983. Cellular basis of defective cell mediated lympholysis in atopic dermatitis. *J. Immunol.* 130:1678–1682.
7. Smolen, J. S., T. M. Chused, E. A. Novotny, and A. D. Steinberg. 1982. The human autologous mixed lymphocyte reaction. III. Immune circuits. *J. Immunol.* 129:1050–1053.
8. Geha, R. S., F. S. Rosen, and E. Merler. 1973. Identification and characterization of subpopulations of lymphocytes in human peripheral blood after fractionation on discontinuous gradients of albumin. The cellular defect in X-linked agammaglobulinemia. *J. Clin. Invest.* 52:1726–1734.
9. Hausman, P. B., H. V. Raff, R. C. Gilbert, L. J. Picker, and J. D. Stobo. 1980. T cells and macrophages involved in the autologous mixed lymphocyte reaction are required for the response to conventional antigen. *J. Immunol.* 125:1374–1379.
10. Leung, D. Y. M., A. R. Rhodes, and R. S. Geha. 1981. Enumeration of T cell subsets in atopic dermatitis using monoclonal antibodies. *J. Allergy Clin. Immunol.* 67:450–455.
11. Byrom, N. A., and D. M. Timlin. 1979. Immune status in atopic eczema: a survey. *Br. J. Dermatol.* 100:491–495.
12. Engleman, E. G., C. J. Benike, R. T. Hoppe, H. S. Kaplan, and F. R. Berberich. 1980. Autologous mixed lymphocyte reaction in patients with Hodgkin's disease. Evidence for a T cell defect. *J. Clin. Invest.* 66:149–158.
13. Sakane, T., A. D. Steinberg, and I. Green. 1978. Failure of autologous mixed lymphocyte reactions between T cells and non-T cells in patients with systemic lupus erythematosus. *Proc. Natl. Acad. Sci. USA.* 75:3464–3468.
14. Sakane, T., and I. Green. 1979. Specificity and suppressor function of human T cells responsive to autologous non-T cells. *J. Immunol.* 123:583–589.
15. Smith, J. B., K. P. Knowlton, and L. S. Koons. 1977. Immunologic studies in chronic lymphocytic leukemia: defective stimulation of T cell proliferation in autologous mixed lymphocyte culture. *J. Natl. Cancer Inst.* 58:579–585.
16. Fjelde, A., and B. Kopeczka. 1967. Cell transformation and histogenic effects in blood leucocyte cultures of atopic dermatitis patients. *Acta Dermato-Venereol.* 47:168–173.
17. Smolen, J. S., T. A. Luger, T. M. Chused, and A. D. Steinberg. 1981. Responder cells in the human autologous mixed lymphocyte reaction. *J. Clin. Invest.* 68:1601–1604.
18. Damle, N. K., J. A. Hansen, R. A. Good, and S. Gupta. 1981. Monoclonal antibody analysis of human T lymphocyte subpopulations exhibiting autologous mixed lymphocyte reaction. *Proc. Natl. Acad. Sci. USA.* 78:5096–5098.
19. Tomonari, K. 1980. Cytotoxic T cells generated in the autologous mixed lymphocyte reaction. I. Primary autologous mixed lymphocyte reaction. *J. Immunol.* 124:1111–1121.
20. Miller, R. A., and H. S. Kaplan. 1978. Generation of cytotoxic lymphocytes in the autologous mixed lymphocyte culture. *J. Immunol.* 121:2165–2167.
21. Chiorazzi, N., S. M. Fu, and H. G. Kunkel. 1979. Induction of polyclonal antibody synthesis by human allogeneic and autologous helper factors. *J. Exp. Med.* 149:1543–1548.
22. Hausmann, P. B., and J. D. Stobo. 1979. Specificity and function of a human autologous reactive T cell. *J. Exp. Med.* 149:1537–1542.
23. Smith, J. B., and R. P. Knowlton. 1979. Activation of suppressor T cells in human autologous mixed lymphocyte culture. *J. Immunol.* 123:419–422.