

CELLULAR IMMUNODEFICIENCY IN ANHIDROTIC ECTODERMAL DYSPLASIA

James R. Davis and Lawrence M. Solomon

*From the Department of Dermatology, Abraham Lincoln School, College of Medicine,
University of Illinois, Chicago, Ill., USA*

Abstract. By using in vitro methods a group of patients with anhidrotic ectodermal dysplasia (AED) was shown to have depressed lymphocyte function when compared with a control group. IgE levels of the AED group were elevated above those of a control group at the $p=0.01$ level of significance. In vivo methods utilizing the application of DNCB demonstrated, in addition, decreased delayed hypersensitivity reactions in the anhidrotic patients. Thus there appears to be some degree of cellular immune hypofunction in patients with AED, all of whom have demonstrated at some time a lichenified dermatitis clinically indistinguishable from atopic dermatitis.

Key words: Cellular immunodeficiencies; Anhidrotic ectodermal dysplasia; T cell dysfunction; A-topic dermatitis

Anhidrotic ectodermal dysplasia (AED) is a disease characterized by recurrent bouts of fever. These febrile episodes have usually been ascribed to the patient's inability to sweat in a hot environment and to a lack of mucus secreting glands in their oropharynx and trachea, leading to recurrent upper respiratory tract infections.

It has recently become apparent (16) that AED patients also frequently suffer from a dermatitis indistinguishable from atopic dermatitis. Since patients with atopic dermatitis may also have varying degrees of an immunodeficiency disorder in both the T- and B-cell systems (3, 5, 7-9, 12, 13, 15, 17, 19, 20, 21) we felt it would be of interest to study some parameters of humoral and cell mediated immunity in a number of patients with AED and atopic dermatitis.

PATIENTS

All individuals in the AED group either were experiencing an exacerbation of what appeared clinically as atopic dermatitis, or had done so over the preceding 1½ years. The

diagnosis of atopic dermatitis in this study was based upon (1) family history (4/6 patients, or 66%, had a relative with either atopic dermatitis or allergic rhinitis), (2) the patient's description of his dermatitis, the distribution of lesions, the absence of contact sensitization preceding exacerbations, aggravating and alleviating factors, and (3) clinical observation.

Of the 6 patients with AED studied, 5 had the complete phenotypic picture. The remaining patient demonstrated decreased numbers of sebaceous and eccrine glands by biopsy, a history of exacerbations and remissions of eczema, and was the sister of another patient (not included in the study) with a more complete phenotypic picture of AED.

The study patients were sex-matched and closely age-matched with those of the control patient group. Control patients had non-eczematous skin disorders such as impetigo and primary irritant reactions.

In addition to the AED group and the control group, a third "miscellaneous" group of 5 patients was formed. In this third group were placed 3 individuals in whom T-cell abnormalities might reasonably be expected to occur. Two of the patients had dermatologic problems which may well have represented a suppression of their cell-mediated immune system, i.e. one had disseminated verrucae (vulgaris) while another had a recurrent *T. tonsurans* infection. A third patient had stage IV Hodgkin's disease. In addition, we studied the mother of 3 of the patients with AED, who herself had atopic dermatitis but no evidence of AED. The fifth member of this miscellaneous group, a female, had dyskeratosis congenita. Informed consent was obtained from all participants after the procedures had been fully explained.

METHODS

Two in vivo methods were used to assess the cell-mediated immune capabilities of those under study. Participants were given an intradermal injection of 0.1 cc of Mumps Skin Test Antigen (Eli Lilly) on the flexural aspect of the forearm. The reaction was read 36-48 hours later and graded for the number of millimeters of erythema (15 mm or more representing a positive reaction). In addi-

tion, 0.05 ml of a sensitizing dose (2%) of DNCB was applied and occluded with Saran Wrap for 5 days. Eight to ten days following the application, 0.05 ml of an eliciting dose (0.1%) of DNCB was applied and occluded for 48 hours. Reading took place 15 minutes after removal of the occlusive wrap to allow for the resolution of any non-specific erythema. The reaction was graded as follows: 0 for no reaction, 1 for erythema less than 10 mm, 2 for erythema greater than 10 mm, 3 for erythema and induration, 4 for vesiculation, and 5 for necrosis.

Five *in vitro* methods were used to assess immunologic status. First, a complete blood count was drawn to determine the total number of WBCs per mm³ present and the differential count. Second, total IgG, IgA, and IgM serum levels were obtained by a radial immunodiffusion technique. Total serum IgE levels were obtained from serum samples maintained at -20°C by the Phadebas Radioimmunoassay. Fourth, the method of Jonda (4, 11) was used to determine the per cent of T-lymphocytes by erythrocyte rosetting.

Fifth, white blood cells obtained from heparinized venous blood (20 units/ml) were cultured and their responses to non-specific T-cell stimulator PHA measured. RBCs were allowed to settle for 1.5 hours in an inverted syringe in a 37°C incubator, after which the overlying leukocyte suspension was forced into a separate sterile tube, centrifuged, and diluted to 350 000 lymphocytes/ml in a 90% suspension medium, 10% autologous plasma mixture. 100 ml of the suspension medium contained the following: 9.1 ml of a 10×Hanks' Balanced Salt Solution, 2 ml minimum amino acid mixture, 2 ml multi-vitamin mixture, 1 ml 200 μM glutamine, 2 ml 7.5% NaHCO₃, 1 ml of penicillin-streptomycin (5 000 units/ml), 1 ml 2 M Hepes Buffer Solution. 4 ml aliquots in 30 ml Falcon Culture Flasks were used, with a total of 10 such flasks being used per participant. Two methods were used to assess the blastogenic response to the mitogen PHA: 1) A morphologic method in which the percentage of lymphoblasts present was determined by counting after Wright's staining. 2) A scintillation counting method in which the amount of uptake of tritiated thymidine was measured. For the morphologic method two flasks served as controls, each containing 4 ml of the participant's lymphocytes in 90% suspension medium+10% autologous plasma mixture. In the two additional flasks 0.1 ml phytohemagglutinin M (Difco) was added per flask and all four flasks were incubated at 37°C in 5% CO₂ for 4 days. The percentage of lymphoblasts present was determined by counting after the culturing period.

In the scintillation counting method the control tubes not containing PHA were cultured in triplicate, as were three flasks each containing 0.1 ml reconstituted PHA. After 78 hours of culture, 0.1 ml tritiated thymidine was added to each of the six flasks and they were then cultured for an additional 18 hours. Next, each of the cell cultures was centrifuged and the resultant pellets washed twice consecutively with each of the following: ice-cold saline, 5% trichloroacetic acid, absolute methanol. Precipitates were left to dry overnight and the following day were digested with NCS (a solubilizer). 10 ml of a scintillation cocktail was added to each tube and the disintegrations per minute per tube determined (2, 22).

RESULTS

The results are summarized in Table I.

IgE values were considered to be accurate within a range of ±10% for the 1:10 dilutions used for the determination of the 11 lowest values in this study, ±20% for the 1:50 dilutions used for the determination of the three highest values. The values of the remaining immunoglobulin classes were also closely reproducible, as shown by the following two samples taken from patient No. 10:

Sample No. 1	Sample No. 2
IgG: 1 560 mg/100 ml	IgG: 1 560 mg/100 ml
IgA: 250	IgA: 260
IgM: 152	IgM: 144

(Control values for both samples were identical)

The Mann-Whitney U Test was chosen to analyze IgE levels found in the control and patient groups because the values were not normally distributed in either group and because the sample size was small (1, 18). The U number derived was associated with a $p < 0.01$ for a one-tailed test. Thus, IgE levels of the patient group were significantly elevated above those of the control group. There was also a statistically significant difference in IgE levels found in the control and the miscellaneous group (with $p < 0.01$). No significant difference in IgE was found, however, when the AED group and the miscellaneous groups were compared. Differences between any two of the three groups were insignificant at the $p = 0.05$ level for each of the remaining immunoglobulin classes (IgG, IgA, IgM).

³H uptake of cultures containing PHA

The ratio of ³H uptake of cultures not containing PHA was used to measure the *in vitro* response of each participant's lymphocytes to the T-cell mitogen, phytohemagglutinin. The numerator and denominator represent an average of 3 values each. By pooling data from all of the white cell cultures it was found that for any one patient's sample each of the three unstimulated ³H-thymidine values deviated by an average of 4.1% from the mean of the three values and that each of the PHA-stimulated ³H-thymidine values deviated by an average of 8.6% from the mean of the three.

We studied morphologically the percentage of blast cells present in stimulated as compared with

Table I. Anhidrotic ectodermal dysplasia with atopic dermatitis immunologic findings

In vitro techniques						In vivo techniques	
Immuno-globulins ^a (IgE units/ml)	Ratio of stim./unstim. tritium uptake	WBC count ×10 ³	Lymphocytes (%)	E-Rosetting, % Lymphocytes equalling T-cells	Mumps test, + or - (mm erythema)	DNCB score between 0 and 5	
<i>Atopics with anhidrotic ectodermal dysplasia</i>							
1	1 328	19.4	6.3	62	39	Neg. (0 mm)	0
2	470	86.1	10.6	55	52	Neg. (5 mm)	1
3	873	204	5.6	33	58	Neg. (9 mm)	2
4	1 095	281	4.6	48	64	Neg. (10 mm)	1
5	310	231	2.7	36	59	Pos. (16 mm)	2
6	25	32.2	4.7	37	51	Neg. (9 mm)	1
<i>Normal controls</i>							
7	25	207	9.8	77	57	Pos. (15 mm)	4
8	13	296			58	Neg. (12 mm)	1
9	27.5	98.4	11.5	35	57	Neg. (5 mm)	1
10		250	5.6	27	62	Neg. (0 mm)	3
11	15.5	291	5.5	32	55	Pos. (15 mm)	2
12		179					
13		433	6.2	27	59	Pos. (15 mm)	2
14		158	6.1	27	62	Pos. (17 mm)	2
<i>"Miscellaneous" group</i>							
15 ^b	55	128	6.4	35	56	Neg. (0 mm)	0
16 ^c	620	67.4	5.2	30	47	Neg. (12 mm)	0
17 ^a	295	93.5	6.7	25	58	Neg. (0 mm)	1
18 ^e	155	144	6.9	31	61	Neg. (11 mm)	4
19 ^f	150	42.5					

^a Differences between any two of the three groups were insignificant at the $p=0.05$ level for each of the IgG, IgA, IgM classes.

^b 9-year-old female, atopic, dyskeratosis congenita.

^c 4-year-old female with verrucae.

^d 9-year-old female with fungal infection.

^e 38-year-old parent of patients 1, 2, 3; atopic.

^f 19-year-old Hodgkins patient.

non-stimulated controls, but found the morphological method not as accurate as the tritium uptake method.

Again making use of the Mann-Whitney U Test the population with AED had a lower response in terms of lymphocyte stimulation ratios with a 0.071 confidence level (one-tailed test). There was a significantly lower ($p<0.01$) response in the miscellaneous group than in the control group. No difference was detected when the miscellaneous and AED patient groups were compared ($p=0.792$ for a two-tailed test).

The mean for T-cells in the patient group was 53.8% and 58.6% in the non-atopic control group. There was no significant difference in T-cell levels between these small groups.

With the in vivo methods of assessing cellular im-

munity, 75% of those patients with prior mumps infections (defined as those patients whose serum demonstrates the ability to neutralize the mumps virus in vitro) should demonstrate an area of erythema of 15 mm at 48 hours. One-sixth (17%) of the atopic-AED group reacted positively as opposed to 4/12 (33%) of the non-atopics tested. An analysis of these findings with the Fisher exact probability test suggested that there was no significant difference between these groups.

Positive reactions to DNCB occurred in 5/6 (83%) of the AED group and 7/7 (100%) in the control group. Although the difference in the incidence of positive reactions is not significant between the two groups, the qualitative differences between the groups in reaction intensity are significant (mean of 1.2 for the AED atopic group,

2.1 of a total of five for the control group) at the 0.05 level using a one-tailed intergroup *t*-test. No differences in reactivity to mumps and DNCB were detected between the control and miscellaneous groups or between the miscellaneous group and the AED group.

The variation in intensity of reactions to DNCB appeared to vary within the atopic group itself. Patient 1, for example, demonstrated no reaction; patient 2, erythema < 10 mm; and patient 3, erythema > 10 mm. The order of severity of atopic dermatitis was patient 1 > patient 2 > patient 3. The mother of these affected patients, who manifested none of the stigmata of AED, as did the children, reacted most strongly (i.e., with vesiculation). There was, therefore, an inverse relationship between severity of atopy and the severity of skin reaction to DNCB in the group of four.

COMMENT

We attempted to answer two questions by studying these patients: Is there a reason other than eccrine and mucus gland deficiency to account for the episodes of hyperthermia these patients suffer? And, secondly, could a study of the immune status in genetically similar groups of patients with AED and atopic dermatitis clarify some of the unusual relationships between T-cell and B-cell function in these patients?

Let us examine the immune capabilities first. We found IgE to be significantly elevated in the AED group when compared with the control group, whereas cell-mediated immunity appeared to be depressed in the AED group.

The number of cutaneous reactions to mumps antigen or DNCB was normal in the AED patients, but their reactions were qualitatively less intense, suggesting depressed cell-mediated immunity in this group. Significant differences were also found to exist between the miscellaneous and normal control groups with regard to both IgE levels and lymphocyte responses to PHA, but no such differences existed between the miscellaneous and AED groups. Our inability to separate the miscellaneous AED groups according to IgE levels and lymphocyte stimulation ratios suggests that perhaps each of these two groups may represent a subgroup of a

larger population of patients with cell-mediated immune disturbances.

Although a rough correlation did exist between the degree of response of lymphocytes to the mitogen PHA (significant at the 0.071 level) and the number of T-cells found present by E-rosetting, there was no significant difference in T-cell counts between the AED and normal control groups. It is possible that the discrepancy between T-cell counts and in vivo immune responses was due to differences in antigen processing or lymphocyte proliferative capabilities between the two groups. In other words, there may be immune differences other than, or in addition to, reduced number of T-cells, which could explain a weakened cellular reaction in response to a given antigen.

The significance of depressed lymphocyte reaction to PHA was found to be even greater when we grouped those patients having elevated IgE levels, whether they had atopy or not. Disregarding the presence of atopic dermatitis, 11 patients who were found to have IgE levels greater than or equal to 150 units/ml had ³H-thymidine stimulation ratios significantly¹ lower (*p*=0.05) than 4 patients who had IgE levels less than 150 units/ml. Thus high IgE values from any cause increased the significance of the difference in lymphocyte proliferation when comparisons were made with controls. Conversely, 2 of the patients in the miscellaneous group, one with widespread verrucae and the other with resistant *T. tonsurans* infection (suggesting depressed cell-mediated immunity), did evidence elevated IgE levels in the absence of atopic dermatitis. These 2 patients also gave signs of decreased ³H-thymidine stimulation ratios and impaired responses to in vivo antigen challenges. These findings suggest that atopic dermatitis is related to disruption of T-cell function as well as of IgE production.

Jones (6) attempted to explain the relationship of increased IgE to depressed cellular immune reactions by hypothesizing that the atopic state may be represented by an "accelerated sequence" of events in which cellular immunity is suppressed by rising IgE levels. An increase in immediate immunologic reactions in the skin (mediated by IgE) could then, perhaps by a dilution of the antigen with edema fluid (10), render delayed hypersensitivity reactions less intense.

Three observations support the contention that IgE itself depresses cellular immunity. First, it has been noted that with pregnancy, when im-

¹ Analysis was accomplished using the one-tailed Mann-Whitney U test.

munoglobulin levels become higher than normal. T-cell function declines. Second, the ability of a supernatant of sensitized white cells to transfer immunity to PPD to a different culture may depend upon growth of the first culture in monautologous serum (14). In other words, growth of a patient's white cells in a serum from another individual may unmask a sensitivity to an antigen by removing "blocking" antibodies. Third, it has been observed that high levels of IgE in lymphoid tissue can be demonstrated by immunofluorescence in atopic patients. It has further been hypothesized that IgE fixation might possibly be the source of lymphocyte suppression (9).

Another possibility is that an "actual" cellular defect may be present. In other words, a cellular immunodeficiency might be more basic to atopy than to elevated IgE levels. In such a situation the elevated IgE levels in patients with low T-cell function could represent a type of "compensatory" immune response. If not all individuals were capable of developing high IgE levels then the coexistence of low IgE levels and low cell-mediated immunity in some atopics could be more easily understood. In such a case low T-cell function (the primary event) could then exist even in the absence of a high suppressant serum IgE level. The presence of patients with serious immune disorders such as agammaglobulinemia (in which there is generally some component of decreased cellular immunity) with atopic dermatitis in the face of normal or decreased IgE levels supports this notion. Conversely, a high IgE level need not necessarily be capable of inducing T-cell depression; indeed, a number of patients (including patients 3 and 4 of the AED group) demonstrate relatively high stimulation ratios of their lymphocytes in the face of increased IgE levels.

Finally, as an additional observation, we found that patients with AED appear to have more sensitive skin. Four of the 6 patients (66%) with AED experienced a significant primary irritant reaction, resulting in erythema >10 mm within 2-3 hours of application of the sensitizing dose of DNCB. None of the controls experienced so intense a reaction. Whether or not such a reaction might have been due to the inability of the patients with anhidrosis effectively to dilute the irritant with sweat, is not known. Such an irritant effect, however, may have played a part in subsequent delayed hypersensitivity reactions to DNCB.

As a result of finding decreased cellular immune competence in our AED group, it may be reasonable to question the current assumption that a patient with AED who experiences fever is suffering from lack of thermal control due to absence of sweat glands. Perhaps some of the recurrent fever in such patients may represent recurrent viral infections.

ACKNOWLEDGEMENT

This investigation was supported by funds from NIDR Grant No. 02872.

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Received April 8, 1975

L. M. Solomon, M.D.
Department of Dermatology
University of Illinois College of Medicine
P. O. Box 6998
Chicago, Illinois 60680
USA