

# Disordered immune regulation in autoimmune rheumatic diseases

## Autologous mixed lymphocyte reactivity in polymyositis/dermatomyositis and systemic lupus erythematosus<sup>1</sup>

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**Polymyositis/dermatomyositis (PM/DM) and systemic lupus erythematosus (SLE) are autoimmune disorders of unknown etiology. In order to study whether immunoregulatory abnormalities might be involved in these autoimmune states, the autologous mixed lymphocyte reaction (AMLR) was investigated in patients with either adult PM/DM, childhood DM (CDM), or SLE. The AMLR was found to be significantly depressed in adult PM/DM regardless of disease activity. By contrast, in CDM the AMLR was normal. In SLE, the AMLR was depressed in patients with both active and inactive disease and the responding T cell population appeared to be defective, as shown in a study on a pair of identical twins. These studies thus underscore the existence of a primary immunoregulatory dysfunction in these two autoimmune diseases.**

**Index terms:** Dermatomyositis • Lupus erythematosus, systemic

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The autologous mixed lymphocyte reaction (AMLR) is the *in vitro* proliferative response of T cells to autologous non-T or activated T cells.<sup>1,2</sup> The response is directed against Class II histocompatibility antigens present on the stimulating cells.<sup>3</sup> The AMLR is capable of generating various immunoregulatory activities including both T cell helper and suppressor function, broadly reactive cytotoxic cells, and lymphokines such as interleukin-2 and interferon- $\gamma$ .<sup>4-10</sup> The AMLR thus represents an *in vitro* model for dissecting cellular and molecular interactions leading to specific immunological functions and can be used to analyze interactions occurring in an aberrant fashion, for example,

in autoimmune disorders and malignancy. Support for this rationale has in fact been obtained in studies of the AMLR in various diseases where it has been shown to be defective.<sup>11-15</sup>

Both polymyositis/dermatomyositis (PM/DM) and systemic lupus erythematosus (SLE) are rheumatic diseases in which autoimmunity plays a direct pathologic role. Muscle destruction in PM/DM is the result of a cell-mediated immune reaction to autologous skeletal muscle<sup>16</sup> whereas in SLE, humoral mechanisms, in the form of antigen-antibody complexes deposited in various tissues, are primarily involved in the pathogenesis.<sup>17</sup> In autoimmune diseases, the presence of an underlying disorder of immunoregulation contributing to the pathogenesis is an important consideration. In this article, we report the results of our study of AMLR reactivity in adult and childhood PM/DM and in SLE.

## Methods

### Patients

*Adult polymyositis/dermatomyositis:* Diagnosis was based on the criteria of Bohan et al<sup>18</sup>; all patients included in the study had "probable" or "definite" disease by these criteria. Patients with malignancy, overlap syndromes (defined as the presence of a second established rheumatologic diagnosis), or childhood DM were excluded. All patients whose disease was described as "active" were studied after diagnosis was established and before treatment was begun. These patients had elevated muscle enzyme levels and weakness at the time of study. All, however, were ambulatory and without intercurrent illness. Patients whose disease was described as "inactive" were studied upon return to their physicians for routine check-up. These latter patients were in clinical and biochemical remission at the time of study. Patients with inactive disease and receiving doses of corticosteroid greater than 7.5 mg/day prednisone equivalent were excluded; patients (Patient 6 and Patient 7) receiving smaller doses of corticosteroid (ie, 7.5 mg/day or less) were studied at least 24 hours after a dose of medication, since it has been demonstrated that the AMLR returns to normal levels within 24 hours of administration of an oral dose of prednisone.<sup>19</sup> Patients receiving cytotoxic immunosuppressive medications were excluded. No patients studied had light- or electron-microscopic criteria for the diagnosis of inclusion body myositis.

*Childhood dermatomyositis:* All patients with childhood dermatomyositis (CDM) fulfilled the criteria of Bohan et al.<sup>18</sup> Other conditions for inclusion in the study were the same as described for patients with adult PM/DM. Patient 1 was being treated with a 2 mg/day course of prednisone.

*Systemic lupus erythematosus:* All SLE patients met the American Rheumatism Association 1982 revised criteria for the classification of SLE.<sup>20</sup> None of the patients was receiving cytotoxic drugs or corticosteroids >7.5 mg/day at the time of the study. As with PM/DM patients, those receiving corticosteroids were studied at least 24 hr after a dose of medication. Of the 16 patients studied, six were receiving an alternate-day course of prednisone (7.5 mg or less). Active or inactive disease was defined by using a modification of a quantitative scoring system of flare severity previously published by Lokshin et al.<sup>21</sup>

### Laboratory methods

*Preparation of lymphocytes and purification of T cells and non-T cells:* Peripheral blood mononuclear cells were isolated by centrifugation on a Ficoll-Hypaque (Pharmacia Fine Chemicals, Piscataway, NJ) gradient. T and non-T cells were separated by rosette technique with sheep erythrocytes (SRBC) using an overnight procedure. T cells recovered from the rosette pellet by lysis with 0.14 M NH<sub>4</sub>Cl were used as responder cells and were 95-97% E-rosette positive. Non-T fractions contained 55-65% SIg-positive cells, 25-35% esterase-positive cells, and less than 5% E-rosette-positive cells.

*Fractionation of T cells into T4 and T8 subpopulations:* This was accomplished by use of a previously described panning procedure.<sup>4</sup> Briefly, 30-40 × 10<sup>6</sup> T cells were treated with optimal dilutions of either OKT4 or OKT8 monoclonal antibodies (Ortho Diagnostics, Raritan, NJ) for 45 min at 4° C. The treated cells were allowed to adhere for 1 hour at 4° C on plates coated with goat anti-mouse IgG (Zymed Labs, San Francisco, CA). Nonadherent cells, after OKT8 or OKT4 treatment, were generally >90% T4 or >90% T8, respectively. Contamination with T8 or T4 cells, respectively, was <2%.

*Autologous mixed lymphocyte reaction:* A total of 1 × 10<sup>5</sup> T cells was cultured with 1 × 10<sup>5</sup> mitomycin C-treated (50 μg mitomycin C/2 × 10<sup>6</sup> cells/mL) non-T cells in RPMI-1640 medium

**Table 1.** Autologous mixed lymphocyte reaction in patients with polymyositis/dermatomyositis and matched controls

Subject*	Age (yr)	Activity of disease†	<sup>3</sup> H-thymidine incorporation (cpm) in culture containing		Δ CPM‡
			Responders alone	Responders + stimulators	
Patient 1	21	I	7,965 ± 3,823	17,098 ± 2,827	9,133
Control 1			750 ± 568	10,012 ± 6,738	9,262
Patient 2	23	A	3,060 ± 591	4,028 ± 2,865	968
Control 2			4,528 ± 888	20,202 ± 3,115	15,674
Patient 3	45	I	363 ± 228	1,145 ± 261	782
Control 3			62 ± 74	1,072 ± 596	1,010
Patient 4	47	A	3,185 ± 233	11,646 ± 1,135	8,461
Control 4			3,924 ± 314	14,546 ± 1,668	10,622
Patient 5	50	A	800 ± 80	1,038 ± 345	238
Patient 6	55	I	698 ± 260	329 ± 193	-369
Control 5			1,148 ± 299	3,893 ± 36	2,745
Patient 7	57	A	1,669 ± 265	425 ± 263	-1,244
Control 6			2,954 ± 188	6,934 ± 226	3,980
Patient 8	62	I	406 ± 468	211 ± 13	-195
Control 6			2,954 ± 188	6,934 ± 226	3,980
Patient 9	67	I	730 ± 670	1,088 ± 722	358
Control 7			594 ± 389	2,253 ± 658	1,659
Patient 10	70	I	472 ± 161	1,704 ± 273	1,232
Control 10			407 ± 150	9,676 ± 615	9,269
Patient 11	58	A	52 ± 13	49 ± 9	-3

\* Normal controls were age-, sex-, and race-matched with each patient and were tested concurrently.

† Assessed as described under Materials and Methods. A = active disease; I = inactive disease.

‡ Significantly depressed in patients ( $p < 0.01$ , Wilcoxon signed rank test for paired data).

supplemented with 10% heat-inactivated pooled human AB serum, 1 mM l-glutamine, 25 mM Hepes buffer, 100 U/mL penicillin, and 100 μg/mL streptomycin. Triplicate cultures were carried out in round-bottom microtiter plates (Linbro Chemical Co., Hamden, CT) in 5% CO<sub>2</sub> and 95% air at 37° C. On day 5, 0.5 μCi (1.85 × 10<sup>4</sup> Bq) <sup>3</sup>H-thymidine (3 Ci/mMol [1.11 × 10<sup>5</sup> MBq/mMol], New England Nuclear, Boston, MA) was added per well and the cultures were harvested 16 hours later using a MASH Harvester. Uptake of <sup>3</sup>H-thymidine was measured by liquid scintillation counting. The results are expressed as Δ counts per minute (Δ cpm) where

$$\Delta \text{ cpm} = (\text{cpm of AMLR cultures}) \\ - (\text{cpm of responders cultured alone})$$

### Statistical analysis

In our study, patients were paired with age- and sex-matched normal subjects. Because our study population was not a random sample from the population at large, there was no presumption that our results would follow a normal distribution. All data were therefore analyzed without assumptions about the distribution; the Wilcoxon signed rank test for paired data<sup>22</sup> was used to test for significant differences between patients and controls, and between patients with active and inactive disease. Results were analyzed for normality by means of the Wilk-Shapiro test, and where results were found to follow a normal distribution, the t test for paired data was also performed; there were no differences between results of the Wilcoxon signed rank test and the

**Table 2.** Allogeneic mixed lymphocyte reaction in patients with polymyositis/dermatomyositis

Subject	Responders alone (cpm)	Responders + autologous stimulators* (cpm)	Responders + allogeneic stimulators† (cpm)
Patient 3	363 ± 228	1,145 ± 261	19,473 ± 618
Control 3	62 ± 74	1,072 ± 596	9,032 ± 1,370
Patient 9	730 ± 670	1,088 ± 722	26,883 ± 2,266
Control 8	594 ± 389	2,253 ± 658	41,082 ± 5,654

\*  $1 \times 10^5$  responding T cells were cultured with  $1 \times 10^5$  mitomycin-C treated autologous non-T cells for 6 days.

†  $1 \times 10^5$  responding T cells were cultured with  $1 \times 10^5$  mitomycin-C treated allogeneic non-T cells for 6 days.

t test for paired data. All data are presented as analyzed with the Wilcoxon signed rank test.

## Results

### Polymyositis/dermatomyositis

The AMLR proliferative response was studied in eleven PM/DM patients and their age-, race-, and sex-matched normal controls. As shown in *Table 1*, this AMLR proliferative response was significantly depressed in PM/DM patients ( $p < .01$ ). A time-course study of the AMLR in normal individuals and in PM/DM patients revealed a continuous low reactivity by PM/DM lymphocytes throughout the culture period (data not shown) indicating that altered kinetics does not account for the poor reactivity of PM/DM lymphocytes. Furthermore, addition of increasing numbers of stimulator cells did not reconstitute the response (data not shown). It is important to note that a nonspecific depression of all T lymphocyte functions was not present in these patients, as they were shown to possess reactivity in allogeneic MLR (*Table 2*).

A comparison of five patients with active disease and six with inactive disease showed a similar impairment of AMLR proliferative responses in both groups.

### Childhood dermatomyositis

Twelve patients with CDM were evaluated for AMLR reactivity. As shown in *Table 3*, the patients as a group responded well in the AMLR and had no statistically significant differences when compared with normal controls, although three of the 12 patients did demonstrate impaired AMLR responses (Patients 1, 2, and 8). The impaired response did not appear to be

related to disease activity since Patients 1 and 8 had clinically inactive disease whereas Patient 2 had active disease.

### Systemic lupus erythematosus

The *Figure* shows the autologous reactivity of T cells as well as plate-separated T4 and T8 cells in SLE patients and normal controls. T cells from 16 SLE patients showed significantly decreased AMLR reactivity when compared with matched normal T cells ( $p < 0.01$ ). When T4 (OKT8<sup>-</sup>) and T8 (OKT4<sup>-</sup>) subpopulations were compared, the T4 cells from SLE patients showed poor proliferation compared with normal T4 cells ( $p < .05$ ). It has been shown that T8 cells will proliferate in an AMLR only in the presence of T4 cells or exogenously supplied IL-2.<sup>23</sup> Thus, in these experiments, neither T8 cells from normal controls nor those from SLE patients showed proliferation when cultured alone in an AMLR system.

When the patient group was divided into groups with active and inactive disease, all nine patients with active disease had decreased responses compared with their matched normal counterparts as well as the normal group as a whole ( $p < 0.01$ ). Of the seven patients with inactive disease, two demonstrated normal AMLR reactivity whereas five patients responded with much lower proliferation. On a statistical basis, therefore, the group with inactive disease did not differ significantly ( $p > 0.05$ ) from the normals, although the majority of these patients (5/7) did not respond well.

We had the opportunity to study a set of identical twins discordant for the expression of SLE. Cross-stimulation studies were performed on responder and stimulator cells from each member of the pair (*Table 4*). There was a major defect in the patient's T cells, which were unable to respond to her own or the twin's non-T cells. A similar lack of response was seen with the patient's T4 cell population. The SLE non-T cells appeared capable of stimulating the normal twin's T cells. The defect in the T cells also extended to the allogeneic MLR where the patient's cells responded poorly to allogeneic third-party non-T cells.

## Discussion

The AMLR, which denotes proliferation of T cells in response to autologous non-T cells, appears to be a complex and important example

**Table 3.** Autologous mixed lymphocyte reaction in patients with childhood dermatomyositis and matched controls

Subject*	Age (yr)	Activity of disease†	<sup>3</sup> H-thymidine incorporation (cpm) in culture containing		Δ CPM‡
			Responders alone	Responders + stimulators	
Patient 1	35	I	161 ± 33	241 ± 160	80
Control 1			1,048 ± 490	6,846 ± 1,574	5,798
Patient 2	15	A	438 ± 60	119 ± 33	-319
Control 2			5,296 ± 1,048	188 ± 68	-5,108
Patient 3	20	I	799 ± 271	3,458 ± 699	2,659
Control 3			5,421 ± 1,411	8,734 ± 1,309	3,313
Patient 4	27	I	5,020 ± 1,601	12,222 ± 675	7,202
Control 4			6,552 ± 2,019	15,288 ± 2,804	8,736
Patient 5	18	A	1,634 ± 1,397	2,849 ± 968	1,215
Control 5			2,191 ± 1,660	13,319 ± 1,600	11,128
Patient 6	27	I	4,582 ± 2,949	21,682 ± 5,008	17,100
Control 6			944 ± 823	3,135 ± 683	2,191
Patient 7	33	I	358 ± 86	2,516 ± 622	2,158
Patient 8	20	I	23,400 ± 6,621	22,878 ± 2,511	-522
Control 8			3,534 ± 732	1,777 ± 625	-1,757
Patient 9	16	I	340 ± 114	2,890 ± 683	2,550
Control 9			58 ± 1	3,678 ± 974	3,620
Patient 10	12	I	739 ± 527	6,448 ± 3,510	5,709
Control 10			2,714 ± 1,469	7,050 ± 1,366	4,336
Patient 11	31	I	224 ± 47	2,694 ± 1,071	2,470
Control 11			2,467 ± 627	16,344 ± 3,836	13,877
Patient 12	11	I	837 ± 71	7,152 ± 2,789	6,315
Control 12			509 ± 245	5,756 ± 1,408	5,247

\* Normal controls were age-, sex-, and race-matched with each patient and were tested concurrently.

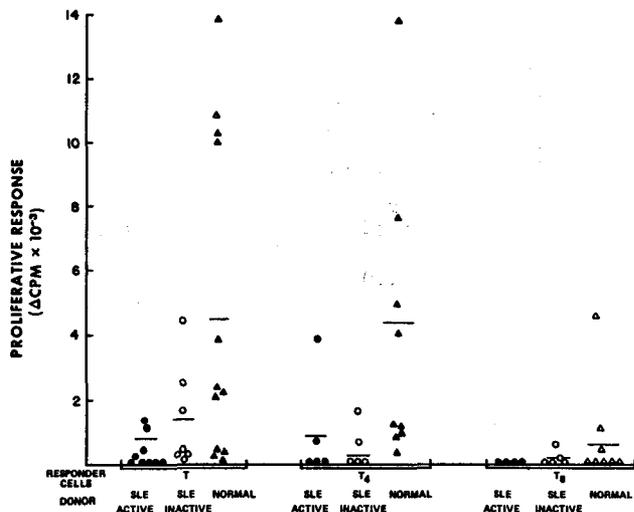
† Assessed as described under Materials and Methods. A = active disease; I = inactive disease.

‡ No significant difference between patients and controls using the Wilcoxon signed rank test for paired data.

of autoreactivity or autorecognition. T cell recognition of cell surface structures encoded by Ia/DR genes of the major histocompatibility complex is the basis of the AMLR, and such recognition has been shown essential for the afferent limb of normal immune responses to foreign antigens. It has further been shown that immunoregulatory activities of T cells are activated in AMLR cultures. It has therefore been hypothesized that T cell recognition of self, as reflected in appropriate AMLR reactivity, is essential for the maintenance of normal immune homeostasis.<sup>24</sup> AMLR defects have been reported in other

autoimmune disorders as well as in neoplastic diseases.<sup>11-15</sup> Autoimmunity-prone strains of mice also demonstrate reduced syngeneic MLR responses.<sup>25</sup> Thus, decreased AMLR may reflect an immunoregulatory imbalance that, together with other factors, may lead to autoimmune disease.<sup>24</sup>

It is likely that cell-mediated autoimmunity to muscle is important in the pathophysiology of PM/DM. The following observations support this hypothesis: (a) lymphocytes sensitized to skeletal muscle are present in patients<sup>26</sup>; (b) patient lymphocytes can mediate injury to muscle cells in



**Figure.** Autologous mixed lymphocyte reactivity (AMLR) activity of T, T4 (OKT8<sup>+</sup>), or T8 (OKT4<sup>+</sup>) cells from normal subjects and individual patients with systemic lupus erythematosus (SLE). Horizontal bars represent the mean.

tissue culture<sup>27</sup>; and (c) lymphocytes from PM/DM patients can elaborate myotoxic lymphokines when incubated with autologous muscle.<sup>28</sup> Analysis of an autoimmune state such as that seen in PM/DM requires consideration of a diversity of etiologic possibilities including genetic predisposition, viral induction, and immunoregulatory abnormalities.<sup>29</sup>

The impaired AMLR response in our group of patients with PM/DM thus suggests the presence of abnormal immunoregulation in this expression of autoimmunity. It is currently unclear whether this defect precedes disease expression or results from it, through the depletion of AMLR-reactive T cell subsets by their commitment as part of the disease process. We do not

think that the decrease in AMLR reactivity could be due to nonspecific illness, as those patients with inactive disease who were clinically well also showed the AMLR defect. Further, in another study, the AMLR has been shown to be unhindered in at least one group of normal individuals who were nonspecifically ill.<sup>30</sup> The cellular basis for this defect is at present unclear. Studies in our laboratory have shown decreased IL-2 production in PM/DM AMLR cultures (unpublished observations). Furthermore, AMLR responses were partially restored following removal of adherent cells (unpublished observations).

In contrast to adult PM/DM, the CDM patients as a group showed no significantly different AMLR responses from normals. CDM differs from adult PM/DM in other respects also. Clinically, there is an increased incidence of calcification of muscle and skin in gastrointestinal vasculitis.<sup>31,32</sup> Conversely, ophthalmologic, pulmonary, and malignant occurrences are less common in CDM than adult PM/DM.<sup>31,33,34</sup> Histologically, most authors agree on the higher incidence of vasculitis affecting small arteries, capillaries, and venules in CDM.<sup>30,35,36</sup> Reticulotubular inclusions within endothelial cells and a reduced capillary/muscle-fibre ratio have both been demonstrated in CDM,<sup>36</sup> whereas they have not been consistently identified in adult PM/DM.<sup>36</sup> Immunologic differences between CDM and adult PM/DM have also been suggested. Unlike adult PM/DM, lymphocytes from CDM patients do not demonstrate significant cytotoxicity.<sup>37,38</sup> Our finding of an immunoregulatory defect in adult PM/DM but not in CDM thus lends further support to the argument that the pathophysiologic basis for CDM differs from that for adult PM/DM.

SLE is an autoimmune disorder in which multiple immune abnormalities are associated with recurrent or continuous inflammatory processes in a variety of organ systems. It is characterized by the presence of autoantibodies to nuclear and other tissue antigens.<sup>39</sup> Disordered immune system control in SLE has been documented by several investigators.<sup>40</sup> The studies reported in this paper and by others<sup>14,41</sup> indicate that the AMLR is impaired in SLE patients. Furthermore, in a serial study of SLE patients, the AMLR was found to correlate with disease activity; ie, it returned to normal when the disease was quiescent.<sup>42</sup> However, other studies, including this one, have found the AMLR to be uniformly depressed regardless of disease activity.<sup>43</sup> Al-

**Table 4.** Autologous mixed lymphocyte reaction in patient with SLE and healthy identical twin

Stimulator cells	Proliferative response (Δcpm)	
	SLE patient	Healthy twin
T responder cells		
SLE non-T	330	5,392
Twin non-T	433	3,173
Third-party non-T	12,887	48,512
T4 responder cells		
SLE non-T	700	5,906
Twin non-T	1,210	5,082

SLE = systemic lupus erythematosus.

though as a group our patients with inactive disease were not statistically different from the controls, the finding of a significantly impaired AMLR response in 5/7 of these patients indicates that in most cases the AMLR response does not normalize with a decline in disease activity. The T4 subpopulation is defective since these cells are unable to respond. Whether the T8 cells also have a defect in responding to autologous signals is not known at present since these cells proliferate in an AMLR only in the presence of a source of IL-2.<sup>23</sup> These questions are currently being studied.

Our results with the set of twins discordant for SLE suggest that the AMLR defect resides in the responding T cells and not in the ability of the non-T cells to act as stimulators. Sakane et al<sup>44</sup> came to the same conclusion in a similar study of two sets of identical twins. However, using HLA, A and B, and MLR-matched normal siblings of patients with inactive SLE, Kuntz et al<sup>45</sup> found the defect to be in the non-T stimulators. In the latter study, it is difficult to know whether the patients were matched at the HLA-D locus because the MLR was used both as the criterion of identity and measure of abnormality. Our interpretation of the AMLR defect in SLE is based on a study of a single twin pair. It is entirely possible, given the heterogeneous nature of SLE, that in some patients a stimulatory defect is also present, as suggested by a recent study.<sup>46</sup>

The AMLR T cell defect in SLE appears to represent an intrinsic abnormality in the T cells since it was present regardless of disease activity. SLE is a heterogeneous disease in both its clinical and laboratory manifestations. Thus far, no correlations have been reported in the literature between the degree of AMLR reactivity and clinical and/or laboratory parameters of autoimmunity.

In summary, we have shown, utilizing the AMLR, that defects in immunoregulation exist in adult PM/DM and SLE. Characterization of these defects would aid in the understanding of the pathogenesis of these and other autoimmune disorders.

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