Clin. exp. Immunol. (1990) **81**, 373–379

Lymphocyte activation markers in idiopathic myositis: changes with disease activity and differences among clinical and autoantibody subgroups

F. W. MILLER, L. A. LOVE, S. A. BARBIERI,* J. E. BALOW* & P. H. PLOTZ *Arthritis and Rheumatism Branch, National Institute of Arthritis and Musculoskeletal and Skin diseases, and *Kidney Disease Section, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD, USA*

(Accepted for publication 12 March 1990)

SUMMARY

We studied the immunologic correlates of disease activity and differences among subgroups of patients with idiopathic inflammatory myopathy by analysing phenotypic and activation marker expression on peripheral blood mononuclear cells (PBMC). Compared with controls, myositis patients with clinically active disease ($n=51$) had significantly lower proportions of $CD8^+$ cells and higher proportions of PBMC that expressed DR, $CD3^-$ DR, $CD14^-$ DR, interleukin-2 receptors, and the late T cell activation markers CD26 and TLiSA1. TLiSA1 expression, a marker for cytotoxic differentiation, correlated significantly with both clinical activity indices and serum levels of muscle-associated enzymes. In serial studies of seven patients, the proportion of PBMC expressing MHC class II antigen and late T cell activation markers decreased as myositis disease activity decreased, independent of type of therapy. Among the clinical subgroups, polymyositis ($n=21$) and inclusion body myositis ($n=11$) were virtually indistinguishable; dermatomyositis patients ($n=19$) showed decreased proportions of $CD3^+DR^+$ and $TLiSA1^+$ cells, and increased proportions of $CD20^+$ and $CD20^+DR^+$ cells compared with the other two groups. Patients with autoantibodies to histidyl-tRNA synthetase (Jo-1 antigen, $n=11$) had significantly lower proportions of $CD3^+$ and $CD4^+$ cells, lower CD4/CD8 ratios, and higher proportions of DR^+ cells expressing CD20, compared with patients without anti-Jo-1 antibodies. These findings support the concept that activated lymphocytes, especially cells undergoing anamnestic responses and cytotoxic differentiation, are important in the pathogenesis of idiopathic myositis. Moreover, taken together with other studies, these data suggest that groups of patients segregated by clinical or autoantibody status have different mechanisms of systemic immune activation and immunopathology.

Keywords idiopathic inflammatory myopathy polymyositis lymphocyte activation markers anti-Jo-1

INTRODUCTION

Patients who develop a syndrome of progressive muscle weakness, elevated serum levels of muscle-associated enzymes, and mononuclear cell infiltrates in muscle—without evidence of muscular dystrophy, toxin exposure, metabolic derangement or infection—are considered to have idiopathic inflammatory myopathies (IIM). These diseases have been traditionally classified into five or six clinical groups (Bohan & Peter, 1975; Cronin, Miller & Plotz, 1988a), and criteria for diagnosis of the major forms, polymyositis (PM) and dermatomyositis (DM), have been proposed (Bohan & Peter, 1975). The rarity and heterogeneity of these disorders, however, have limited our knowledge of their pathogenetic mechanisms.

The association of myositis with other autoimmune diseases in some patients (Miller, 1989) and the many humoral and cellular immune abnormalities described (Cronin, Plotz & Miller, 1988b), suggest an autoimmune pathogenesis. On the humoral immune side, patients develop a wide variety of autoantibodies, have circulating immune complexes and abnormal complement components, with deposition of immunoglobulins and the complement membrane attack complex in the muscles of DM patients (Cronin *et al.*, 1988b). Among the abnormalities of cellular immune function in IIM are decreased lymphocyte proliferative responses to antigens, altered mixed leucocyte responses (Cronin *et al.*, 1988b), increased peripheral lymphocyte responses to (Kalovidouris *et al.*, 1989) and trafficking to (Miller *et al.*, 1988) skeletal muscle, and increased numbers of activated lymphocytes within muscle on biopsy of these patients (Engel & Arahata, 1986).

Presented in part at FASEB meetings, Las Vegas, Nevada, May, 1988.

Correspondence: Frederick W. Miller, MD, Ph.D. NIH Building 10, Room 9N228, Bethesda, MD 20892, USA.

One approach to delineate pathophysiology is the functional and phenotypic characterization of peripheral blood mononuclear cells (PBMC) in myositis patients. The availability of a large well-defined patient population has allowed a detailed analysis of the expression of lymphocyte phenotypic and activation markers at different phases in these patients' disease process. Here we describe increased immune activation *in vivo* in idiopathic myositis and demonstrate alterations with disease activity as well as differences among the major clinical and autoantibody subgroups. These findings have immunopathogenetic as well as possible therapeutic implications.

PATIENTS AND METHODS

Patient population

Adult patients with definite PM (16 women, five men, aged 25–59 years) and DM (13 women, six men, aged 21–52) according to the Bohan & Peter (1975) criteria were studied. Data from 11 patients (four women, seven men, aged 52–68) with inclusion body myositis (IBM), a pathologically distinct, more slowly progressive variant of PM predominantly affecting older men and reported to be more resistant to therapy (Mikol, 1986), were also analysed. Normal blood donors without known disease (12 women, eight men, aged 25–60) served as controls. All patients were being evaluated for treatment protocols and were either not receiving therapy for their myositis or receiving oral steroid therapy, which was withheld for at least 24 h prior to cell collection. Patients who had received cytotoxic therapy within 1 month before cell collection were excluded from the study.

PBMC isolation

PBMC, obtained by apheresis or collection of 50 ml of heparinized blood, were isolated on Ficoll–Paque (Pharmacia/LKB, Piscataway, NJ) by standard methods (Boyum, 1968). Cells were then frozen in RPMI 1640 containing 10% dimethyl sulphoxide and 20% fetal calf serum (FCS) using a programmable freezer (The Virtis Co., Gardiner, NY) and stored in liquid nitrogen for up to 6 months until use. Cells showed >95% viability by trypan blue staining after this treatment.

Identification of PBMC surface antigens

PBMC were thawed in RPMI 1640 containing 1% FCS and washed twice in HBSS supplemented with 0·1% FCS, 0·1% sodium azide. Cells were blocked with 30 μg of human immunoglobulin by incubating for 15 min at room temperature and were directly stained with fluorescein-conjugated or phycoerythrin-conjugated monoclonal antibodies, and isotype-matched labelled control antibodies, following the manufacturers' instructions. The antibodies used were: Leu4 (CD3), Leu16 (CD20, a B cell marker), Leu3 (CD4), Leu2 (CD8), Leu11 (CD16, a natural killer cell marker), LeuM3 (CD14, a monocyte/macrophage marker), HLA-DR, and interleukin-2 receptor (IL-2R, CD25) antibodies (all from Becton Dickinson, Mountain View, CA); Ta1 (CD26, Coulter Immunology, Hialeah, FL); and VLA-1 and TLiSA1 (T Cell Sciences, Cambridge, MA). Stained cells were analysed with a FACS 440 (Becton Dickinson) by standard methods. The FACS operator was unaware of the diagnosis or status of the patients from whom samples were taken. Fluorescent signals were processed by Consort-40 software (Becton Dickinson) using a PDP-11/23 based computer system (Digital Equipment Corps). Gating of monocytes was performed after collecting data on 25000 cells and all data, except those pertaining to LeuM3 staining, were analysed using the gated analysis (Miller *et al.*, 1978).

Clinical assays

Routine complete blood counts, leucocyte differentials, serum creatine kinase and aldolase levels, and autoantibody identification were determined in the NIH Clinical Pathology Department by standard techniques. Anti-Jo-1 antibody status was confirmed by ELISA and histidyl-tRNA synthetase activity inhibition studies (Biswas *et al.*, 1987). Patients were clinically graded for myositis disease activity at the time of cell collection by their treating physician using a 0–4 scale of 0, no disease activity; 1, mild; 2, moderate; 3, severe; and 4, extremely severe disease activity. This global clinical assessment was based on a physical examination, including formal manual muscle strength testing, an activities of daily living questionnaire, laboratory data and patients' subjective assessments of disease activity.

Statistical analysis

Cell surface marker data, autoantibody data, and routine clinical data were analysed using SAS (SAS Institute, Cary, NC) on an IBM 370 computer. Significance levels were set at $P < 0{\cdot}05$, and were determined using Wilcoxon rank sums with Bonferroni corrections for multiple comparisons and Spearman correlation coefficients.

RESULTS

Differences in PBMC antigen expression between IIM patients and controls

Table 1 shows the proportions (mean ± s.e.m.) of PBMC expressing the listed phenotypic and activation antigens, and reveals many significant differences between patients with clinically active myositis and controls. Patients with IIM have significantly lower proportions of circulating $CD8^+$ cells, but higher proportions of cells bearing MHC class II antigens (DR). Double-labelling experiments showed that the higher DR expression is within populations of $CD3^+$, $CD20^+$ and $CD14^+$ cells, but significant differences between patients and controls were noted only in the $CD3^+$ DR^+ and $CD14^+$ DR^+ groups. Both early (IL-2R) and late (CD26 and TLiSA1) T lymphocyte activation marker expression on peripheral cells is also increased in the IIM patients, but not the very late activation antigen (VLA-1). These results were not due to differences in proportions of $CD3^+$ cells available for counting as shown by $CD26^+/CD3^+$, and $TLiSA1^+/CD3^+$ ratios, which were also significantly higher in patients than controls (Table 1). The proportion of DR^+ cells expressing CD3 was also significantly increased in the patients.

Because many of the patients, but none of the controls, were receiving corticosteroids and had received cytotoxic agents, we were initially concerned that some of these differences may be due to these drugs. Analysis of concurrent steroid doses *versus* the individual surface marker data showed no significant correlations. Furthermore, comparisons between patients receiving corticosteroids ($n = 43$), with or without prior cytotoxic therapy, and patients newly diagnosed and never treated with either group of agents ($n = 8$) revealed no significant differences in any of the parameters (data not shown). These data strongly suggest that cytotoxic therapy over 1 month prior

Table 1. PBMC subset profiles in IIM patients and healthy controls

	All IIM ($n=51$)		Controls ($n=20$)		
PBMC subset	Mean %	s.e.m.	Mean %	s.e.m.	P*
Single staining					
$CD3^+$	54	2·3	61	3·0	NS
$CD4^+$	34	2·1	38	1·5	NS
$CD8^+$	21	1·2	27	1·5	<0·01
$CD20^+$	18	1·7	15	1·4	NS
$CD16^+$	15	1·5	15	2·3	NS
DR^+	42	2·2	25	1·3	<0·001
IL-$2R^+$	2·5	0·4	0·9	0·2	<0·005
$CD26^+$	50	2·7	23	2·8	<0·001
$TLiSA1^+$	60	2·5	41	4·8	<0·005
VLA-1^+	1·0	0·1	2·0	1·2	NS
Double staining					
$CD3^+DR^+$	12	1·6	3·4	0·6	<0·001
$CD20^+DR^+$	14	1·8	9·5	1·8	NS
$CD14^+DR^+$†	46	2·9	35	3·9	<0·05
Ratios					
$CD4^+/CD8^+$	2·0	0·2	1·5	0·1	NS
$CD3^+DR^+/DR^+$	0·37	0·02	0·1	0·0	<0·01
$CD20^+DR^+/DR^+$	0·34	0·03	0·6	0·1	NS
$CD26^+/CD3^+$	1·0	0·1	0·4	0·1	<0·001
$TLiSA1^+/CD3^+$	1·1	0·1	0·7	0·1	<0·005

All patients with clinically active disease.

PBMC, peripheral blood mononuclear cell; IIM, idiopathic inflammatory myopathy; NS, not significant; IL-2R, interleukin-2 receptor.

* Statistical probability that the proportion of positive PBMC in IIM patients differs from controls.

† Data generated without gating.

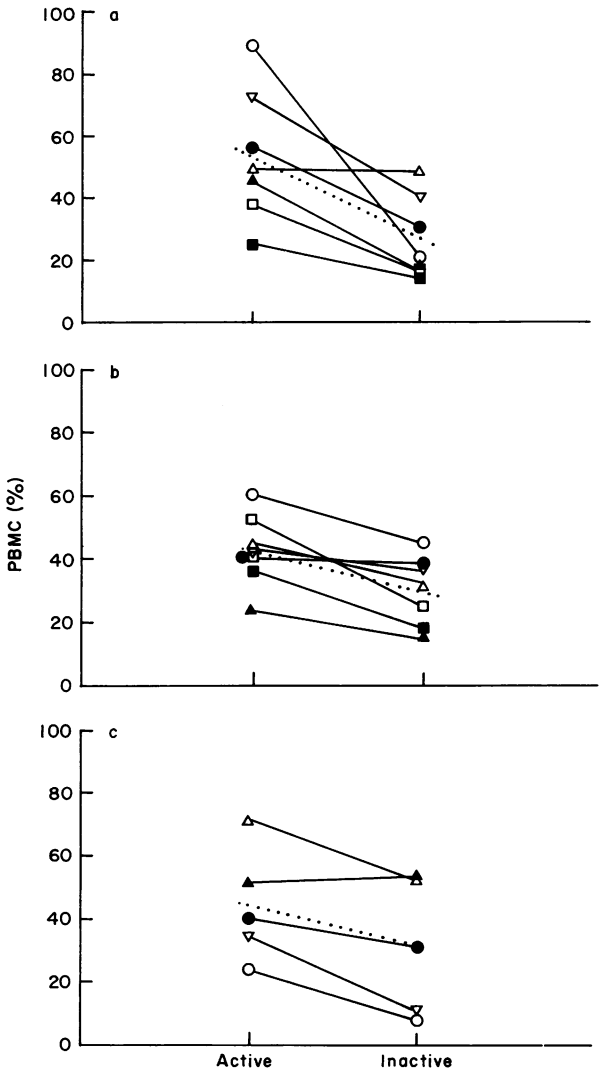

Fig. 1. Serial analysis of peripheral blood mononuclear cells (PBMC) from seven myositis patients during periods of active and relatively inactive disease showing the significant decreases ($P<0·03$ in each) in the proportion of cells expressing DR (a), CD26 (b), and TLiSA1 (c). Only five of the patients had TLiSA1 measured. Each patient is represented by the same symbol in each panel. Three patients received only oral prednisone alone (□, ■, △); two patients were re-evaluated in the inactive state more than 1 month after discontinuing apheresis (●) or i.v. cyclophosphamide (○). The dotted line represents the mean decrease in expression of each of the listed cell surface markers.

to study, and corticosteroid treatment over 24 h prior to study, did not account for the significant differences noted between the patient and control populations.

In the IIM patients there were significant positive correlations between proportions of PBMC positive for CD20 and DR, CD26 and CD3, and CD26 and CD4. Conversely, there were significant negative correlations between proportions of cells expressing CD3 and CD20, CD3 and DR, and TLiSA1 and DR. Additionally, the proportions of $TLiSA1^+$ cells positively correlated with concurrent serum creatine kinase ($P<0·04$), and clinical activity index ($P=0·03$) data.

Further evidence that the increased MHC class II antigen and T cell activation marker expression are related to myositis disease activity comes from the serial evaluation of seven IIM patients studied during relatively active and less active periods in their disease. Figure 1 shows the proportion of cells expressing DR, CD26, and TLiSA1, and depicts the significant decrease in all three when comparing the active with inactive disease state. No significant differences were noted, however, in any of the other markers studied, although the same trend was true for IL-$2R^+$, $CD3^+$ DR^+, $CD20^+$ DR^+ and $CD14^+$ DR^+ PBMC. That the differences seen are not simply due to the therapy for myositis is suggested by several lines of evidence. Firstly, these seven patients were treated by many different therapies with different modes of action. Three of the seven patients received oral prednisone alone, and the remaining four received prednisone plus another therapy: oral methotrexate, high-dose i.v. methotrexate with leucovorin rescue, apheresis, and monthly bolus i.v. cyclophosphamide. Secondly, re-evaluation was performed in the three patients receiving prednisone alone at a time when their prednisone dose was discontinued ($n=1$) or much reduced ($n=2$) compared with the dose taken at the time of active disease. Follow-up PBMC subset studies of two of the four other patients were performed when they were taking much lower prednisone doses, more than 1 month after either apheresis or pulse i.v. cyclophosphamide was discontinued (Fig. 1).

Table 2. PBMC subset profiles among IIM clinical subgroups

	DM ($n=19$)		PM ($n=21$)		IBM ($n=11$)		P*	
PBMC subset	Mean %	s.e.m.	Mean %	s.e.m.	Mean %	s.e.m.	DM-PM	DM-IBM
Single staining								
$CD3^+$	46	3·5	56	3·5	66	3·8	NS	<0·005
$CD4^+$	29	2·8	34	3·5	42	4·3	NS	NS
$CD8^+$	17	1·7	23	1·8	24	2·7	NS	NS
$CD20^+$	25	3·0	15	1·9	11	2·1	<0·01	<0·005
$CD16^+$	14	2·4	14	3·2	18	4·8	NS	NS
DR^+	52	3·2	36	2·9	34	4·0	<0·001	<0·005
IL-$2R^+$	3·2	0·6	1·2	0·2	3·6	1·2	<0·01	NS
$CD26^+$	54	5·6	41	3·2	60	3·4	NS	NS
$TLiSA1^+$	54	4·1	68	3·5	59	4·8	<0·05	<0·05
VLA-1^+	1·1	0·2	0·8	0·2	1·1	0·4	NS	NS
Double staining								
$CD3^+DR^+$	7·1	1·4	15	3·1	16	2·8	<0·05	<0·01
$CD20^+DR^+$	25	3·4	11	1·6	5·6	1·5	<0·01	<0·001
$CD14^+DR^+$†	45	5·2	46	4·1	47	6·6	NS	NS
Ratios								
$CD4^+/CD8^+$	2·0	0·3	1·8	0·3	2·3	0·5	NS	NS
$CD3^+DR^+/DR^+$	0·1	0	0·4	0·1	0·6	0·1	<0·01	<0·05
$CD20^+DR^+/DR^+$	0·5	0·1	0·3	0	0·2	0	<0·05	<0·05
$CD26^+/CD3^+$	1·3	0·1	0·7	0	0·4	0·1	<0·01	<0·05
$TLiSA1^+/CD3^+$	1·2	0·2	1·2	0·2	0·9	0·1	NS	NS

PBMC, peripheral blood mononuclear cells; IIM, idiopathic inflammatory myopathy; DM, dermatomyositis; PM, polymyositis; IBM, inclusion body myositis; all patients with clinically active disease; NS, not significant; IL-2R, interleukin-2 receptor.

* Statistical probability that the proportion of positive PBMC in DM differs from that in PM (DM-PM) or that the proportion of positive PBMC in DM differs from that in IBM (DM-IBM).

† Data generated without gating.

Differences among clinical and autoantibody subgroups of IIM patients

Since different immunopathogenetic mechanisms may operate among different subgroups of IIM (Miller, 1989), we were interested in determining whether these subgroups, based upon clinical and pathologic criteria, showed differences in PBMC subsets. Table 2 lists the data when patients were divided into the three major clinical groups of PM, DM, and IBM, and shows that significant differences do exist among these groups. DM patients have significantly higher proportions of circulating $CD20^+$ B cells than do either PM or IBM patients, and this is responsible for the increased proportions of DR^+ cells in these patients. Conversely, PM and IBM patients as groups each have higher proportions of peripheral $CD3^+$ T cells than DM patients, and the DR^+ fraction of these cells is also higher. Regarding T cell activation markers specifically, the PM and IBM groups each have significantly higher proportions of $TLISA1^+$ cells and higher $CD26^+/CD3^+$ ratios than the DM group. These differences are not due to differences in total *in vivo* leucocyte or lymphocyte counts, which were not significantly different among any of the patient subgroups. The PM and IBM groups were virtually indistinguishable. Only the proportions of $CD26^+$ cells were significantly higher in IBM patients than in PM patients.

Patients with anti-Jo-1 autoantibodies differed in many respects from those without those antibodies (Table 3). The presence of anti-Jo-1 antibody was associated with significantly lower proportions of PBMC expressing CD3 and CD4, lower CD4/CD8 ratios, and higher proportions of DR^+ cells displaying CD20.

DISCUSSION

The IIM are a clinically and pathologically diverse group of diseases which share the common feature of chronic muscle inflammation of unknown cause. Although evidence suggests a certain genetic predisposition with subsequent environmental triggers including viral infection as possible aetiologic factors (Love, 1989), the final pathologic events in these diseases appear to be autoimmune in nature (Miller, 1989). Little is known, however, about the specific immune abnormalities of the IIM because of their rarity and heterogeneity. Therefore, we initiated these studies as first steps in the attempt to understand which peripheral lymphocytes become activated, traffic abnormally to muscle, and result in myofibre destruction.

Three principal findings are described in this study: (i) Myositis patients have significantly decreased proportions of

Table 3. PBMC subset profiles in IIM patients with and without anti-Jo-1 antibody

PBMC subset	Anti-Jo-1$^+$ ($n=11$) Mean %	s.e.m.	Anti-Jo-1$^-$ ($n=40$) Mean %	s.e.m.	P*
Single staining					
CD3$^+$	44	5·4	57	2·4	<0·05
CD4$^+$	24	3·7	36	2·2	<0·02
CD8$^+$	21	2·2	21	1·4	NS
CD20$^+$	20	3·1	18	1·9	NS
CD16$^+$	17	4·2	15	1·6	NS
DR$^+$	45	5·6	41	2·3	NS
IL-2R$^+$	2·5	0·5	2·5	0·5	NS
CD26$^+$	43	5·2	52	3·0	NS
TLiSA1$^+$	60	5·2	60	2·8	NS
VLA-1$^+$	0·8	0·2	1·0	0·2	NS
Double staining					
CD3$^+$DR$^+$	10	1·8	13	2·0	NS
CD20$^+$DR$^+$	18	3·7	13	2·0	NS
CD14$^+$DR$^+$†	49	4·2	46	3·5	NS
Ratios					
CD4$^+$/CD8$^+$	1·2	0·2	2·2	0·2	<0·03
CD3$^+$DR$^+$/DR$^+$	0·3	0·1	0·4	0·1	NS
CD20$^+$DR$^+$/DR$^+$	0·5	0·1	0·3	0	<0·04
CD26$^+$/CD3$^+$	1·1	0·2	1·0	0·1	NS
TLiSA1$^+$/CD3$^+$	1·3	0·3	1·1	0·1	NS

PBMC, peripheral blood mononuclear cell; IIM, idiopathic inflammatory myopathy, all patients with clinically active disease; NS, not significant; IL-2R, interleukin-2 receptor.

* Statistical probability that the frequency of positive cells in anti-Jo-1$^+$ patients differs from that in anti-Jo-1- patients.

† Data generated without gating.

circulating CD8$^+$ cells, and increased proportions of DR$^+$, CD3$^+$ DR$^+$, CD14$^+$ DR$^+$, IL-2R$^+$, CD26$^+$ and TLiSA1$^+$ PBMC compared with controls; (ii) The abnormally high MHC class II antigen and the early and late T cell activation marker expression in these patients decreases in conjunction with decreasing myositis disease activity; and (iii) Significant differences exist in certain PBMC subsets among clinical and autoantibody subgroups of the IIM.

Previous studies have been equivocal in defining peripheral surface lymphocyte markers in myositis patients; some have shown decreased CD8$^+$ cell populations and increased CD4/CD8 ratios (Behan *et al.*, 1983; Behan & Behan, 1984) and some have not (Iyer, Lawton & Fenichel, 1983). Most have agreed upon increased DR expression, but have not defined the specific cell types involved. Our study demonstrates a significant decrease in the proportion of CD8$^+$ cells in myositis patients as a whole (Table 1) and suggests that this decrease is greater in DM than PM or IBM patients (Table 2). This implies that some of the discrepancies in previous studies may be due not only to different methodologies but also to different patient populations. Low proportions of CD8$^+$ cells are commonly identified in similar studies of other presumed autoimmune conditions, but its significance is not clear. We did detect an increased CD4/CD8 ratio in our patients over controls but this difference did not reach significance.

Since there is evidence of active PBMC trafficking to muscle in IIM patients (Miller *et al.*, 1988) and activated lymphocytes in those muscles (Engel & Arahata, 1986), we determined the proportion of PBMC expressing activation markers. As T lymphocytes are stimulated and undergo activation, they begin a predictable series of changes in surface antigen expression. 'Early' T cell activation markers, such as the transferrin receptor and IL-2R (CD25), which appear within 1–2 days on *in vitro* activated T cells, have been found to be at least transiently expressed in the periphery and target organs in many lymphomas and several autoimmune diseases (Greene *et al.*, 1986). Elevated IL-2R$^+$ PBMC in active myositis patients is consistent with the demonstration of soluble IL-2R levels as an indicator of myositis disease activity (Miller *et al.*, 1989b) and suggests a role for IL-2-responsive cells in the pathophysiology of IIM.

The more recently described 'intermediate' or 'late' activation markers, which are expressed 4–7 days after *in vitro* T cell activation, are less well understood. One of these, known as Ta1 (CD26), is a 105-kD surface protein (Fox *et al.*, 1984) which has been shown to be present on PBMC and/or T cells infiltrating tissues in several human diseases (Hafler *et al.*, 1985; Shen *et al.*, 1987; Dienes *et al.*, 1987; Kelly *et al.*, 1987; Williams *et al.*, 1987; Eguchi *et al.*, 1989). The finding that virtually all of the peripheral anamnestic response to recall antigens resides in the CD26$^+$ population (Hafler *et al.*, 1986, 1989) suggests that the increased CD26$^+$ population in myositis patients may represent an increased population of activated cells undergoing anamnestic responses.

Another late activation cell marker is the 'T lineage specific antigen', TLiSA1. This is a 70-kD protein which has been suggested to be a receptor for a differentiation factor important in establishing cytotoxicity, since anti-TLiSA1 antibody can inhibit the development of this function *in vitro* (Burns *et al.*, 1985). As in the case of CD26, in many DM and anti-Jo-1$^+$ patients, the proportion of PBMC expressing TLISA1 was higher than the proportion of PBMC expressing CD3, suggesting that these activation markers may be present on CD3$^-$ cell populations. The increased proportion of PBMC expressing TLiSA1 in myositis patients over controls, the correlation of the expression of TLiSA1 with clinical and biochemical indicators of disease activity, and the decrease in this population of cells in serially studied patients in proportion to myositis activity, all imply that cells undergoing cytotoxic differentiation may play an important role in the pathophysiology of the IIM. The finding that patients with rheumatoid arthritis and systemic lupus erythematosus also have increased proportions of TLiSA1$^+$ PBMC (Tabata *et al.*, 1989) suggests that this may be a phenomenon common to many systemic rheumatic diseases.

VLA-1 is one of a family of cell surface glycoprotein heterodimers which appear 2–3 weeks after initial T cell stimulation and may be involved in cell adhesion functions (Dedhar, Hagg & Gray, 1989). VLA-1 expression *in vivo* occurs on T cells in the lung (Saltini, Hemler & Crystal, 1988) and rheumatoid synovium (Laffon *et al.*, 1989), but has not been described to be increased in expression in the peripheral circulation of any disease, consistent with our results showing no significant difference in the proportion of VLA-1$^+$ circulating cells between myositis patients and controls.

Several lines of evidence suggest that immunopathogenetic mechanisms may be qualitatively or quantitatively different among clinical subgroups of the IIM. The finding of relatively greater numbers of $CD8^+$ $CD11^-$ ($Leu15^-$) T cells surrounding and invading morphologically normal myofibres in PM and IBM patients compared with DM patients, suggests that PM and IBM are diseases primarily mediated by MHC-restricted cytotoxic T cells (Engel & Arahata, 1986). In DM muscle biopsies, however, more $CD20^+$ and $CD4^+$ cells are found, primarily in perivascular locations, suggesting that DM is a more 'humorally mediated' disease than PM or IBM (Engel & Arahata, 1986). This is consistent with studies showing increased deposition of immunoglobulins (Whitaker & Engle, 1972) and the complement membrane attack complex (Kissel, Mendell & Rammohan, 1986) on myofibres in muscle biopsies from DM patients much more frequently than from PM patients. In addition, these same clinical subgroups differ significantly in certain epidemiologic features (Leff *et al.*, 1988), response to therapies, and mortality (Miller *et al.*, 1989a). These findings have interesting parallels with the PBMC data reported in this study. While PM and IBM patients are virtually indistinguishable in all the parameters analysed, DM patients differ significantly from these two (Table 2). DM patients have higher proportions of circulating $CD20^+$ cells, DR^+ cells, and $CD20^+$ DR^+ cells consistent with the concept of more 'humorally mediated' disease in these patients. However, the hypothesis that T cells have a greater role in disease pathogenesis in PM and IBM is consistent with the higher proportions of $CD3^+$, $CD3^+$ DR^+, and $TLiSA1^+$ PBMC in these patients compared with DM patients.

Another means of subsetting IIM patients, which appears to be useful in predicting therapeutic responses and prognosis, is on the basis of autoantibodies. The most commonly defined autoantibody in myositis patients is anti-Jo-1, found in about one-third of IIM patients (Biswas *et al.*, 1987). Anti-Jo-1 antibodies, which are only found in a genetically restricted, clinically distinguished subset of myositis patients (Miller, 1989), also describe a different epidemiologic group of patients (Leff *et al.*, 1988) with relatively poor responses to therapy and high mortality (Miller *et al.*, 1989a). Interestingly, these patients also differ in having lower proportions of circulating $CD3^+$ and $CD4^+$ cells, lower CD4/CD8 ratios, and higher proportions of DR^+ cells expressing CD20, compared with those without these autoantibodies. How these differences relate to the genetic and clinical aspects of these patients remains to be elucidated.

Our data further emphasize that idiopathic myositis is a syndrome of immunologically heterogeneous disorders, but similar in some respects to other chronic inflammatory diseases. Contrasting clinical and autoantibody subgroups can be defined that exhibit characteristic *in vivo* immunologic differences. The further delineation of these subgroups and their related immune profiles may lead to better understanding of the aetiology, pathogenesis, and possible immunotherapy of the idiopathic inflammatory myopathies.

ACKNOWLEDGMENTS

We wish to thank Drs Richard Leff, David Fraser and Mary Cronin for their clinical assistance in the completion of this study.

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