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F W Miller, … , T Biswas, P H Plotz

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Research Article

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Origin and Regulation of a Disease-specific Autoantibody Response

Antigenic Epitopes, Spectrotype Stability, and Isotype Restriction of Anti-Jo-1 Autoantibodies

Frederick W. Miller, Sharon A. Twitty, Tapas Biswas, and Paul H. Plotz
Arthritis and Rheumatism Branch, National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institutes of Health, Bethesda, Maryland 20892

Abstract

Anti-Jo-1 antibodies (AJoA), which bind to and inhibit the activity of histidyl-transfer RNA synthetase (HRS), are found in a genetically and clinically distinct subset of myositis patients. This specificity suggests that understanding the antigenic epitopes and immunoregulation governing the production of AJoA may result in clues to disease pathogenesis. Limited digestion of human HRS by V8 protease resulted in four major antigenic polypeptides of 35, 34, 21, and 20 kD; digestion with subtilisin gave four fragments of the same sizes and two additional major antigenic polypeptides of 28 and 17 kD. Sera from 12 AJoA positive patients reacted indistinguishably with these proteolytic fragments by Western blotting, and AJoA elution studies suggested a common epitope(s) on all six. Isoelectric focusing showed a different polyclonal pattern of AJoA in each patient, although serial analyses in individual patients revealed stable AJoA spectrotypes over years of observation. Enzyme-linked immunosorbant assays showed that the AJoA response was mainly restricted to the IgG_1 heavy chain isotype. The levels of IgG_1 AJoA varied in proportion to disease activity over time but were independent of total IgG_1 levels, and three patients became AJoA negative as their myositis remitted after treatment. These findings suggest that AJoA are induced by an antigen-driven mechanism, bind to a common epitope or epitopes on HRS, and are modulated by an immune response closely linked to that which is responsible for myositis in these patients. (*J. Clin. Invest.* 1990. 85:468–475.) anti-histidyl-tRNA synthetase • autoantibody subclass • dermatomyositis • immunomodulation • polymyositis

Introduction

Unique autoantibodies are characteristic of a subset of patients with idiopathic inflammatory myopathy. These autoantibodies bind to cytoplasmic elements involved in protein synthesis and are directed against evolutionarily conserved regions critical to the recognized function of the target autoantigen. The known autoantigens include at least five aminoacyl-transfer RNA (tRNA)[1] synthetases (1–4), $tRNA^{ALA}$ (3, 5) several unidentified tRNAs (6), elongation factors (6–8), and the signal recognition particle (9). The autoantigen to which myositis autoantibodies are most frequently directed is histidyl-tRNA synthetase, also known as Jo-1 antigen, which catalyzes the esterification of histidine to its cognate tRNA (1). Patients who develop anti-Jo-1 antibodies (AJoA) are a genetically restricted group (10) with a high incidence of interstitial lung disease, arthritis, and Raynaud's phenomenon (11). AJoA are seen in ~ 33% of patients with primary polymyositis, 25% with primary dermatomyositis, and 15% with myositis associated with another connective tissue disease, and have not been detected in other subgroups of patients with idiopathic inflammatory myopathy (12). Patients with AJoA also tend to be younger with an acute onset of myositis in the first half of the year (13).

The reasons for the remarkable association of these autoantibodies with certain forms of myositis are unknown. It has been suggested, however, that they might arise as a result of the interaction of translational components with infecting positive-stranded RNA viruses, notably picornaviruses (1), which are also associated with myositis through ultrastructural (14), serological (15, 16), animal model (17–19), and complementary nucleic acid hybridization studies (20).

The role of AJoA in the pathogenesis of inflammatory myopathy remains speculative, yet the high specificity of these autoantibodies for the diagnosis of myositis and the closely associated clinical features and human leukocyte antigen markers suggest that the immunoregulation of AJoA is closely linked to genetic and/or environmental factors responsible for the development of muscle inflammation.

In order to understand the in vivo induction and regulation of the production of these unique autoantibodies, we have studied their antigenic epitopes, spectrotypes, class and subclass distribution, as well as the variation in their levels, in a population of well-characterized myositis patients followed over a long period of time. Our data show that AJoA from all patients appear to react with a common epitope or epitopes on HRS. Additionally, we found stable polyclonality of the AJoA response, in spite of its marked isotype restriction to IgG_1, and correlation of AJoA levels with myositis disease activity.

Methods

Sera. All myositis sera were obtained from patients with definite polymyositis or dermatomyositis (21) and were stored at −20°C before use. AJoA positive sera were initially identified by double immunodiffusion assays using prototype AJoA positive sera (1) and were confirmed by inhibition of histidyl-tRNA synthetase activity (12). Control sera were collected from blood donors without known disease.

Clinical evaluations. The relative disease activity of myositis patients being evaluated for or entered into treatment protocols was prospectively assessed by their treating physicians, without knowledge of AJoA levels, using a subjective global activity index (AI), where 0 = no disease activity, 1 = mild, 2 = moderate, 3 = severe, and 4 = extremely severe disease activity. These assessments were based upon both objective and subjective data from physical examinations,

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Address reprint requests to Dr. Miller, NIH Building 10, Room, 9N228, Bethesda, MD 20892.

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1. *Abbreviations used in this paper:* AJoA, anti-Jo-1 autoantibodies; GBBS, 10% goat serum in borate-buffered saline; HRS, histidyl-tRNA synthetase; PBST, 0.05% Tween 20 in PBS.

including formal manual muscle strength testing, activity of daily living questionnaires, laboratory studies, and patients' reports.

Protease digestions. *Staphylococcus aureus* V8 protease and subtilisin (Boehringer-Mannheim Biochemicals, Indianapolis, IN) were incubated with biochemically isolated human histidyl-tRNA synthetase (HRS) (22) in the manner of Cleveland et al. (23). Limited proteolysis was achieved by boiling 500 μl of HRS (50 μg/ml) in 0.15 M potassium phosphate, 10 mM $MgCl_2$, 5 mM DTT, pH 7.5, with 11 μl of 20% SDS for 2 min, then adding 10 μl of protease at 0.1 μg/μl and incubating at 37°C for 20 min. Then 200 μl of 6× sample buffer was added to the mixture and the mixture was boiled for 2 min, after which 25-μl aliquots were loaded per lane and electrophoresed into 15% acrylamide gels along with undigested HRS and molecular weight standards (Bio-Rad Laboratories, Richmond, CA) (23). Samples were transferred to nitrocellulose sheets (22), which were then blocked with 10% goat serum in borate-buffered saline (GBBS), and incubated with patient serum samples diluted 1:10 in GBBS overnight at 4°C. The sheets were washed with PBS containing 0.05% Tween 20 (PBST), and then incubated with peroxidase-conjugated affinity-purified goat anti-human IgG (Jackson Immunoresearch Laboratories, Avondale, PA) and developed as previously described (22).

To assess the relationship and complexity of epitopes on the major proteolytic fragments of HRS, we eluted AJoA from the major bands and reprobed other nitrocellulose strips containing all the proteolytic polypeptides. For these experiments, proteolytic fragments of HRS were localized on nitrocellulose sheets using AJoA positive serum and affinity-purified ^{125}I-protein A (Amersham Corp., Arlington Heights, IL) following the manufacturer's specifications. The bands of interest were cut out and AJoA were eluted by washing twice, 10 min each, with a buffer containing 500 mM NaCl, 0.5% Tween 20, 100 μg/ml immunoglobulin-free bovine serum albumin (BSA) (Sigma Chemical Co., St. Louis, MO), 50 mM glycine, pH 2.3. Eluted AJoA were neutralized in 50 mM $NaHPO_4$, pH 7.5, and applied to selected nitrocellulose strips containing proteolytic fragments of HRS that had been previously blocked with BSA. The strips were washed in PBST, incubated with a 1:1,000 dilution of ^{125}I-protein A in 2% BSA for 2 h at room temperature, rewashed, and then processed for autoradiography.

Spectrotype analysis of AJoA. Isoelectric focusing and nitrocellulose blotting was performed by the method of Knisley and Rodkey (24) using precast gels (pH 3.5–9.5; Pharmacia LKB Biotechnology, Piscataway, NJ).

Sera were diluted 1:5 to 1:25, depending on the AJoA concentration, with 0.015 M borate-buffered saline containing 10% sucrose, 0.5% Tween 20, and 2% ampholines (pH 3.5–9.5; Pharmacia LKB Biotechnology) and 10 μl was applied to LKB sample template strips placed near the anode. Isoelectric focusing was performed on 10 cm × 10 cm gels with cooling (4°C) at 100 V for 15 min, 200 V for 45 min, 5 W for 6 h, and then 15 W until the rate of voltage increase doubled (usually 1–2 h). The adequacy of focusing was determined by monitoring both prestained marker (LKB) migration and changes in voltage over time. When the focusing was complete, the pH gradient was confirmed by a surface microelectrode. Focused antibodies were passively transferred to nitrocellulose sheets, which had been previously wetted with water, incubated with 1 μg/ml of HRS (22) in 0.5 M sodium bicarbonate overnight at 4°C, and blocked with 1% BSA in PBST. The nitrocellulose sheets were carefully layered onto the gel and incubated in a humid environment for 15 min at 37°C (24). After washing in PBST, the nitrocellulose sheets were exposed to ^{125}I-labeled protein A at a 1:1,000 dilution in PBS/BSA. The sheets were washed in PBST and antibody bands were located by autoradiography.

ELISA determinations. Total IgG subclass determinations were performed using the Human IgG Subclass Monoclonal ELISA Kit (ICN Immunobiologicals, Lisle, IL) following the manufacturer's instructions. Purified monoclonals used in this kit are: SG-11 (anti-IgG_1); HP-6014 and SH-21 (anti-IgG_2); HP-6050 and SJ-33 (anti-IgG_3); and HP-6025 (anti-IgG_4) (25). Total IgA and IgM levels were quantitated similarly using affinity-purified alkaline phosphatase-labeled goat IgG anti-human IgA (ICN code 61-617) or IgM (ICN code 61-666). Affinity-purified human IgA or IgM (ICN Immunobiologicals) were used as standards in the ELISA. Extensive preliminary ELISA experimentation was performed using myeloma proteins supplied by Dr. William Yount (University of North Carolina, Chapel Hill, NC) to confirm the specificity of the assays. Cross-reactivity to unrelated immunoglobulin classes and subclasses was determined to be < 1.5% in all assays (26, and data not shown).

Class and subclass AJoA quantitations were accomplished using a modification of an AJoA-specific ELISA (12) with human HRS as antigen (22). IgA and IgM AJoA determinations were performed by incubating microtiter wells (Immulon 1 plates; Dynatech Laboratories, Alexandria, VA) with 100 μl of antigen at 2.5 μg/ml overnight at 4°C. Wells into which standards were to be placed remained empty. All other steps were carried out at room temperature and, except where noted, 100 μl of reagent or sample was used. The next day the plates were washed with PBST and each well, except those into which standards were to be placed, was blocked with 200 μl of 1% BSA in PBS (PBS/BSA) for 1 h. After washing the plates in PBST, human IgA or IgM standards, or AJoA positive sera in appropriate dilutions in PBS/BSA, were added in duplicate to the plates and incubated for 2 h. The plates were washed again with PBST, followed by distilled water, and alkaline phosphatase labeled goat anti-human class- or subclass-specific antibodies, diluted 1:1,000 in PBS/BSA, were added to the plates. After incubation for 2 h, plates were washed with PBST and then distilled water, developed using a phosphatase substrate system (Kirkegaard and Perry Laboratories Inc., Gaithersburg, MD), and read at 410 nm at appropriate time points using a microplate reader (Dynatech, model MR 700). The data were analyzed by the Immunosoft program (Dynatech). Correlation coefficients of the standard curves exceeded 0.97 for each assay and data was calculated only from samples from which absorbance readings fell within the linear portion of the standard curves.

IgG subclass AJoA determinations were performed similarly, using the human IgG subclass monoclonal ELISA kit (ICN Immunobiologicals) according to the manufacturer's instructions except for the substitution of antigen for coating antibody as the first step.

Protein sequence analysis. Amino acid sequence analysis of human HRS (27) was performed using the programs ANTIGEN and FLEXPRO and comparisons of regions for homology were performed using the default parameters of FSTPSCAN (28) on PCGENE (IntelliGenetics, Mountain View, CA).

Data analysis. Statistical analysis of data was performed using SAS (SAS Institute Inc., Cary, NC) on an IBM 370 computer. Grouped data were compared by Wilcoxon rank sums and Spearman correlation coefficients were determined using Bonferroni corrections for multiple comparisons (29).

Results

Autoantibodies of a given specificity are characteristically produced by a diversity of lymphocyte clones, yet they often target a common site on an autoantigen molecule. We performed a series of experiments, therefore, to determine the heterogeneity of antigenic epitopes seen by AJoA as well as the complexity and stability of different patients' B cell responses to HRS.

Epitope analyses. When probed with AJoA positive serum by Western blotting, undigested HRS showed two antigenic proteins with apparent molecular sizes of 110 and 55 kD, the former presumably representing undissociated HRS homodimers as previously described (22). Limited proteolysis of HRS by *S. aureus* V8 protease reproducibly produced one major, relatively protease resistant, antigenic polypeptide of 35 kD, and three minor polypeptides of 34, 21, and 20 kD. The pattern observed usually included minor bands of antigenicity trailing the 35-kD fragment and overlapping the 34-kD fragment (Fig. 1 *A*). Digestion of HRS by subtilisin gave four

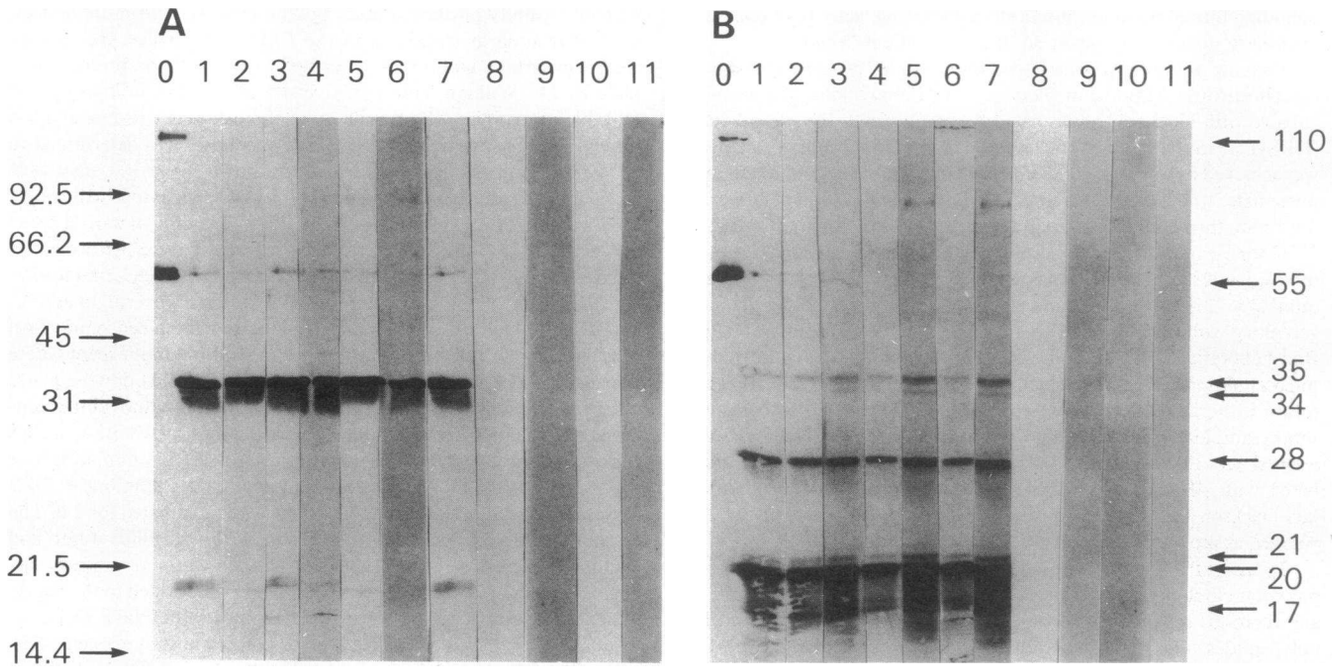

Figure 1. Immunoblot of biochemically isolated Hela cell HRS before (0) and after (1-11) partial protease digestion by *S. aureus* V8 protease (*A*) and subtilisin (*B*). The positions of molecular weight markers (phosphorylase B 92.5 kD, BSA 66.2 kD, ovalbumin 45 kD, carbonic anhydrase 31 kD, soybean trypsin inhibitor 21.5 kD, and lysozyme 14.4 kD) are given by arrows at the left. The positions and apparent molecular weights in kilodaltons of undigested HRS and the six major antigenic fragments of HRS are given at the right. All major antigenic fragments reacted indistinguishably with sera from 12 different AJoA-positive patients (seven are shown in lanes *1–7*). No reaction was seen after incubation with serum from myositis patients containing anti-ribonucleoprotein (8) or anti-La antibodies (9), or with myositis patient serum without antibodies to extractable nuclear antigens (10), and normal control serum (11).

major antigenic fragments of the same sizes and two additional major polypeptides of 28 and 17 kD, with minor bands between these two polypeptides (Fig. 1 *B*). Other minor bands, possibly the result of varying degrees of proteolysis from sample to sample, were occasionally observed after protease digestion. Prolonged digestion with increased amounts of protease resulted in the complete loss of all antigenic polypeptides. Sera from all 12 AJoA positive patients tested reacted indistinguishably with the major proteolytic fragments of HRS in each experiment, and no sera from normal controls nor from patients without AJoA recognized any of these polypeptides (Fig. 1).

In order to assess the relationship and complexity of epitopes on each of the proteolytic fragments, AJoA were eluted from the major polypeptides above and were used to reprobe nitrocellulose strips onto which all proteolytic fragments were blotted. Fig. 2 shows representative results from such an experiment and depicts the common binding of AJoA to all major polypeptides regardless of which fragment was used to elute AJoA. Similar data were obtained from such reprobing experiments after V8 protease digestion of HRS and from two other AJoA positive patients tested.

In the simplest view, these data suggest that the smaller antigenic polypeptides are generated from the larger ones, that a common epitope or epitopes are present on all the polypeptides, and that AJoA from all positive patients react with this (these) same epitope(s). Analysis of the linear amino acid sequence of human HRS was performed using the PCGENE package to determine whether there are repeated sequences on HRS that could serve as multiple antigenic epitopes. The analysis did not reveal any duplicated regions of greater than four amino acids in length and no regions that were predicted to be similar enough to be related antigenically. We cannot exclude the unlikely possibility, however, that several conformational epitopes exist on the protein that all AJoA see identically.

Spectrotype analysis of AJoA. To determine the complexity of the AJoA response, we analyzed AJoA spectrotypes by isoelectric focusing sera and allowing AJoA to transfer passively to nitrocellulose sheets previously coated with antigen. All 12 AJoA positive patients' sera studied during active myositis gave positive patterns, whereas normal sera and myositis sera without AJoA gave no detectable bands by this sensitive and specific method (Fig. 3). Although discrete AJoA bands were often present, all spectrotypes showed a diffuse polyclonal pattern, with each serum having an apparently distinct spectrotype. Fig. 3 shows representative serial spectrotypes from four patients who maintained an AJoA response over the period of observation and from one who became AJoA negative. Although individual bands varied in intensity from sample to sample, when antibody was present, the pattern was qualitatively stable over time.

Isotype analysis of total immunoglobulins and AJoA. Because autoantibodies tend to show isotype restrictions, we investigated the class and subclass of AJoA and total immunoglobulins to learn if a pattern was present in our patients. ELISA assays showed that in each of 12 AJoA positive patients studied during periods of active myositis, the most abundant AJoA isotype was IgG_1, representing on average 94% of the total AJoA (Table I). IgG_1 AJoA represented up to 3.7% of the total IgG_1 in one patient (data not shown), although in most

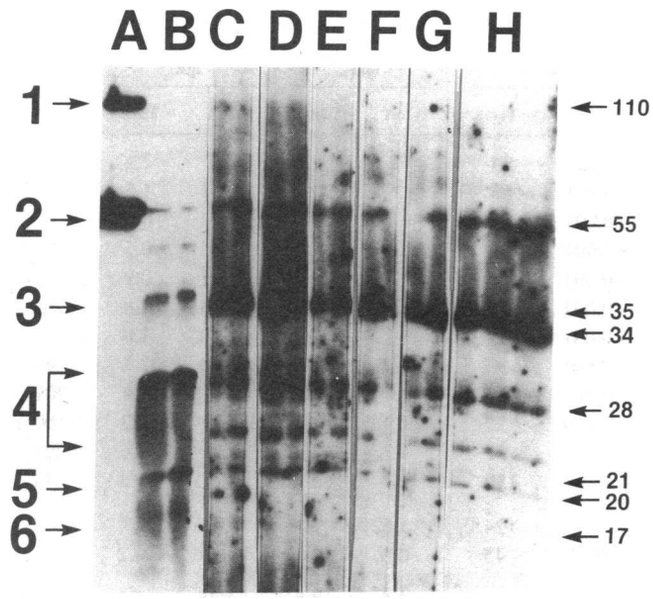

Figure 2. An autoradiograph of a representative immunoblot before (*A*) and after (*B–H*) limited digestion of HRS by subtilisin demonstrating that the AJoA binding to intact HRS and to the major antigenic polypeptides (with the position and apparent molecular weight in kilodaltons labeled at the right) can be eluted and will rebind to the other major antigenic polypeptides. A piece of nitrocellulose, on which AJoA from patient 1 were localized by ^{125}I-protein A and prior autoradiography (*A–B*), was cut into six regions labeled by numbers at the left. AJoA were eluted separately from each of these regions, neutralized and exposed to undeveloped nitrocellulose strips (labeled at the top with a letter corresponding to the region from which AJoA were eluted such that C = 1, D = 2, E = 3, F = 4, G = 5, and H = 6). The smaller HRS antigenic fragments, not well visualized after photographic reproduction, were clearly present on autoradiographs exposed for longer periods of time. Similar data were obtained after repeating this experiment with two other AJoA positive patients and after limited digestion of HRS with *S. aureus* V8 protease (data not shown).

cases the proportion was less than 1% (Table I). 7 of the 12 patients had detectable IgG_3 AJoA, and these seven also had significantly higher IgG_1 AJoA levels (mean IgG_1 AJoA = 87 μg/ml) than those from the five patients without detectable IgG_3 AJoA (mean IgG_1 AJoA = 23 μg/ml). No patient had detectable IgG_4 AJoA, despite analysis of sera from all patients at multiple time points. Only one patient had detectable IgG_2 AJoA, and only two patients had IgA AJoA. 11 patients had IgM AJoA at one or more time points. To interpret the AJoA levels of a given subclass within the context of the relative abundance of total immunoglobulins in that subclass, we calculated an AJoA index (Table I). These calculations showed that AJoA were significantly ($P < 0.03$) overrepresented by IgG_1 relative to all other classes and subclasses.

Regression analyses of all patient data showed that there were significant ($P < 0.001$ for each) correlations between: total AJoA and IgG_1 AJoA, $r = 0.99$; IgG_1 AJoA and IgG_3 AJoA, $r = 0.96$; total IgG_1 and total IgA, $r = 0.89$; and total IgG_1 and total IgG_3, $r = 0.91$. No other significant correlations were found. Given the high specificity of our ELISA assays, these data suggest the possibility of coordinate regulation of several isotypes of both total immunoglobulins and AJoA.

Changes in AJoA levels over time. The analysis of AJoA levels in sera from the 12 patients, who have been followed for periods of 2–13 yr, showed changing IgG_1 AJoA levels in proportion to changes in myositis disease activity but unrelated to total IgG_1 levels. Fig. 4 depicts representative data from four patients and demonstrates the major patterns observed. AJoA levels became undetectable by double immunodiffusion and ELISA assays in 3 of the 12 patients (patients 2, 4, and 10) whose therapy induced the most complete and prolonged remissions of myositis.

Further evidence that IgG_1 AJoA levels were proportional to disease activity comes from the analysis of four patients for whom more detailed data were available. During periods of less disease activity, these patients (with clinical activity indices of 0–2) had significantly ($P < 0.04$ for each) lower IgG_1

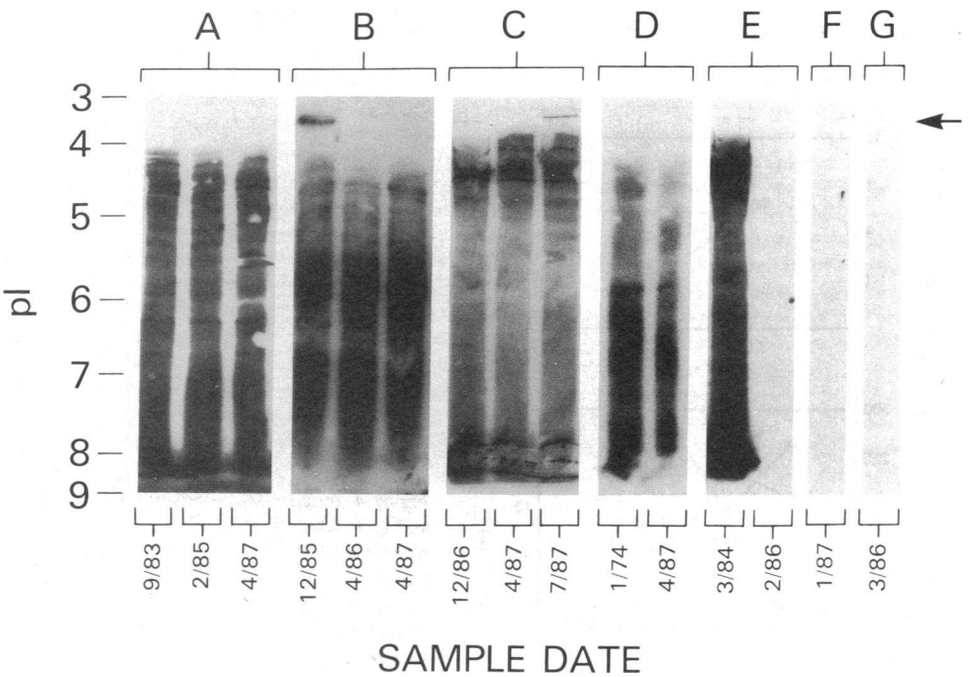

Figure 3. Autoradiography demonstrating spectrotypes of AJoA detected after isoelectric focusing of serum samples, passive blotting to nitrocellulose-immobilized HRS, and probing with ^{125}I-labeled protein A. Data are presented from patients 1 (*A*), 11 (*B*), 9 (*C*), 10 (*D*), and 4 (*E*) and shows the distinct pattern of AJoA from each patient and the spectrotype stability in those patients who sustained an AJoA response (patient 4 became AJoA negative by 2/86 [*E*]). Sera samples from myositis patients without AJoA (*F*) and normal controls (*G*) containing similar concentrations of IgG gave no detectable bands. The area of sample application is shown by the arrow at the right, the approximate pI is indicated at the left and the numbers at the bottom indicate the month/year of serum collection.

Table I. Isotype Distribution of Total Immunoglobulins and Anti-Jo-1 Antibodies (AJoA) from 12 Patients during Active Myositis

	IgG1		IgG2		IgG3		IgG4		IgM		IgA		
Patient	AJoA*	Total*	AJoA	Total	AJoA	Total	AJoA	Total	AJoA	Total	AJoA	Total	AJoA sum
1	92	25	0.2	3	7	2	<0.001	94	1.2	1.3	0.7	2	101
2	6	11	<0.03	5	0.5	0.7	<0.001	65	1.6	2.5	<0.01	1.6	8.1
3	12	10	<0.03	1	<0.01	0.6	<0.001	98	1.8	2.2	<0.01	0.4	13.8
4	7	6	<0.03	1	<0.01	0.5	<0.001	40	3.8	0.7	<0.01	0.6	10.8
5	23	13	<0.03	1	0.06	2	<0.001	150	1.6	0.9	<0.01	0.9	25
6	52	25	<0.03	0.3	<0.01	1.7	<0.001	27	1.7	4.8	<0.01	1.8	54
7	6	4	<0.03	2.2	<0.01	0.7	<0.001	83	1.1	0.6	<0.01	1.0	7
8	39	53	<0.03	0.9	<0.01	6.3	<0.001	50	<0.01	1.2	<0.01	3.2	40
9	198	19	<0.03	2.9	10	0.5	<0.001	602	1.1	1.8	0.03	1.4	209
10	56	32	<0.03	0.5	2	2.4	<0.001	55	1.9	5.3	<0.01	2.2	60
11	213	15	<0.03	0.2	9	0.9	<0.001	95	1.7	4.1	<0.01	1.6	224
12	21	13	<0.03	1.2	2	0.4	<0.001	130	1.3	2.1	<0.01	0.4	24
Mean ±	60	19	0.017	1.6	2.5	1.6	0	124	1.6	2.3	0.06	1.4	(64)
SEM	21	4	0.017	0.4	1.1	0.5	0	45	0.3	0.5	0.06	0.2	(26)
% of sum‡	93.5	73	0.026	6.1	3.9	6.1	0	0.5	2.5	8.8	0.09	5.3	(100)
AJoA index§	1.28		0.004		0.64		0		0.28		0.017		

* AJoA values are μg/ml; total Ig values are mg/ml except IgG4 total values which are μg/ml. ‡ Sum of all classes and subclasses shown.
§ AJoA index, (AJoA % of sum)/(Total Ig % of sum), for each isotype.

AJoA (mean 48 μg/ml) and serum creatine kinase (CK) levels (mean 902 U/liter) than during periods of increased disease activity (with activity indices of 3–4, mean IgG_1 AJoA = 123 μg/ml, mean CK = 2,657 U/liter). Analyses of concurrent CK levels, myositis activity indices and IgG_1 AJoA levels showed significant correlations among all three (Fig. 5).

Discussion

The specificity with which AJoA, which are directed against the very sparse and unstable enzyme HRS (30), are associated with a genetically restricted, clinically distinguished subgroup of myositis patients is remarkable. The origin of AJoA antibodies has been the subject of a number of hypotheses, all of which revolve around three "antigen-driven" scenarios. One is that AJoA result from the direct interaction of the enzyme with picornaviral RNA, thereby rendering it "foreign" to the host immune system (1). A second suggests an antiidiotype network is responsible for AJoA, with the portion of viral RNA which interacts with enzyme being the primary immunogen (31). A third hypothesis relates to "molecular mimicry" suggesting that picornaviral proteins may serve as the primary immunogen and that regions homologous to the viral proteins are present on HRS (32).

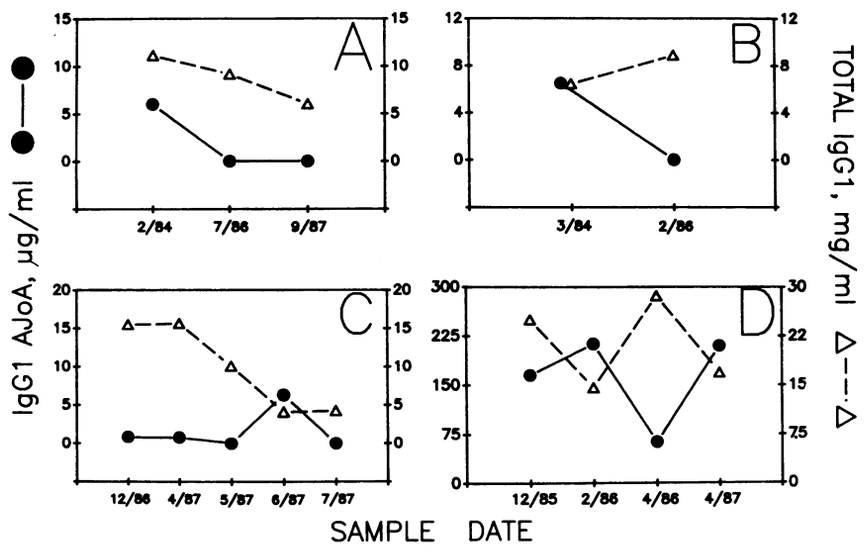

Figure 4. Independent regulation of total IgG_1 and IgG_1 AJoA levels over time as determined by enzyme-linked immunosorbant assays. Data from patients 2 (*A*), 4 (*B*), 7 (*C*), and 11 (*D*) are presented. The numbers at the bottom of each panel indicate the month/year of serum collection.

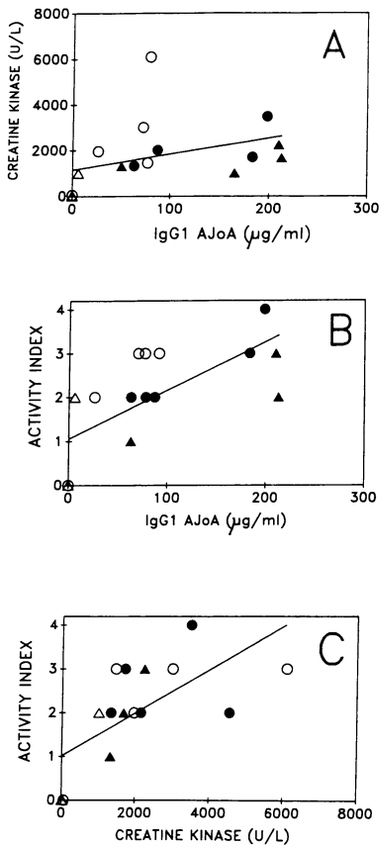

Figure 5. Comparisons of four patients' serial data (patient 1 (○), 9 (●), 2 (△), and 11 (▲)) showing significant ($P < 0.001$ for each) correlations between: (*A*) serum creatine kinase (CK) and IgG_1 AJoA ($r = 0.80$); (*B*) clinical myositis activity indices (AI) and IgG_1 AJoA ($r = 0.71$); and (*C*) CK and AI ($r = 0.78$).

Our data are consistent with the underlying premise of these hypotheses regarding the role of antigen in the induction and regulation of AJoA. That AJoA are probably antigen-driven is supported by the serial appearance of clonally restricted IgM AJoA before IgG_1 and IgG_3 AJoA, all occurring months before myositis onset in a patient observed through the development of myositis (33), and the complexity and polyclonality of the AJoA immune response as shown by their class and subclass distribution and diffuse spectrotypes in this paper. The antigen-driven nature of AJoA and other autoantibody systems seems inconsistent with hypotheses suggesting that autoimmunity results from generalized (polyclonal) B cell activation (34, 35), which also occurs in many circumstances not leading to autoimmune disease. Furthermore, generalized B cell stimulation alone would not seem sufficient to explain: (*a*) the phenomenon of disease-subset specific autoantibodies; (*b*) the frequent finding of subclass-restricted autoantibodies (36); (*c*) the fact that one does not find autoantibodies to all proteins indiscriminately in autoimmune diseases (37) (in the idiopathic inflammatory myopathies they are directed against a group of cytoplasmic proteins which are often functionally related); (*d*) the tendency for autoantibodies to target evolutionarily conserved epitopes and to inhibit the function of autoantigens (38); and (*e*) the independent regulation of multiple autoantibodies in the same individual (39, 40). It is even possible that the apparent polyclonal activation of immunoglobulin-producing cells and the hypergammaglobulinemia observed in some autoimmune conditions represents the result rather than the cause of autoimmunity, perhaps secondary to antigen stimulation and T cell activation (41).

The lack of correlation of AJoA levels with total immunoglobulin levels of any class or subclass suggests that their regulation is specifically mediated by factors that appear to differ in quantity or quality from those which regulate other immunoglobulins. Therefore, the selective disappearance of AJoA with therapy, without a significant decrease in total immunoglobulin levels, reflects this specific immune regulation and not a more general immunosuppressive effect.

While each patient has a distinguishable AJoA spectrotype, presumably resulting from unique genetic and/or environmental factors, the AJoA spectrotype profile within an individual can remain remarkably stable for many years. Although the resolution of the technique cannot exclude the possibility of minor AJoA spectrotype changes over time, this finding is similar to the spectrotype stability of thyroglobulin autoantibodies, which also appear to have idiotypic stability as well (42). These data in autoantibody systems parallel the pattern observed in standard secondary immune responses to antigens, in which an initial rapid diversification of B cell clones is followed by the maintenance of stable polyclonality (43–45). The persistence of high titer polyclonal AJoA responses and stable spectrotypes are therefore consistent with an antigen-driven response and persistence of antigen (46).

Differences in methodology and patient populations make an overview of the scant, and sometimes conflicting, published data on autoantibody isotype distributions difficult. The isotype restriction of AJoA primarily to IgG_1, with small contributions by IgG_3 and IgM, is similar to that reported for a number of other systems. In systemic lupus erythematosus the marker antibodies anti-Sm, anti-ribonucleoprotein, and anti-double-stranded DNA antibodies (47) as well as anti-nuclear antibodies in general (48), anti-acetylcholine receptor (49), anti-La (50), and anti-Ro (51) antibodies have been reported to be similar to AJoA in this respect. The relative isotype restrictions in these diverse autoantibody systems to IgG_1 and IgG_3 suggest that T cells are important in their production and immunoregulation and that this may be a general phenomenon in many human autoimmune diseases (52).

In other autoantibody systems, however, such as rheumatoid factors (53), drug-induced anti-histone (52), anti-thyroid (54), and anti-skin (55) autoantibodies, there appears to be a predominance of IgG_4 isotypes; while in others, such as anti-thyroglobulin (56), anti-ribosomal P (57), and anti-phosphocholine (58) autoantibodies, IgG_2 represents a prominent subclass.

The marked changes in the quantity of AJoA over time appear to reflect changes in myositis disease activity inasmuch as they correlate significantly with serum CK levels and disease activity as judged by the patients' treating physicians. Although these data do not support a pathogenetic role for AJoA, they do suggest that AJoA regulation is closely linked to factors responsible for the disease process in these patients. Many lines of evidence imply that autoimmune phenomena are important as pathologic mechanisms in the idiopathic inflammatory myopathies (59), and that T cell responses are particularly relevant to disease (60, 61). If T cells and/or their lymphokines are responsible for modulating both AJoA responses and muscle inflammation, then one might expect to find correlations between the two as a result of interventions known to affect T cells. In fact, all the patients who clinically improved after therapy showed decreases in AJoA levels, and the three patients who experienced prolonged complete remissions, in-

duced by oral prednisone and methotrexate or chlorambucil therapy, were the three whose AJoA became undetectable by both ELISA and double immunodiffusion assays. Of these three patients, two have had all medications discontinued and have remained clinically well with no AJoA for up to 4 yr. The finding that patients can change AJoA status from positive to negative as reported here and vice versa (33) also has implications for the appropriate timing of screening for AJoA, which would appear to be best performed during a period of active myositis and before therapy.

Further understanding of the factors responsible for AJoA may be obtained from knowledge of the precise antigenic epitopes of HRS. The antigenic polypeptide data presented here, the observation that all AJoA inhibit HRS enzyme activity (12, 62), and the finding that AJoA do not bind to heat-denatured HRS (unpublished observations) nor to any hexapeptide of the linear protein sequence (63), taken together imply that the epitopes are conformational and limited to a small number of functionally critical sites on the molecule. Studies are underway to identify these sites as a further step to understanding the induction of AJoA and idiopathic myositis.

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