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Research Article

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Suppression of Anti-DNA Antibody Synthesis In Vitro by a Cross-reactive Antiidiotypic Antibody

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Abstract

An understanding of the regulatory mechanisms that govern the humoral immune response will be facilitated by the availability of techniques for the measurement of specific antibody production in vitro. We have developed an enzyme-linked immunosorbent assay system for the measurement of in vitro anti-DNA antibody production by peripheral blood mononuclear cells (PBMC) from patients with systemic lupus erythematosus. Using this technique, the PBMC from 74% of serologically active SLE patients produced levels of anti-DNA antibodies that were > 2 SD above the mean of 18 ± 9 IU/ml for normal subjects. Furthermore, the addition of 3I, a monoclonal anti-idiotypic antibody that recognizes a cross-reactive determinant on anti-DNA antibodies, was shown to specifically inhibit anti-DNA production in vitro.

Introduction

The concept that interactions between idiotypic and antiidiotypic play a role in the regulation of the immune response was initially proposed by Jerne (1). Since his original hypothesis was put forward antiidiotypes have been shown in many experimental animal systems to regulate the expression of idiotypic antibodies. Studies on human cells likewise have shown that antiidiotypic can inhibit the in vitro production of idiotypic antibodies (2, 3).

To investigate what regulatory mechanisms might govern the humoral immune response in patients with systemic lupus erythematosus (SLE) it is necessary to be able to measure the synthesis of specific autoantibodies. We have adapted an enzyme-linked immunosorbent assay (ELISA) system originally developed to measure anti-DNA antibodies in serum for the measurement of anti-DNA antibodies spontaneously produced by cultured peripheral blood mononuclear cells (PBMC)¹ obtained from patients with SLE. Using a cross-reactive mouse monoclonal antiidiotypic to human anti-DNA antibodies (4) we have

examined the effect of the antiidiotypic on the synthesis of anti-DNA antibodies in vitro.

Methods

Patients. All patients with SLE met at least four American Rheumatism Association criteria for the diagnosis of SLE (5). All but one patient had serologically active SLE, and most had clinically active disease as well. The majority of these patients were receiving prednisone at the time of study, and several were receiving azathioprine in addition. The control group were healthy subjects without evidence of SLE or other rheumatic disorder and were not taking any drug known to induce a positive antinuclear antibodies.

Assay for serum anti-DNA antibodies. Serum anti-double-stranded DNA (ds-DNA) antibodies were measured by an ELISA system marketed as Cordia-N by Cordis Laboratories, Miami, FL. Plastic disks containing isothiocyanate groups coupled to ds-DNA by incubation in 0.1 M carbonate buffer at pH 9.6 followed by extensive washing were incubated with 5 μ l of test serum diluted in 0.5 ml of 0.01 M phosphate-buffered saline, pH 7.4, containing 6% bovine serum albumin and 0.05% Tween 20 (ICI United States, Inc., Wilmington, DE) for 45 min at 37°C with shaking. Each disk was then washed five times with 0.002 M Tris-saline, pH 7.8. A mixture of antigen-purified, alkaline phosphatase-labeled goat anti-human IgG and IgM antibodies was added for 45 min at 37°C. The disks were again washed five times, transferred to clean, unused vials, and incubated with 1.0 ml of the *p*-nitrophenyl phosphate (PNPP) substrate for 20 min at 37°C. The reaction was terminated by the addition of 0.1 ml of 3 N NaOH and the absorbance at 405 nm was measured against a distilled water blank. The characteristics of this assay system have been described (6).

Assay for in vitro anti-DNA production. 40 ml of blood were obtained from normal subjects and from patients with SLE. PBMC were isolated by standard methods of centrifugation over Ficoll-Hypaque, followed by several washes with HB-101 medium (Hana Media, Berkeley, CA), a serum-free lymphocyte growth medium containing 1 mg/ml of protein. The PBMC were incubated in HB-101 for 4 h to remove any adsorbed immunoglobulin, then centrifuged, resuspended in HB-101, and incubated at a concentration of 2.5×10^6 PBMC/ml for 60 h on plastic disks containing covalently bonded ds-DNA (Cordis Laboratories) in an atmosphere of 6% CO₂ at 37°C.

After incubation, anti-DNA antibody bound to the disks was measured in the same ELISA system described above for measuring anti-DNA antibodies. Briefly, the PBMC were washed off the disks using a washing device that insures vigorous washing with Tris-buffered saline followed by vacuum aspiration. The disks were then incubated for 45 min at 37°C with a goat antiserum to human IgG and IgM conjugated with calf alkaline phosphatase (Cordis Laboratories). After six washes the disks were incubated for 45 min at 37°C with PNPP (Cordis Laboratories). The enzyme activity was terminated by the addition of sodium hydroxide and the absorbance was measured at 405 nm. For each assay a standard curve was constructed using serial dilutions of a positive control serum, and the absorbance was converted to international units per milliliter.

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1. Abbreviations used in this paper: ds-DNA, double-stranded DNA; PBMC, peripheral blood mononuclear cells; PNPP, *p*-nitrophenyl phosphate.

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To determine the amount of 3I-reactive anti-DNA antibody synthesized in vitro, PBMC were incubated as described on disks coated with ds-DNA. The disks were incubated with the 3I antiidiotype or with an isotype matched murine myeloma protein, followed by an affinity-purified alkaline phosphatase-linked goat anti-mouse IgG H+L +IgM (human serum absorbed) antiserum (Kirkegaard & Perry Laboratories, Gaithersburg, MD) and PNPP.

Assay for the 3I idiotype in human sera. Sera were diluted 1:1,000 in Pi/NaCl and absorbed to polystyrene wells. 3I culture supernatant or a dilution of 3I ascites was added. After incubation, the plates were washed and incubated with (35 S) methionine-labeled rat anti-mouse kappa light chain. The plates were again washed and assayed for radioactivity.

3I suppression studies. A mouse monoclonal antiidiotype (3I) that was cross-reactive with anti-DNA antibodies from a majority of patients with SLE was used in these studies. This antiidiotype is not anti-binding site. Its characteristics have been previously reported (4). The 3I IgG was purified from the ascitic fluid of BALB/c mice by ammonium sulfate precipitation followed by ion exchange chromatography on a DE-52 column. 3I at 14 or 28 µg/ml was added to PBMC that were incubated for 60 h in 1 ml of HB-101. Anti-DNA activity was detected with an affinity-purified, phosphatase-labeled goat anti-human IgG, A, M (mouse serum absorbed) antiserum (Kirkegaard & Perry Laboratories) and PNPP. The irrelevant monoclonal antibodies MOPC21 (γ1), AR13 (γ1), and MPC11 (γ2b) isolated from the ascitic fluid of BALB/c mice were used as controls. For each experiment disks to which mouse monoclonal antibody was added but no lymphocytes were used as blanks. In some experiments 3I was added to the PBMC at the end of the incubation just before the assay to determine if 3I demonstrated any interference with the ELISA system.

F(ab')₂ suppression studies. F(ab')₂ fragments of 3I were produced by pepsin digestion for 24 h at 37°C and then isolated by Sephadex G-100 gel chromatography. These were added to PBMC at concentrations varying from 5–20 µg/ml. A control containing the F(ab')₂ but no lymphocytes was included.

Assay for in vitro production of IgG. PBMC obtained from patients with active SLE were preincubated for 4 h at 37°C in HB-101 medium without supplementation. The cells 2×10^6 /ml were centrifuged, resuspended in the same medium with and without the 3I antiidiotype at a concentration of 28 µg/ml, and added to glass fiber disks covalently bonded to goat anti-human IgG. The cells were incubated for 3 d, then removed from the disks by vigorous washing, and the disks developed in an ELISA assay using goat anti-human IgG, A, M (mouse serum absorbed) conjugated with alkaline phosphatase (Kirkegaard & Perry Laboratories) and PNPP.

Results

Anti-DNA production in vitro. 23 patients with SLE were studied, 21 of them women and 2 men. All but one of the patients had serologically active disease. The mean serum anti-DNA for seven control subjects was 286 ± 81 IU/ml. 22 of 23 SLE patients had a serum titer of anti-DNA activity > 2 SD above the mean of the controls.

As shown in Fig. 1, when 2.5×10^6 PBMC/ml from the patients with SLE were cultured in vitro for 60 h the mean anti-DNA activity produced was 96.6 ± 89.7 IU/ml, as compared with 18 ± 9 IU/ml for PBMC from the control subjects ($P < 0.001$). 17 of the 23 patients (74%) produced levels of anti-DNA antibodies in vitro that were > 2 SD above the mean for normal subjects. A number of factors were found to influence these results. Media containing serum reduced the measurable anti-DNA antibodies, perhaps due in part to the presence of DNAase in the serum. The amount of anti-DNA produced was dependent on the duration of incubation and the number of PBMC added.

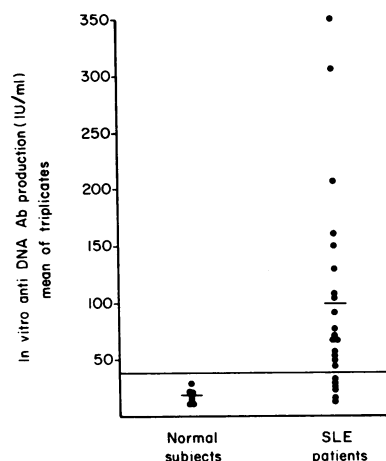


Figure 1. Production of anti-DNA antibodies (Ab) in vitro by PBMC from normal subjects and patients with SLE. Each point represents the mean of triplicate incubations. The mean of each group is indicated by a short horizontal line and 2 SD above the normal mean by the long horizontal line.

For example, in one experiment 5×10^5 PBMC produced 60 ± 5.8 IU (SE) while 1×10^6 PBMC produced 119 ± 4.1 IU and 2×10^6 PBMC produced 223 ± 7.2 IU, respectively. The length of incubation was limited to 72 h due to the fact that the ELISA disks could not be sterilized. When the media and disks were cultured for the presence of contamination, however, it was shown that they remained sterile up to 72 h.

The anti-DNA activity detected reflects de novo synthesis. The addition of cycloheximide (100 µg/ml) to the culture system reduced the in vitro antibody response by > 85% (mean of four experiments). Furthermore, 24-h preincubation of PBMC in medium alone did not alter their anti-DNA activity (297 IU after 4 h vs. 329 IU after 24 h of preincubation), demonstrating that de novo synthesis was being measured and not simply release of preformed anti-DNA antibodies.

Finally, as shown in Table I, a proportion of the anti-DNA antibody secreted by SLE PBMC in vitro bears the 3I idiotype. The 3I antiidiotype binds to the anti-DNA antibodies produced in vitro. Monoclonal murine antibodies of the same isotype as

Table I. Detection of Idiotype on Anti-DNA Antibodies Synthesized by PBMC from a Patient with SLE

Incubation	1st antiserum	2nd antiserum	Mean absorbance	SE	IU
Medium alone	None	Anti-human	215	±7	
Medium alone	3I, 1:5	Anti-mouse	157	±7	
PBMC	None	Anti-human	795	±23	108
PBMC	3I, 1:5	Anti-mouse	1,100	±131	
PBMC	3I, 1:10	Anti-mouse	825	±18	

Disks were incubated in triplicate with and without 2.5×10^6 PBMC in 1 ml of HP-101 medium for 60 h. After washing to remove the PBMC, some disks were first incubated as indicated with mouse antiidiotype (3I) for 45 min and washed. The disks were then incubated as indicated for 45 min with either a goat anti-human IgG,A,M (mouse serum absorbed) antibody or a goat anti-mouse IgG,M H+L (human serum absorbed) antibody both affinity purified and alkaline phosphatase labeled. After the addition of PNPP for 45 min absorbance at 405 nm was measured. In other experiments using the irrelevant monoclonal MOPC as the first antiserum the mean absorbance did not increase above that of medium alone. IU, international units.

3I do not bind, showing a specific interaction of 3I with the anti-DNA antibodies rather than rheumatoid factor activity in the culture supernatant.

Suppression of antibody synthesis by antiidiotypic antibody. 13 SLE patients were studied to determine if production of anti-DNA antibodies could be suppressed by the 3I antiidiotype (Table II). All 13 patients had detectable anti-DNA antibodies in their serum and all had a baseline anti-DNA synthesis in vitro of > 40 IU. 12 patients had 3I-reactive anti-DNA antibodies. The addition of 3I at concentrations of 14 or 28 µg/ml resulted in suppression of > 20% of anti-DNA synthesis by PBMC from 11 of 13 patients with SLE. The anti-DNA antibodies in the serum of these 11 patients demonstrated 3I reactivity, although there was not a linear correlation between the amount of 3I reactivity in serum and the degree of suppression by the 3I antiidiotype. Of the two patients whose antibody production was not suppressed by 3I, one did not demonstrate elevated 3I reactivity in serum.

To determine whether the 3I antiidiotype caused a nonspecific suppression of IgG production or a specific suppression of anti-DNA antibodies, PBMC from five SLE patients were as-

sayed for in vitro production of IgG in the presence and absence of the 3I antiidiotype. In all five patients tested, 3I induced no decrease in secreted IgG. Cells from three of these five patients were simultaneously tested for secretion of anti-DNA antibodies; anti-DNA antibodies decreased by 20–56%.

To determine whether the suppression noted above was the result of a macrophage-mediated antibody-dependent cytotoxicity we used F(ab')₂ fragments of 3I in the test system at concentrations of 5–20 µg/ml. There was no significant difference between the amount of suppression with the intact immunoglobulin and the F(ab')₂ fragment. Table III illustrates one experiment comparing suppression by 3I, the F(ab')₂ fragment of 3I and three irrelevant mouse monoclonal antibodies. As can be seen, both intact 3I and the F(ab')₂ fragment suppressed anti-DNA antibody production, while the irrelevant monoclonal antibodies produced < 20% inhibition.

Discussion

Our results confirm earlier observations that PBMC from patients with SLE will spontaneously produce anti-DNA antibodies in vitro (7). The anti-DNA antibodies produced in vitro bear an idiotype found on the anti-DNA antibodies present in serum. Using a short-term culture system we have also shown that the addition of a mouse monoclonal antiidiotype can specifically suppress the in vitro synthesis of anti-DNA antibodies by PBMC from patients with SLE. This finding supports previous work that implicates antiidiotypes among the regulatory mechanisms that can control synthesis of anti-DNA antibodies in patients with SLE and previous studies reporting the inhibition of idiotypic antibodies by the addition of antiidiotype to in vitro cultures of human PBMC.

Abdou et al. (8) reported that the binding of human anti-DNA antibodies to DNA could be blocked by autologous sera obtained from patients with SLE when their disease was in remission and by the F(ab')₂ and Fab fragments derived from these sera. The inhibition of binding was interpreted as being due to the presence of antiidiotypes in the inactive sera that reacted

Table II. Effect of Antiidiotypic Antibody (3I) on the Synthesis of Anti-DNA Antibodies by PBMC from Patients with SLE

Pt.	Serum* anti-DNA	Control	In vitro anti-DNA production			
			3I added		MOPC added	
			IU†	% Sup- pression	IU	% Sup- pression‡
3I added at 28 µg/ml	MOPC added at 28 µg/ml					
	IU/ml	IU†				
C.J.	954	106	60	43		
R.S.	600	71	53	26		
J.M.	2,140	49	32	35		
V.B.	1,241	309	173	44		
A.O.	1,010	147	74	50	107	27
E.D.	1,055	49	33	32	45	8
			3I added at 14 µg/ml		MOPC added at 28 µg/ml	
L.C.	1,995	45	18	59		
S.N.	736	162	99	39	164	+1
K.M.	759	110	34	69		
A.R.	867	198	98	51	156	21
R.B.	1,423	66	14	79	66	0
M.D.	1,793	48	48	0	47	2
F.K.	1,050	55	51	7		
Normal values	<450	<40				

IU, international units.

* Values from serum obtained at the same time as the PBMC used in the in vitro study.

† Values represent the mean of triplicate incubations.

‡ Mean percent suppression by all irrelevant mouse monoclonal antibodies at 28 µg/ml was 14.1±5.1 SE, n = 10.

^{||} Serum anti-DNA was 3I negative.

Table III. Effect of a Series of Mouse Monoclonal Antibodies on Anti-DNA Antibody Synthesis

Patient	Additions	Anti-DNA antibody produced		Suppression
		IU	%	
R.B.	None	66	—	
	3I (14 µg)	14	79	
	3I F(ab') ₂ (10 µg)	28	56	
	MOPC ²¹ (28 µg)	64	3	
	AR ¹³ (28 µg)	73	+10	
A.R.	MPC ¹¹ (28 µg)	52	21	
	None	198	—	
	3I (14 µg)	98	51	
	MOPC ²¹ (28 µg)	156	21	
	MPC ¹¹ (28 µg)	224	+12	

Each value represents the mean of triplicate incubations of 2.5 × 10⁶ PBMC in 1 ml of HP-101 medium for 60 h with the additions indicated minus a blank incubated without PBMC. The range of blanks was from 12 to 15 international units (IU).

with the binding site of the anti-DNA antibodies present in the sera of patients when their disease was active. Hahn and Ebling (9) reported the suppression of idiotype bearing anti-DNA antibodies in the sera of young mice given repeated inoculations of a monoclonal cross-reactive antiidiotype before the appearance of the idiotypic IgG in their serum. Inoculations begun after the appearance of idiotype bearing anti-DNA antibodies led to a diminution but not an absence of such antibodies. We have not quantitated the degree of suppression of the 3I idiotype bearing anti-DNA antibodies in our study system. Since an undetermined but presumably variable portion of the anti-DNA antibodies produced by each SLE patient bears the 3I reactive idiotype, it is probable that the 44% mean suppression seen with the addition of 14 μ g of 3I represents a higher percent inhibition of 3I-reactive anti-DNA antibodies. We have previously shown in four patients that from 40% to over 90% of the anti-DNA antibody is reactive with the 3I antiidiotype. The antiidiotype used in our experiments differs from the autologous antiidiotype described by Abdou et al. (8) as it is not binding site specific. It has been proposed that antiidiotypes directed to framework determinants may have a role in regulation.

There are two well-documented reports of the inhibition of synthesis of specific idiotypic rheumatoid factor by PBMC in vitro after incubation with antiidiotype. Koopman et al. (2), using PBMC from a patient with B cell lymphoma that synthesized an IgM kappa rheumatoid factor, were able to suppress the synthesis of the paraprotein by the addition of a rabbit polyclonal antiidiotype. Both IgG and F(ab')₂ but not Fab fragments of the IgG antiidiotype were effective, but the concentration of the F(ab')₂ required to produce 50% inhibition was five times greater than the concentration of IgG. At 10 μ g of anti-idiotypic IgG per 2×10^5 PBMC/ml, the appearance of the idiotype bearing rheumatoid factor in the culture medium after 5 d of incubation was virtually abolished. Takeuchi et al. (3) also examined the effect of a cross-reactive mouse monoclonal antiidiotype on the synthesis of idiotype bearing (K_a) rheumatoid factor by human peripheral blood lymphocytes from patients with rheumatoid arthritis in culture. They could suppress rheumatoid factor synthesis 81% by the addition of 10 μ g of IgG antiidiotype per 10^6 cells in 200 μ l incubated with pokeweed mitogen for 5 d, while F(ab')₂ fragments of the antiidiotype had no effect on this system. We found significant inhibition by the F(ab')₂ fragment of our antiidiotype.

Our results with anti-DNA antibodies and those described above with rheumatoid factor demonstrate that it is possible to suppress the synthesis of a specific idiotypic human autoantibody in vitro by the addition of antiidiotype. It is not yet clear what regulatory mechanisms are responsible for this suppression, nor

the possible physiologic role of a cross-reactive antiframework antiidiotype as opposed to an antiidiotype directed at the combining site. It remains to be determined whether manipulations of the immune response by such antiidiotypes has any utility in the management of diseases of altered immunity.

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