Raji Cell Assay for Immune Complexes

EVIDENCE FOR DETECTION OF RAJI-DIRECTED IMMUNOGLOBULIN G ANTIBODY IN SERA FROM PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS

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ABSTRACT We asked whether binding of human immunoglobulin (Ig)G antibody reacting with Raji cells could be distinguished from binding of IgG immune complexes. Using a standard Raji assay employing ^{125}I-IgG goat anti-human Fcγ, we found that digestion of Raji cells with pronase reduced by 95% their ability to bind complement-fixed aggregated human γ globulin and complement-fixed tetanus toxoid-antitetanus toxin complexes. However, binding at 37°C of IgG from the sera of 16 patients with systemic lupus erythematosus (SLE) to pronase-digested Raji cells was reduced much less consistently and extensively (9–100% reduction; mean reduction of 51%). In more detailed studies of two SLE sera, sucrose density gradient centrifugation showed that >50% of the IgG binding to undigested Raji cells sedimented in the 7S region. Pepsin digestion of immunoglobulin fractions from four SLE sera caused a reduction in SLE IgG binding to undigested Raji cells when detected with ^{125}I anti-Fcγ, but an increase when binding was detected with ^{125}I-anti-Fab, suggesting that substantial SLE IgG can bind through F(ab')$_2$ regions. Binding of IgG from SLE sera was not directed at neoantigenic sites induced by pronase digestion because binding activity was adsorbed with undigested cells as readily as with digested cells. Moreover, sera from 10 SLE patients that had negative Raji assays contained no IgG that bound to pronase-digested Raji cells. We conclude that much of the IgG bound at 37°C to Raji cells from the sera of many patients with SLE does not represent immune complexes but is probably antibody directed toward sites on the Raji cell.

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INTRODUCTION

The Burkitt lymphoma-derived human lymphoblastoid cell line, Raji, which bears receptors on its plasma membrane for immunoglobulin (Ig)[1]G and several complement components including C3-C3b, C3d, C1q, has been used for the past several years to detect small amounts of circulating immune complexes (IC) (1). The receptors for C3 have been thought to be primarily involved in IC binding (1), although recent evidence suggests that C1q receptors may play a larger role (2). Binding to the Fc receptors has been shown to be negligible (3). A clinical assay for circulating IC using this cell line has enjoyed wide use (1). A number of diseases have been shown to be associated with circulating IC, and clinical correlation of disease activity with Raji cell reactivity has been demonstrated in some cases (for review, cf. 4). The cell line has additionally been used to purify IC for purposes of antigen identification (5).

In the course of evaluating a number of antisera for usefulness in detecting IC on the surface of Raji cells, we discovered that goat IgG antibodies directed against human γ, μ, and α chains were all capable of detecting material adsorbed to Raji cells from sera from patients with systemic lupus erythematosus (SLE). Because antibodies to a number of lymphocyte determinants are known to circulate in the sera of these patients (6) it seemed reasonable to consider that such antibodies might be responsible for at least a part of the Raji cell activity.

Such antilymphocyte antibodies, detected by cyto-

[1] *Abbreviations used in this paper*: AHG, aggregated human IgG; HSA, human serum albumin; Ig, immunoglobulin; NHS, normal human serum; PBS; phosphate-buffered saline; SLE, systemic lupus erythematosus.

toxicity, had been thought to be chiefly of the IgM class and to react with lymphocytes in the cold (7, 8). Early evidence indicated that these antibodies were not detected by the Raji assay (1). But sucrose density gradient centrifugation of SLE sera has demonstrated that much of the Raji-binding material in SLE serum has a sedimentation coefficient equivalent to that of monomeric IgG (9). Moreover, IgG antilymphocyte antibodies capable of binding at 37°C have recently been described in the sera of patients with SLE (6).

The present study was undertaken, therefore, to evaluate the extent to which the Raji-binding material in the sera of patients with SLE is antibody directed against determinants on the Raji cell, as opposed to IC that bind to the receptors present on the Raji cell. Our results indicate that a considerable amount of the reactivity in the sera of these patients is, in fact, Raji-directed antibody.

METHODS

Raji cell line. Raji cells obtained from Dr. Argyrios Theofilopoulos of The Scripps Clinic and Research Foundation, La Jolla, Calif., were maintained in continuous spinner culture in RPMI 1640 medium supplemented with fetal calf serum (10%), penicillin (100 U/ml), and streptomycin (100 μg/ml), all from Gibco Laboratories, Grand Island Biological Co., Grand Island, N. Y. The culture was replenished from frozen stock at 6-mo intervals. Cells were maintained at concentrations of 0.5–1 × 10^6/ml. Before use, the cells were centrifuged at 200 *g* for 10 min at 4°C, were resuspended in spinner's medium (spinner salt solution, Eagle, Gibco Laboratories, catalogue No. 310-4230, pH 7.3), and were then adjusted to a concentration of 10^7 cells/ml. More than 95% of these cells excluded trypan blue.

Sera. Sera were collected from 26 patients with SLE who met the provisional diagnostic criteria of the American Rheumatism Association (Atlanta, Ga.). These patients were unselected except for attending the clinics of the University of Rochester Medical Center. As a source of complement, normal human sera (NHS) were collected in glass tubes from eight healthy laboratory workers and were pooled. The blood was allowed to clot for 30 min at room temperature followed by another 30 min at 0°C. Serum was separated by centrifugation at 2,100 *g* for 20 min at 4°C and stored at −70°C. Before each experiment, an aliquot was thawed at room temperature and centrifuged at 2,100 *g* for 15 min at 4°C.

Antisera. The IgG fraction of goat antiserum specific for human Fcγ chains was obtained commercially (catalogue No. 012-03, Atlantic Antibodies, Westbrook, Maine). The preparation was a DEAE cellulose-purified preparation of goat serum from animals immunized with the Fc fraction of a papain digest of human IgG. The goat IgG fraction had been further purified by immunoadsorption with insolubilized human IgM and IgA. Double immunodiffusion performed by us showed reactivity with human IgG but not human IgM nor IgA. The goat IgG was radiolabeled with ^{125}I (Amersham Corp., Arlington Heights, Ill.) by the chloramine T method to a specific activity of 8 μCi/μg (1 Ci = 3.7 × 10^7 Becquerels) and was frozen at −70°C (10). Immediately before use, an aliquot was thawed and the specific activity adjusted to 0.04–0.08 μCi/μg by the addition of unlabeled goat IgG anti-Fcγ. The concentration of goat IgG anti-Fcγ was then adjusted to 1 mg/ml in spinner's medium containing 1% human serum albumin (HSA; Fraction V, Sigma Chemical Co., St. Louis, Mo., A2386). For some experiments, a similar goat IgG anti-human Fab reagent, obtained from the same supplier (Atlantic Antibodies, catalogue No. 017-03), was labeled as above.

Raji cell assay. The assay for detecting circulating IC was performed essentially as described by Theofilopoulos and Dixon (11). In brief, 2 × 10^6 Raji cells in polypropylene 12 × 75-mm test tubes (Fisher Scientific Co., Pittsburgh, Pa.) were resuspended by vortexing in 50 μl of spinner's medium. Next, 25 μl of serum (1:4 in saline) or solution to be tested for the presence of IC were added, and the suspension was incubated for 45 min at 37°C, shaking every 5 min. Cells were then washed free of unbound protein and resuspended in 50 μl of spinner's medium containing 1% HSA. Cell-bound IgG was measured by incubating the cells for 30 min in an ice bath with 25 μl (an amount determined to be in excess of the maximum amount of IgG bound) of ^{125}I goat anti-human Fcγ. Cells were washed free of unbound radiolabeled protein by centrifugation and resuspension in spinner's medium containing 1% HSA. The amount of radioactivity associated with the cell pellet was quantified by gamma scintillometry. Duplicate determinations varied no more than 5%.

Proteins. Human IgG was purified from Cohn fraction II (catalogue No. 82-310-2, Miles Laboratories Inc., Elkhart, Ind.) by ion exchange chromatography on DEAE cellulose, and was dialyzed against phosphate-buffered saline (PBS) (0.15 M NaCl, 0.02 M sodium phosphate, pH 7.0). Aggregated human IgG (AHG) was prepared by incubating a 6.5-mg/ml solution for 70 min in a 63°C water bath. Aliquots were stored at −70°C for up to 4 mo and thawed on the day of each experiment. The preparations were centrifuged at 2,100 *g* for 15 min immediately before use. After fixation of complement aggregates stored in this manner showed negligible variation in their ability to bind to complement receptors on Raji cells. To prepare complement-fixed aggregates, 25 μl of AHG were incubated for 30 min at 37°C with 25 μl of NHS and 50 μl of 0.15 M NaCl. Of this material, 25 μl was assayed for binding to Raji cells. In the absence of complement (NHS) or in the presence of heat-inactivated complement, AHG binding was negligible (Fig. 1).

Pronase (Sigma Chemical Co., P5130, lot No. 19C-0388, 4.4 U/ml) was dissolved immediately before use in spinner's medium. Raji cells at 10^7/ml were incubated with pronase for 25 min at 37°C and washed free of the enzyme by centrifugation and resuspension in spinner's medium. The cell concentration was readjusted to 10^7/ml. Viability and cell yield were unchanged by pronase digestion; cellular debris, however, diminished.

Tetanus toxoid (4 mg/ml) and human globulin antitetanus toxin (145 mg/ml) were obtained from the Massachusetts Public Health Biologic Laboratories, Boston, Mass. IC were prepared by incubating 0.25 ml of antitetanus toxin with an equal volume of tetanus toxoid serially diluted in PBS for 1 h at 37°C and for 18 h at 4°C. Insoluble complexes were removed by centrifugation at 2,100 *g* for 30 min. 100 μl of the supernates were incubated with 600 μl of normal saline and 100 μl NHS, the source of complement, for 30 min at 37°C. The binding of these complement-fixed IC to Raji cells was then evaluated by incubating 25 μl of these solutions with 2 × 10^6 Raji cells and proceeding with the Raji assay. Maximum binding was obtained with IC prepared from tetanus toxoid diluted 1:4 and 1:32. Because insoluble IC were noted after the overnight incubation in those tubes containing tetanus toxoid diluted 1:8 and 1:16, IC prepared from tetanus toxoid diluted 1:4 and 1:32 are referred to, respectively, as "relative antibody excess" and "relative antigen excess."

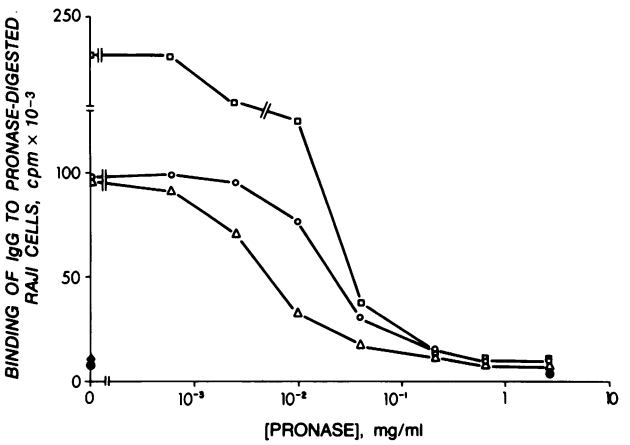

FIGURE 1 Loss of binding capacity for IC after pronase digestion of Raji cells. Complement-fixed IC comprised of tetanus toxoid antitetanus toxin in relative antigen excess (open circles) and in relative antibody excess (squares) and complement-fixed AHG (triangles) were incubated with Raji cells that had been digested previously with various amounts of pronase. Cells were separated from unbound material and the amount of cell-bound IgG was quantified with ^{125}I-goat anti-Fcγ. The counts per minute for IC prepared with tetanus toxoid-antitetanus toxin were corrected for binding produced by the antitetanus toxin alone. Pooled NHS that served as the source of complement (solid circles) and AHG that had been incubated with heat inactivated NHS (solid diamond) contained negligible amounts of material capable of binding to Raji Cells.

To determine the amount of binding of IC to pronase-treated Raji cells, 25 μl NHS was incubated for 30 min at 37°C with 75 μl of titered amounts of complement-fixed IC or AHG (prepared as described above). 25 μl of this mixture was then incubated with 2×10^6 Raji cells as described in the Raji assay.

Sucrose density gradients. A 0.2-ml sample of serum was placed on a 5.3-ml continuous gradient of 10–40% sucrose in PBS. The gradient was centrifuged for 15 h at 100,000 *g* in a Beckman SW65K rotor (Beckman Instruments, Inc., Fullerton, Calif.) at 4°C and was fractionated from the bottom into 21 equal fractions. Sucrose concentrations of the fractions were monitored by refractometry. Trace quantities of ^{125}I-IgG (purified murine myeloma IgG_{2a}, PC5) and ^{125}I-IgM (purified human macroglobulin) served as markers. Of each fraction, 25 μl was assayed for binding to 2×10^6 Raji cells.

Pepsin digestion. The Ig fractions of sera from three patients with SLE and one normal subject were precipitated with 50% saturated ammonium sulfate, dialyzed against 0.1 M acetate buffer, pH 4.5, and corrected to concentrations of 10–20 mg/ml. Digestion with thrice-crystallized pepsin (Schwartz/Mann Div. Becton, Dickinson & Co., Orangeburg, N. Y., catalogue No. 2095), added in a ratio of 2 mg pepsin for every 100 mg Ig, was performed at 37°C for 36 h (12). The protein was dialyzed against PBS and concentrated to 25–30 mg/ml. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis showed >95% disappearance of the IgG band, whereas a dominant $F(ab')_2$ band appeared (13). To evaluate the binding of the pepsin-digested Ig fractions of SLE sera to Raji cells, 25 μl of the digests (neat, 1:2, 1:4) was incubated with 2×10^6 Raji cells in the manner described above for the usual Raji assay. The amount of Ig bound to the Raji cells was detected using two reagents, ^{125}I-IgG goat anti-Fab and ^{125}I-IgG goat anti-Fcγ. The amounts of binding of the pepsin digests detected by these two reagents were compared with the binding seen with the starting sera assayed by the routine Raji test.

RESULTS

Making use of the observation of Lobo et al. (14) that pronase digestion destroys the ability of lymphocytes to bind IC yet preserves the antigenic determinants to which antilymphocyte antibodies are directed, we first asked whether the complement receptors of Raji cells withstood digestion by pronase. As Fig. 1 indicates, treatment of Raji cells with pronase reduced by 99% their ability to bind complement-fixed IC of tetanus toxoid-antitetanus toxin and AHG. The effect of pronase was dose dependent and complete at a concentration of 0.7 mg/ml. Only minimal binding of AHG to undigested Raji cells was seen when the source of complement (NHS) had been heat-inactivated, indicating that binding was complement-dependent.

Because the amount of IC incubated with the Raji cells in Fig. 1 was less than a saturating dose, the total amount of IgG bound would predictably be enhanced by increasing the input. Therefore, we incubated pronase-digested Raji cells with those same IC used in Fig. 1 in concentrations ranging from 0.06 to 1.6 mg IgG/2×10^6 pronase-digested Raji cells. Nevertheless, detection of bound IgG with ^{125}I-anti/Fcγ showed only a modest threefold increase from 4,500 to 13,500 cpm. These amounts of Ig cover the range of concentrations present in the sera of normal individuals (~10 mg/ml), patients with moderate hyperglobulinemia (20 mg/ml), and patients with multiple myeloma (>50 mg/ml). Thus, high IgG inputs alone, even when associated with in vitro prepared IC, do not result in significant binding to pronase-digested Raji cells.

Moreover, the binding of IC to pronase-digested Raji cells was independent of complement; no more than a 10% difference in counts per minute was seen when the three IC analogs were pretreated with heat-inactivated human serum rather than fresh serum (data not shown). Thus, because complement caused only minimal augmentation of IC binding to pronase-treated cells, Fc receptors are likely responsible for most of the binding.

We next asked whether IgG from serum of patients with SLE would bind to pronase-treated Raji cells. In the experiment described in Fig. 2, using conditions identical to those in Fig. 1, we found that Raji cells treated with sufficient pronase to eliminate binding of complement-fixed AHG were, nevertheless, capable of binding IgG from the serum of this patient with SLE. Raji cells digested with 0.1 mg/ml pronase bound only 5% of the AHG bound to untreated Raji cells, whereas

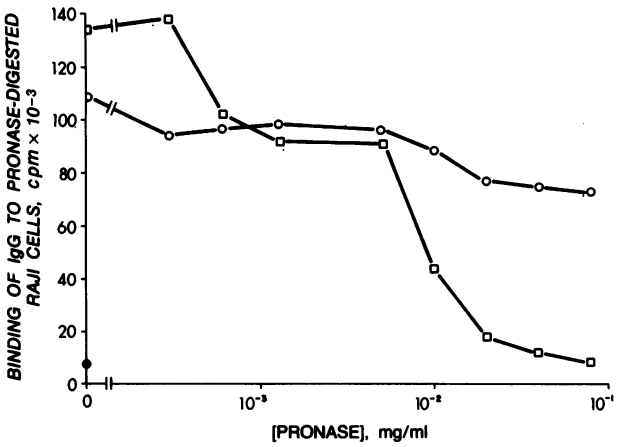

FIGURE 2 Pronase-digested Raji cells that do not bind complement-fixed AHG are able to bind IgG from serum of patients with SLE. As in Fig. 1, Raji cells that had been digested with various amounts of pronase were incubated with complement-fixed AHG (squares) or with serum from a patient with SLE (open circles). After separation of the cells from unbound material, the amount of cell-bound Ig was quantified by incubation of the cells with ^{125}I-goat anti-Fcγ. Binding of NHS alone (solid circle) is shown.

the treated cells bound ~70% of the amount of IgG bound to normal Raji cells from the SLE serum. Changes in cell viability and yield were not responsible for these changes (see Methods).

We next asked how commonly IgG in SLE sera would bind to pronase-digested Raji cells. Table I displays the data obtained by testing sera from 26 patients with SLE. In experiment 1, 13 patients who were known to have elevated Raji assay levels were evaluated. Considerable variation was seen in the ability of pronase to diminish Raji cell binding of IgG from SLE serum. Some patients (numbers 7 and 11) showed no decrease in the ability of digested cells to bind IgG, whereas others (numbers 2, 4, 14–16) showed marked reduction of binding after pronase digestion. These same cells showed a 95% reduction in their ability to bind complement-fixed AHG.

In experiment 2 of Table I, several SLE patients with normal Raji assay values were included to determine whether pronase digestion might result in increased binding of noncomplexed SLE IgG to the Raji cell membrane, perhaps indicating the appearance of cryptic antigens uncovered by pronase digestion

TABLE I

Study of SLE Sera for IgG Binding to Pronase-digested and Untreated Raji Cells

	^{125}I-anti-Fcγ bound to Raji cells, cpm × 10^{-3}							
	Experiment 1				Experiment 2			
Control	Patient No.	Undigested cells	Digested cells	R	Patient No.	Undigested cells	Digested cells	R
				%				%
	1	38.2	15.9	42	14	19.0	6.9	34
	2	20.2	6.9	28	15	14.5	4.9	25
	3	44.5	21.0	49	16	17.4	4.3	9
	4	34.4	13.0	36	17	11.7	7.6	N*
	5	47.7	20.9	44	18	7.5	5.4	N
	6	28.8	11.0	37	19	10.4	5.2	N
	7	23.6	23.5	>100	20	9.3	5.0	N
	8	16.2	7.2	47	21	8.9	4.7	N
	9	31.3	19.2	69	22	8.6	3.2	N
	10	39.2	18.8	50	23	9.2	5.4	N
	11	34.5	30.9	>100	24	11.3	5.6	N
	12	65.9	38.0	60	25	9.3	4.0	N
	13	29.0	11.8	40	26	7.6	4.4	N
AHG + NHS		102.1	8.8	6		100.6	8.1	5
NHS		8.8	3.7			9.2	3.6	
Saline		2.5	1.6			3.2	1.5	

The two experiments were performed as in Fig. 2 with sera from 26 patients with SLE, complement-fixed AHG (AHG + NHS), the source of complement alone (NHS), and the diluent alone (saline) being incubated with both undigested Raji cells and with Raji cells digested with pronase at 1 mg/ml. R%, percent binding remaining to Raji cells after pronase digestion, ([SLE digested − NHS digested] ÷ [SLE undigested − NHS digested]) × 100.

* N, normal Raji assay value.

to which the IgG in SLE serum might be binding. This explanation is not likely because none of the patients with normal Raji assays exhibited higher counts per minute using digested cells. These patients were not evaluated directly by other means for detecting antilymphocyte antibodies. However, it seems likely that if neoantigens were responsible, we would have found at least 1 serum out of 26 showing increased binding to pronase-treated Raji cells. Furthermore, if IgG antibody in SLE sera were binding to neodeterminants on pronase-treated Raji cells, those antibodies would not be removed by adsorption with undigested Raji cells. Table II summarizes three experiments that indicate that undigested Raji cells were as capable as digested cells of removing from SLE sera IgG that bound to pronase-digested Raji cells. Why all of the binding activity was not adsorbed from the sera is unanswered at the present time. The temperature (37°C) may not have maximized binding of low avidity antibody; multiple adsorptions may need to be used. Conditions were not altered to pursue this point.

To determine the size of the material in SLE serum that binds to pronase-digested Raji cells, we subjected two SLE sera to sucrose density gradient centrifugation and assayed the fractions for binding activity to Raji cells. Data from one of the patients is shown in Fig. 3; gradient fractions from the other patient gave

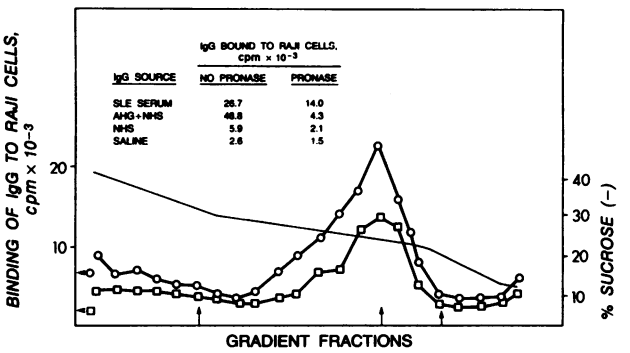

FIGURE 3 Binding to Raji cells and pronase-digested Raji cells of SLE IgG from sucrose density gradient fractions. An SLE serum was centrifuged on a 10–40% sucrose density gradient and fractions were evaluated for binding to Raji cells (circles) or to pronase-digested Raji cells (squares). Horizontal arrows refer to binding with NHS alone. Vertical arrows refer to molecular weight markers IgM, IgG, and albumin, from left to right. Inset indicates binding to cells of the unfractionated serum and controls.

similar results. Virtually all of the IgG bound to untreated or pronase-digested Raji cells was noted to sediment with the 7S marker. A fast moving shoulder that may indicate the presence of a small amount of IC is seen on the 7S peak. Recovery of Raji-binding material applied to the gradient was complete.

To test whether the SLE IgG was bound to the Raji cells via its Fab region, as would be the case with antibodies to Raji cells, we prepared pepsin digests of the Ig fractions of three SLE sera (one serum was digested twice). The quantity of Ig bound to undigested Raji cells from the SLE sera was compared with the binding of the pepsin digests using two radiolabeled goat antibody preparations, one with anti-Fab specificity and the other with anti-Fcγ specificity (Table III). Raji cells clearly bound pepsin-digested SLE IgG. This was most evident when the uptake of ^{125}I-anti-Fab was studied. As expected, uptake of ^{125}I-anti-Fcγ by other aliquots of these same cells was much less marked, in contrast to the consistent superiority of ^{125}I-anti-Fcγ in the detection of the Raji cell-bound IgG from SLE serum. All four SLE pepsin digests showed similar data, whereas a pepsin digest of normal Ig showed no preferential detection by either anti-Fab or anti-Fcγ (Table III). The fact that binding of the SLE pepsin digests detected by anti-Fcγ is not reduced to background levels likely results from the presence of some anti-Fd antibodies in the anti-Fcγ reagent, allowing some reactivity with cell-bound F(ab')$_2$ fragments.

TABLE II

Adsorption by Undigested and Pronase-digested Raji Cells of the IgG in Systemic Lupus Erythematosus Serum that Binds to Pronase-digested Raji Cells

Adsorbing cell	Assay target cell	IgG bound to Raji Cells, cpm × 10^{-3} anti-Fcγ, Patient 1	Patient 2	Patient 3
×10^{-6}				
None	U	85	78	33
None	D	59	29	14
16 D	D	34	21	12
32 D	D	37	22	11
64 D	D	32	16	8
16 U	D	34	26	9
32 U	D	33	14	8
64 U	D	29	9	6

Sera from three patients with SLE were incubated at 37°C with various numbers of pronase-digested (D) or undigested (U) Raji cells and were then evaluated for their ability to bind to pronase-digested Raji cells (D) as in Fig. 2. The adsorption ratio of serum to 16 × 10^6 cells was identical to that used in the usual Raji assay, as was temperature and time of the adsorption incubation.

DISCUSSION

A frequently voiced concern about the Raji assay as a means for detecting circulating IC is the theoretical

TABLE III

*Binding to Raji Cells of Pepsin-digested SLE IgG Detected with ^{125}I-anti-Fcγ and ^{125}I-anti-Fab**

Source of Raji-binding Ig		Experiment 1			Experiment 2		
Subject	Serum or digest	[Digest]	Anti-Fab bound	Anti-Fcγ bound	[Digest]	Anti-Fab bound	Anti-Fcγ bound
		mg/ml	*cpm × 10⁻³*		*mg/ml*	*cpm × 10⁻³*	
SLE 1	S		16.8	43.5		ND	
	D	31.4	47.9(+185)‡	30.1(−31)			
	D	15.7	27.6	18.0			
	D	7.9	20.1	10.6			
SLE 2	S		24.3	34.6		ND	
	D	26.3	31.8(+31)	23.4(−32)			
	D	13.2	20.3	14.0			
	D	7.6	15.5	9.4			
SLE 3	S		20.1	46.5		41.5	64.7
	D	26.3	32.3(+61)	20.1(−57)	8.5	38.0(−8)	19.4(−70)
	D	13.2	24.3	14.1	4.3	26.1	11.8
	D	6.6	16.6	10.6	ND		
Normal	S		8.5	7.4		13.9	10.3
	D	29.2	16.1(+89)	11.3(+52)	8.5	15.8(+14)	10.2(−1)
	D	14.6	11.5	7.8	4.3	11.6	7.3
	D	7.3	8.6	5.5	ND		
AHG + NHS			104.2	211.8		95.1	136.5
NHS			8.0	6.8		13.9	10.3
Saline			3.4	2.6		5.2	4.2

* Four SLE sera (S) and the pepsin digests (D) of the Ig fractions of these sera were incubated (25 μl of the listed concentrations) with 2 × 10⁶ unmodified Raji cells. The amount of Ig bound was quantified with ^{125}I-IgG goat anti-Fab and with ^{125}I-IgG goat anti-Fcγ.

‡ Numbers in parentheses represent the percent change (negative and positive) relative to counts per minute bound from serum.

possibility that the cells could bind antilymphocyte antibody. Unfortunately, the very diseases in which one might expect to find circulating IC are often those manifesting autoantibodies. So the distinction between binding of the two entities, antilymphocyte antibodies and IC, is an important one. Little investigation has been done, however, to resolve this issue. Therefore we undertook to determine whether such antibodies were at least partly responsible for the binding of IgG to Raji cells in a major IC disease, SLE.

Four lines of evidence were obtained, conjointly indicating that a major portion of the Raji-binding IgG in most of the SLE sera tested is antibody. First, when Raji cells were stripped of their complement receptors by pronase digestion so that they could no longer bind complement-fixed IC of known composition, they retained a substantial part of their ability to bind IgG from SLE sera. The SLE IgG that bound to pronase-treated cells was not binding pronase-induced neoantigens.

Second, binding to pronase-digested Raji cells could not be explained by binding to Fc receptors that are weakly expressed on Raji cells (3). For Fc receptors to account for the uptake seen with SLE IgG, the IC in SLE sera would have to be qualitatively different from those seen on our three IC models, binding either more efficiently than our models or to Fc receptors not measured by our models. We think these possibilities are unlikely.

Third, in representative SLE sera, most of the Raji-binding material sedimented with the 7S marker in sucrose density gradients. The asymmetric shape of the IgG peak suggests that small molecular weight IC are also being detected, but the fact that our 7S molecular weight marker (murine myeloma PC5, IgG2a) gave a similar profile prevents our definitely ascribing this shoulder to complexes. Likewise, the presence of IC in the lower half of the gradients is hard to evaluate because the levels of binding activity are very close to background levels. Woodroffe et al. (9) also evaluated SLE sera by sucrose density gradient fractionation and showed that much of the Raji binding material had a sedimentation coefficient of 7S.

One might ask whether the decrease in binding of SLE IgG induced by pronase digestion is a rough indication of the ratio of IC binding to total binding,

and that the remainder represents binding solely as a result of antibody. At the present time the data do not yield a clear-cut distinction between the two binding species of IgG, monomeric antibody vs. IC. Preliminary experiments, in fact, indicate that the sera of those patients whose binding dropped to baseline with pronase digestion have a significant amount of 7S IgG binding to untreated Raji cells. Thus, pronase may be destroying antigenic sites as well.

Fourth, pepsin-digested SLE IgG binds to Raji cells by its $F(ab')_2$ fragment, as would be expected for antilymphocyte antibodies. If the IgG molecules were to bind by means of their Fc fragments, with or without bound components, one would not see greater binding with anti-Fab than with anti-Fcγ after pepsin digestion of the IgG.

Whether these findings in SLE have implications for interpretations of Raji assays in other immunologic diseases, we cannot say. Certainly, the spectrum of diseases with antilymphocyte antibody is wide and includes several, such as viral infections and subacute bacterial endocarditis, in which circulating IC have been detected by the Raji assay (15). Preliminary experiments with sera from patients with mixed connective tissue disease, scleroderma, and Sjögren's syndrome show significant binding to pronase-digested Raji cells.

Possibly, the particular Raji cell line that we are using is unusual in its ability to bind antibody from SLE patients. The data presented by Woodroffe et al. (9) suggest that it is not. Moreover, our cell line was obtained from the laboratory where the Raji assay was developed (1), and its characteristics have not changed substantially in our hands. It manifests complement-dependent IC binding quite comparable with the original description of the Raji cells under conditions that do not differ significantly from those first described. We have surveyed other groups of patients to determine how our assay compares with published experience. The results indicate positive Raji cell binding with sera from patients with mixed connective tissue disease, various vasculitides, rheumatoid arthritis, scleroderma, subacute bacterial endocarditis, hyperglobulinemic purpura, and hepatitis. We believe that our overall results will not be too dissimilar from published reports.

Given data from other sources, it is not surprising to find IgG antilymphocyte antibody in SLE sera binding to Raji cells. Winfield et al. (6) indicated that most SLE sera contain antilymphocyte IgG that binds to 10–30% of the lymphocytes of all subjects tested. These antibodies have a somewhat greater specificity for B cells, the lineage of the Raji line, and they bind with equal facility at 37°C, the Raji assay temperature, as at 4°C. Searles et al. found antilymphocyte antibodies of all classes in SLE sera, although their antigenic substrate was acetone-fixed lymphocytes that likely bind not only membrane-directed antibody but antibody directed toward intracellular constituents as well (16). Our own preliminary work indicates that several of the SLE sera described herein contain IgM and IgA that binds to Raji cells under the conditions of the assay. Work is in progress to define whether this material is antibody or IC or both.

If these antibodies in SLE sera were directed against membrane constituents, the exact antigenic structures involved would be important to define. Prior incubation of untreated Raji cells with pepsin digests of SLE Ig did not inhibit subsequent binding of our model complement-fixed complexes (data not shown), indicating that the antibodies are probably not specific for the complement receptors. Other membrane determinants have been suggested as the specific binding sites for antilymphocyte antibody. For example, several workers have shown inhibition of the mixed lymphocyte reaction and antibody-dependent cell-mediated cytotoxicity with IgG antibodies from SLE patients (17–19).

The results of this study compel us to urge caution in ascribing Raji positivity to the presence of circulating IC in patients with SLE.

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