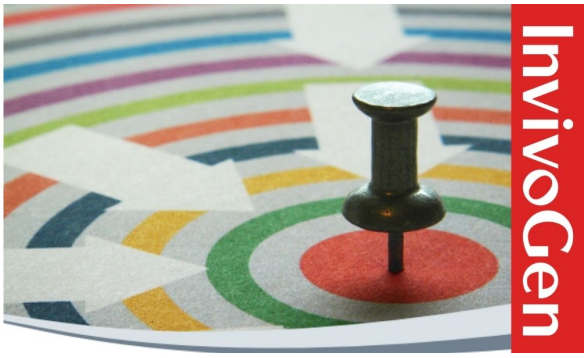

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A HYBRIDOMA FROM AN AUTOIMMUNE NZB/NZW MOUSE PRODUCING MONOCLONAL ANTIBODY TO RIBOSOMAL-RNA

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Spleen cells from an unimmunized autoimmune NZB/NZW F_1 female mouse were fused to the BALB/c MPC-11 drug-resistant clone 45.6 TG 1.7. One hybrid clone (D4) produced IgG-3 immunoglobulin that bound ribosomal RNA. A high concentration of this antibody was produced in the ascitic fluid of NZB/NZW male mice. DNA, tRNA, and synthetic single- and double-stranded polynucleotides could not bind significantly to the antibody. A Scatchard analysis showed that the rRNA-binding immunoglobulin is monoclonal and has a high affinity (10^9 liter/mole) for the RNA antigen.

Female NZB/NZW F_1 mice develop an autoimmune disorder resembling human systemic lupus erythematosus (SLE)[3] (1). The sera of NZB/NZW mice and SLE patients contain autoantibodies that show a unique ability to bind nucleic acids and are believed to play a role in the development of glomerulonephritis (2). The mechanisms that lead to the formation of autoantibodies are not well understood. For example, it is not known whether or not an antigenic stimulation is required; nor is it clear whether autoantibodies are the products of specific genes that are unique to the diseased animal or if they result from deregulation of normally suppressed clones due to viral transformation (3) or polyclonal activation (4). One approach to answering these questions is a detailed immunochemical and structural analysis of individual autoantibodies. Such an approach has now become possible with the development of the cell fusion technique that enables one to produce large quantities of homogeneous, monoclonal antibodies that recognize distinct antigenic specificities (5). We report here the production of RNA-specific, monoclonal antibody from unimmunized autoimmune NZB/NZW mice.

MATERIALS AND METHODS

Cell fusion. Spleen cells (about 10^8) from a 7-month-old female NZB/NZW mouse were fused with 10^7 cells of the 45.6 TG 1.7, 6-thioguanine-resistant clone (kindly provided by Dr. M. D. Scharff) derived from the BALB/c MPC-11. Fusion was facilitated by 35% polyethylene glycol essentially as described by Margulies *et al.* (6, 7). After fusion, the cells were seeded into the wells of two Linbro plates and selected, 1 day later, by using a growth medium containing 100 μM hypoxanthine, 1 μM aminopterin, and 30 μM thymidine.

Antigen binding assay. Radiolabeled nucleic acid antigens (Miles, Kankakee, Ill.) were incubated with cell supernatants or ascitic fluid for 1 hr at 4°C. Reaction conditions were chosen that minimize nonspecific antigen binding to the filter and/or normal serum proteins. Thus, native RNA were incubated in phosphate-buffered saline (PBS), pH 7.4, containing 0.01 M $MgCl_2$; synthetic single-stranded polynucleotides were incubated in 0.05 M borate buffered saline, pH 9.2; and double-stranded polynucleotides (including DNA) were incubated in 0.2 M borate buffered saline, pH 8.0. After incubation, the mixtures were filtered through nitrocellulose Millipore filters (8). The filters were dried and counted in toluene based scintillation liquid.

Affinity chromatography. IgG fractions of goat anti-mouse IgG2 and goat anti-mouse IgG3 antisera (9) were attached to preactivated Sepharose (Pharmacia, Piscataway, N. J.) according to the instructions of the manufacturer. Samples (0.1 ml) of cell supernatants from the RNA-binding hybrid clone were chromatographed on the affinity columns, and the binding of the effluent fractions to *Escherichia coli* ^{3}H-rRNA was compared to the binding of a control sample.

RESULTS

NZB/NZW F_1 female mice were monitored until they produced a fully developed SLE-like disease (1). Sera reached peak levels (5 to 20 μg nucleic acid/ml serum) of antibody binding to double-stranded DNA, poly(rI)·poly(rC), tRNA, and rRNA, at the age of 7 months. Spleen cells from the unimmunized female mice were then hybridized with the drug resistant mutant clone 45.6 TG1.7 (6) producing IgG2b immunoglobulin. The resulting hybrids were screened 4 weeks later by the nitrocellulose filter assay (8) for binding of the following radioactive nucleic acids: ^{14}C-DNA, ^{3}H-poly(rI)·poly(rC), ^{3}H-tRNA, and ^{3}H-rRNA. In two experiments, supernatant samples were drawn from 60 wells of Linbro plates, which contained 2 to 10 visible clones per well. No consistent binding was observed for DNA, poly(rI)·poly(rC), or tRNA. One supernatant (D4), however, bound *E. coli* ribosomal RNA. A representative screening experiment for rRNA binding is shown in Table I. Ouchterlony double diffusion in agar with class-specific antisera (9) showed that all supernatants contained IgG2b, the protein that is secreted by the original myeloma cell. Most hybrids secreted, in addition, IgG2a or IgM. No IgA immunoglobulin was identified in our experiments. The D4 cell supernatant contained IgG2b and an IgG3,

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[3] Abbreviations used in this paper: SLE, systemic lupus erythematosus; ss, single strand; ds; double strand.

TABLE I

Screening of hybrid cell supernatants for immunoglobulin production and binding to ribosomal RNA[a]

Well	Immunoglobulin	^{3}H cpm
A1	IgG2b, IgG2a, IgM	203
A2	IgG2b, IgG1, IgG2a	202
A4	IgG2b	103
A5	IgG2b, IgM	502
A6	IgG2b, IgM	449
B1	IgG2b	126
B3	IgG2b, IgG2a	229
B4	IgG2b, IgM	180
B5	IgG2b	78
C1	IgG2b, IgG2a	187
C2	IgG2b, IgM	92
C3	IgG2b, IgG2a	125
C4	IgG2b	266
C5	IgG2b, IgG2a	116
C6	IgG2b, IgM	263
D1	IgG2b, IgM	315
D2	IgG2b, IgM	170
D3	IgG2b	204
D4	IgG2b, IgG3	5868
D5	IgG2b, IgM	179
D6	IgG2b, IgM	153
NZB/NZW serum (5 μl)	All classes	2045

[a] The binding reaction contained in a final volume of 0.2 ml: 100 μl of cell supernatant, 10 μl (100 ng, 8000 cpm) of ^{3}H-23S + 16S ribosomal RNA mixture (Miles Laboratories) and phosphate buffered saline, pH 7.4, containing 0.01 M $MgCl_2$ Ig classes were determined by double diffusion in agar, using goat antisera (9) specific for IgG1, IgG2a, IgG2b, IgG3, IgM, and IgA.

TABLE II

Affinity chromatography of D4 cell supernatants on different immunoadsorbents

D4 Supernatant	Column Effluent	
	^{3}H-rRNA (*E. coli*) bound to filter	rRNA binding activity
	cpm	%
Control	2100	100
After chromatography on Sepharose-anti-mouse IgG2	2000	95
After chromatography on Sepharose-anti-mouse IgG3	0	0

a mouse immunoglobulin that is present in mouse serum in only minute quantities (10). κ but not λ light chains could be demonstrated in this supernatant (not shown). Absorption of the D4 supernatant on a Sepharose column, to which goat anti-mouse IgG2 antiserum had been attached, depleted the IgG2b immunoglobulin (as determined by Ouchterlony analysis) with retention of the RNA binding activity (Table II). In contrast, a Sepharose column, to which goat anti-mouse IgG3 antiserum had been covalently linked, eliminated all the binding activity (Table II). These results indicate that the rRNA binding activity is an IgG3 type κ-antibody. The D4 culture continued to produce this activity after 3 months growth in culture and cloning. Cells from the D4 clone were injected i.p. into six 3-month-old, NZB/NZW F_1 male mice; 6 weeks later, one of these mice developed an ascitic tumor that produced the rRNA binding IgG3 immunoglobulin. The tumor was then transferred to several mice that produced 15 to 25 ml each of the ascitic fluid. Table III shows the binding of different radiolabeled nucleic acid antigens to the ascitic fluid obtained from a male NZB/NZW mouse carrying the D4 tumor cells. Only ribosomal RNA was significantly bound. Some binding activity (10% or less of the rRNA binding) was also observed with other RNA antigens like poly(rU), poly(rI)·(rC), and tRNA, but very little, if any, with DNA or poly(dA)·(dT).

A saturation curve of ribosomal RNA binding to NZB/NZW D4 ascites is shown in Figure 1. Increasing amounts of 23S rRNA were bound to a fixed volume of NZB/NZW ascitic fluid, and the results were drawn as a Scatchard plot (11). The straight line thus obtained indicated that the rRNA binding immunoglobulin is monoclonal and that it has a high affinity for the RNA antigen (Ko = 10^9 liters/mole). Approximately 1 mg of RNA was bound at saturation to 1 ml of mouse ascites.

TABLE III

Binding of radiolabeled nucleic acid antigens to mouse ascites containing D4 hybrid cells[a]

Antigen	Nanograms Bound
^{3}H-tRNA (*E. coli*)	2.8
^{3}H-16S rRNA (*E. coli*)	54.9
^{3}H-23S rRNA (*E. coli*)	70.1
^{3}H-poly(rA)	0.0
^{3}H-poly(rU)	3.4
^{3}H-poly(rC)	0.5
^{3}H-poly(rA)·poly(rU)	0.0
^{3}H-poly(rI)·poly(rC)	5.6
^{3}H-poly(dA)·(dT)	0.3
^{14}C-dsDNA (*E. coli*)	1.1

[a] Each reaction mixture contained in a volume of 0.1 ml, 10 μl of NZB/NZW D4 ascites diluted 1:100 and 300 ng of the appropriate radiolabeled antigen. Control reactions were run in parallel for each antigen, where BALB/c ascites containing the IgG3 myeloma FLOPC-21 was used in the same protein concentration as the D4 ascites. The controls (about 10% of the specific binding) were subtracted from each value of the D4 binding.

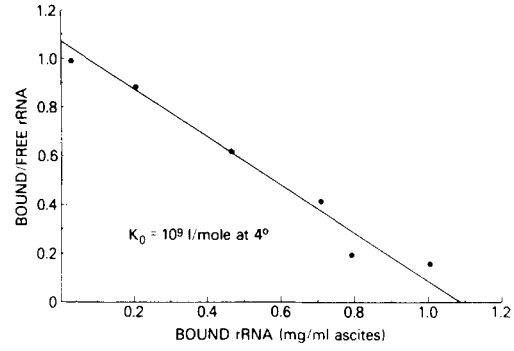

Figure 1. A saturation curve of rRNA binding to NZB/NZW D4 ascites. The reaction mixture contained 10 μl of NZB/NZW D4 ascites diluted 1:100, and increasing concentrations (10 to 1000 ng) of *E. coli* 23S rRNA in a total volume of 0.1 ml phosphate-buffered saline, pH 7.4, containing 0.01 $MgCl_2$. The tubes were incubated and processed as described in *Materials and Methods*. The results are drawn as a Scatchard plot (11). Each point represents the average of a duplicate measurement. Control reactions (in duplicates) were run in parallel for each rRNA concentration, where BALB/c ascites containing the IgG-3 myeloma FLOPC-21 was used in the same protein concentration as the D4 ascites. The controls were subtracted from each value of the D4 binding. The solid line is the least square best fit of the experimental points. Bound RNA in the Scatchard plot is expressed in milligrams per milliliter of original ascites. The association constant (Ko) was calculated on the basis of concentration of bound RNA in the reaction mixture (0.1 ml). Thus, 1 mg RNA/ml ascites is equivalent to 10^{-9} mole RNA/liter of reaction mixture. (The m.w. of 23S rRNA is ~ 10^6 daltons.)

This is about 100-fold higher than the binding capacity of the total anti-rRNA antibody population in NZB/NZW serum.

DISCUSSION

The present work demonstrates that NZB/NZW spleens contain lymphoid cells that are able to produce antibodies with high specificity and affinity for RNA. These findings corroborate our previous results obtained by analysis of the RNA binding properties of total IgG fractions from NZB/NZW mice and SLE patients (12, 13). The presence of high serum levels of anti-nucleic acid antibodies in the NZB/NZW donor mouse and not in nonautoimmune mice, coupled with the fact that the donor mouse was unimmunized, strongly suggest that the RNA binding cell fusion product is a true autoantibody.

A detailed immunochemical, idiotypic, and structural analysis of nucleic acid-specific monoclonal antibodies would help to elucidate the genetic origin of these immunoglobulins and the type of lymphoid cells, that participate in their production. A study of immune complexes composed of autoantibodies and individual nucleic acids would help to clarify their role in the induction of glomerulonephritis and the pathogenesis of SLE. These studies may also provide improved therapeutic approaches to SLE, such as the design of specific tolerogens (14) and the use of anti-idiotypic sera to monoclonal autoantibodies as specific suppressor agents (15).

The importance and utilization of nucleic acid-specific autoantibodies also extends to basic biochemical research. Antibodies to simple or modified nucleotides have been successfully elicited in experimental animals, but antibodies to native dsDNA or native ssRNA are very difficult to produce. Monoclonal antibodies like the anti-rRNA described here may be used in a variety of structural and functional studies involving nucleic acids, such as the elucidation of the structure of ribosomes by immunoelectron microscopy (16).

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