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DNA–Protein Complexes

Natural Targets for DNA-Hydrolyzing Antibodies

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ABSTRACT

Sera of patients with different types of leukemia and acquired immune deficiency syndrome (AIDS) have been examined for the presence of the anti-DNA antibodies. DNA-hydrolyzing activity of antibodies was detected in the sera of patients with chronic lymphoid leukemia (CLL), pre-B-cell acute lymphoid leukemia (pre-B-All), acute myeloleukosis (AML), and AIDS in stages III and IV of the disease. In immunofluorescence tests, the DNA-hydrolyzing antibodies reacted preferentially with proliferating cell nuclei compared with resting cells. A 35-kDa factor was identified as the target for the DNA antibodies in the cell nuclei. The DNA-hydrolyzing antibody fraction from the serum of an AIDS patient crossreacted with HIV I virus proteins gp160, gp120, and p65.

Index Entries: Catalytic antibodies; DNA; crossreactions; nuclear staining; leukemia; AIDS.

INTRODUCTION

The antigen-binding property of antibodies has been successfully utilized to imitate catalysis by enzymes (*1*). The concept applied for this purpose was to generate antibodies against structural analogs of the transition state of various reactions. Thus, antibodies showing strong binding

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for one group of compounds can catalyze the chemical conversion of related molecules, the substrates (2). The antibody repertoire present in mammals may contain similar antibodies that bind a given class of antigens strongly, but catalyze the chemical conversion of structurally related antigens. This concept is supported by recent observations of specific hydrolysis of vasoactive intestinal peptide by antibodies from asthma patients (3) and DNA hydrolysis by antibodies from sera of systemic lupus erythematosus (SLE) patients (4).

Antibodies found in SLE patients are characterized by their ability to interact with cell nuclei (5). This includes interactions with DNA, nucleoprotein complexes, and proteins involved in DNA and RNA processing. Antigen-dependent antibody maturation in SLE may result in appearance of antibodies with different specificities to nuclear antigens. Nuclear DNA has a range of conformations determined by its interaction with various nuclear factors found in chromatin. Maturation of the anti-DNA antibodies may result in appearance of antibodies to single-stranded DNA (ssDNA), double-stranded DNA (dsDNA), and polyreactive antibodies, which can bind various molecules, including DNA (6).

METHODS AND MATERIALS

Fluorescein isothiocyanate (FITC)-labeled and peroxidase-labeled anti-human antisera were from Sigma, Triton X-100 and paraformaldehyde from Bio-Rad, phytohemagglutinin from Welcome, Durtford, England, propidium iodide from Beckton-Dickinson, CA, and cell-culture medium and serum from Gibco. Cell lines were from All Russian Cardiologic Center, Moscow. All other chemicals used were of reagent grade.

DNA-Hydrolyzing Antibodies

DNA-hydrolyzing antibodies were purified from sera of SLE patients and AIDS patients according to ref. (4). The antibodies were electrophoretically homogenous.

Cell Culture

Cells were grown in RPMI 1640 supplemented with 2 m*M* L-glutamine and 20 % calf serum.

Immunofluorescence

Cells (5×10^3) were plated in eight-chamber slides, fixed with 4% paraformaldehyde solution, washed twice with isotonic phosphate-buffered saline (PBS), and permeabilized with 0.5% Triton X-100 for 15

min at 37°C. Fixed cells were then incubated with the DNA-hydrolyzing antibodies fraction (10 μm/mL) for 1 h at 37°C. After washing three times with PBS, the slides were treated with FITC-labeled antihuman IgG (1 h, 37°C) and then washed thrice with PBS. Microscopic evaluation of stained cells was done using a Zeiss fluorescence microscope.

Flow Cytometry

Mononuclear cells were separated from fresh blood by centrifugation in Ficoll-Hypaque gradients (Pharmacia) (400*g* 30 min, room temperature) as described in ref. (*8*). Lymphocytes were then activated by incubation in RPMI 1640 supplemented with 2 m*M* L-glutamine, 20% fetal calf serum, and 3 μg/mL phytohemagglutinin for 72 h; 10^6 cells were treated with the antihuman antisera, washed with PBS, and fixed with 0.5% paraformaldehyde for 10 min at 4°C. Fixed cells were permeabilized with 0.1 Triton X-100 (4 min), washed twice with PBS, and incubated in 100 μL DNA hydrolyzing antibodies solution (10 μg/mL) in PBS with 5% bovine serum albumin (BSA) (30 min 4°C). After washing in PBS, cells were treated with FITC-labeled antihuman IgG (30 min at 4°C), washed again, and treated with 1 μg/mL propidium iodide and 5 U/mL RNase in PBS (1 mL). Cells were analyzed using a FACScan (Becton-Dickinson, CA) sorter fitted with argon ion laser adjusted to emit 500 mW at 480 nm. Green fluorescence was measured using a 530-nm filter and red flurorescence using a 585-nm filter. Bivariant fluorescence distribution patterns were displayed in dot-plot (256 × 256) channel area. Analysis was carried out for 1000 events.

Western Blot Analysis

Nuclear proteins were extracted from nuclei of Raji cells using NaCl as described in (*7*). For Western blot analysis, 50 μg of the extracted proteins were fractionated by SDS-polyacrylamide gel electrophoresis and transferred to Trans-Blot membranes (Bio-Rad). The membrane was blocked using 1% BSA and incubated with PBS containing 0.05% Tween (PBS-T) and the DNA-hydrolyzing antibody fraction (1 μg/mL). After washing thrice with PBS-T, bound antibodies were stained with peroxidase-labeled antihuman IgG. Western blot analysis for staining of the HIV I virus proteins by the DNA-hydrolyzing antibody fraction (1 μg/mL) was done using a Biotech/Du Pont HIV I Western blot kit according to protocol described above.

Determination of the DNA-Hydrolyzing Activity

DNA hydrolysis was measured by incubation of 1 μg of supercoiled DNA pUC19 with IgG (10 μg, 37°C) overnight. The reaction products were analyzed by electrophoresis in 1% agarose gel (*4*).

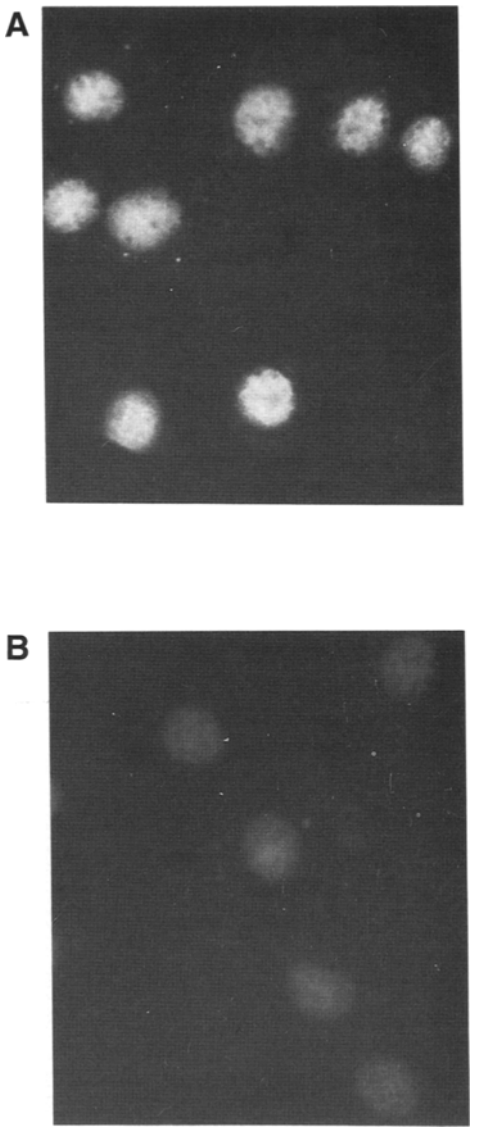

Fig. 1. Nuclear staining of cells with affinity-purified DNA-hydrolyzing antibody fraction. (**A**) Jurkat cell line; (**B**) peripheral blood lymphocytes.

RESULTS AND DISCUSSION

Staining of Nuclei by DNA Antibodies

We have studied the interaction of an affinity-purified DNA-hydrolyzing antibody fraction from SLE sera with Jurkat cells (Fig. 1A) by an immunofluorescent assay. Intense nuclear staining with this proliferative cell line was observed. In contrast, the staining with resting cells (peripheral blood lymphocytes) was essentially equivalent to background fluo-

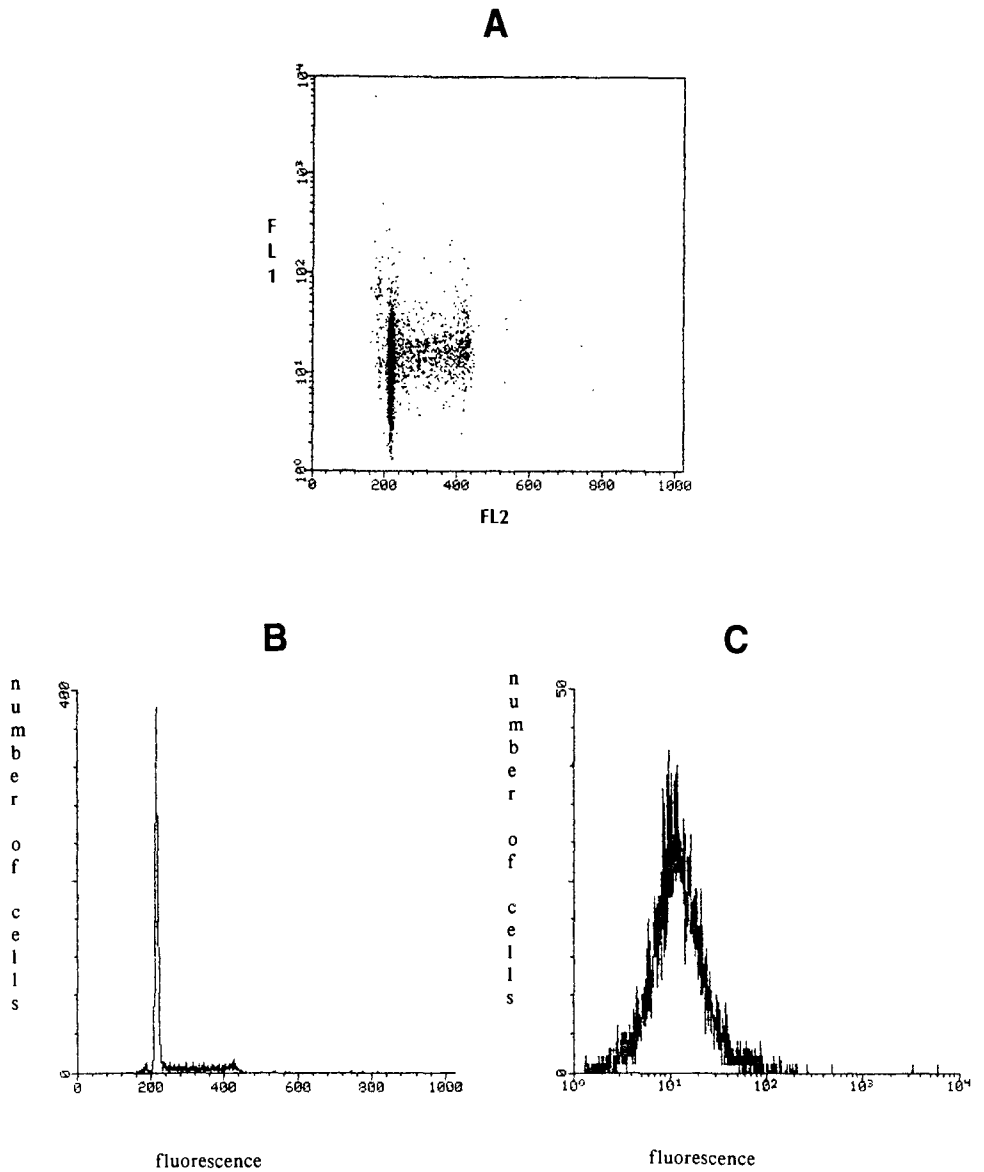

Fig. 2. Dual-parameter flow cytometry of lymphocytes from a healthy human donor 72 h after stimulation with phytohemagglutinin. (**A**) Dot-plot distribution of nuclear staining with affinity-purified DNA antibody fraction (FL1) vs DNA content measured by staining with propidium iodide (FL2). (**B**) Distribution histogram for DNA content measured by staining with propidium iodide. (**C**) Distribution histogram for nuclear staining with affinity-purified DNA-antibody fraction.

rescence (Fig. 1B). The level of staining was increased by activation of the cells by mitogens. Flow cytometric analysis of phytohemagluttin-activated peripheral blood lymphocyte showed nuclear staining with the DNA-hydrolyzing antibody fraction (Fig. 2A–C). The data on proliferating cells from several additional lymphcytic cell lines are shown in Table 1.

Table 1
Nuclear Staining of Different Cell Types
by Affinity-Purified DNA-Hydrolyzing Antibodies
Measured by Indirect Immunofluorescence[a]

Cell line	Origin	% of stained cells
MOLT[b]	T-ALL	60
HUT[b]	T-ALL	80
CEM[b]	T-ALL	90
Jurkat[b]	T-cell lymphoma	93
L 929[c]	Mouse fibroblast	92
OH 1[c]	Mouse lymphoma	95
Blood cells[d]	-	1
Blood cells	T-ALL	34–84

[a]*See text* for methods. T-All, T-cell lymphoid leukemia.
[b]Human.
[c]Mouse.
[d]Healthy human donor.

The percent of cells stained with the DNA-hydrolyzing antibody fraction was 34–95%. The staining was localized in the nucleus in each case.

Crossreaction with a Nuclear Protein

To identify proteins that bind DNA-hydrolyzing antibodies, we stained the nuclear protein extract from Raji cells with these antibodies. The nuclei were extracted with different concentrations of sodium chloride and the extract separated by SDS-polyacrylamide gel electrophoresis. Blots of the gels were stained with the DNA-hydrolyzing antibody fraction (Fig. 3). Intense staining of a 35-kDa protein extracted at 0.3–0.5*M* sodium chloride was evident. The 35-kDa band was not observed in an extract prepared by treatment with 2*M* NaCl and DNase I, and the disappearance of this band was accompanied by staining of a number of bands at 14–17 kDa. Active chromatin contains numerous proteins. We suggest that the 35-kDa protein is a nonhistone nuclear protein bound to DNA in chromatin.

DNA-Hydrolyzing Antibodies in Leukemia and AIDS

Catalysis by the anti-DNA antibodies could be associated with diseases characterized by B-cell activation. We have searched for antibodies with DNA-hydrolyzing activity in the sera of patients with AIDS and different forms of leukemia. IgG from a majority of patients with CLL, pre-B-ALL, or AML was positive for DNA-hydrolyzing activity (Table 2). IgG from four of 13 AIDS patients at stages II–IV of the disease expressed DNA-hydrolyzing activity (Table 3). Previously, we have demonstrated the

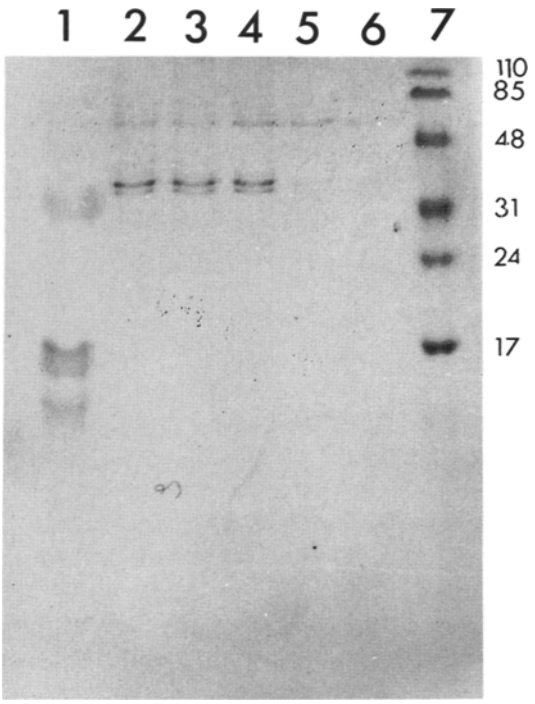

Fig. 3. Immunoblot of an SDS-polyacrylamide gel of proteins extracted from nuclei of Raji cells stained with an affinity-purified DNA-hydrolyzing antibody. Extracts were prepared using: (1) 2*M* NaCl followed by DNase I (50 μg); (2) 0.5*M* NaCl; (3) 0.4M NaCl; (4) 0.3*M* NaCl; (5) 0.2*M* NaCl; (6) 0.1*M* NaCl. Lane 7, marker proteins.

Table 2
Detection of DNA-Hydrolyzing Antibodies
(IgG Fraction) in Sera of Leukemia Patients[a]

Leukemia type	Number of sera tested	Number of positive reactions
CLL	11	7
Pre-B-ALL	4	3
Pre-T-ALL	2	-
AML	4	3
CML	2	1

[a]CLL, chronic lymphoid leukemia; pre-B-ALL, pre-B-cell acute lymphoid leukemia; pre-T-ALL, pre-T-cell acute lymphoid leukemia; AML, acute myeloleukosis; CML, chronic myeloleukosis. A positive reaction was defined as a specific DNA-hydrolytic activity > 100 U/mg as described (4).

Table 3
Detection of DNA-hydrolyzing Activity of Antibodies (IgG Fraction) in Sera of HIV-Infected Patients[a]

Patient code	Age	Sex	Stage of disease	Anti-HIV antibody, ELISA	Antigen set tested	Antigen presence in blood	DNA hydrolysis by IgG
1	39	M	II C	1:8100	p24,55; gp 120/160	No	-
2	36	M	IV	1:24000	p55,65; gp 120/160	Yes	+ +
3	30	F	II B	1:24000	p55; gp120/160	No	-
4	29	F	III A	1:2700	p24, 55,65; gp 41/120/160	No	-
A	35	M	II C	1:8100	p24; gp120	No	+
B	26	M	II C	1:8100	p24; gp120	No	+
C	28	M	II B	1:2700	p17, 24, 55, 65; gp 41/120	No	-
D	18	M	III B	1:24000	p24, 55, 65; gp 41/120/160	Yes	+ +
E	32	M	II C	1:8100	p24, 55; gp 41, 120/160	Yes	-
F	31	M	II C	1:2700	p24, 55; gp 120/160	Yes	-
G	32	F	III A	1:8100	p24, 55; gp 120/160	Yes	-
H	32	F	II B	n.d.	p24; gp120	No	-
I	27	F	II B	n.d.	p24, 55; gp 41/120	No	-

[a]Each serum studied was examined for titer of antibodies characteristic to HIV antigens by ELISA, presence of the HIV antigens in blood, and DNA-hydrolyzing activity of IgG fraction as in (4). + +, 300 U/mL serum; +, >100 U/mL serum; -, <100 U/mL serum; n.d., not detectable.

presence of DNA-hydrolyzing antibodies in SLE patients (4). Based on the data reported here, we concluded that these antibodies could also be formed in other diseases characterized by immunologic dysfunction.

Crossreaction with HIV Proteins

The crossreactivity of DNA-hydrolyzing antibodies isolated from the serum of an AIDS patient with HIV I-specific proteins was examined by immunoblotting analysis. Figure 4 shows an intense staining of gp160, gp120, and p65 HIV proteins by the antibody fraction. Unfractionated AIDS patient serum, in contrast, displayed reactions with several additional HIV proteins. The crossreaction of the DNA-specific fraction appears to be restricted to a few proteins.

In conclusion, we have shown in this article that:

1. DNA-specific antibodies stain the nuclei of proliferating cells preferentially compared to resting cells;

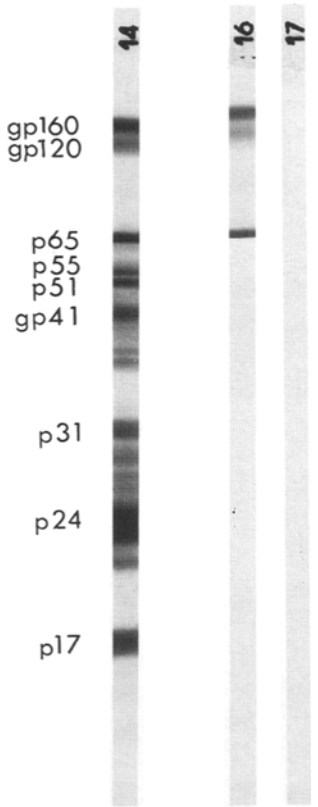

Fig. 4. Crossreaction of DNA-hydrolyzing antibody fraction from the serum of an AIDS patient with HIV I proteins. *See text* for method. An immunoblot stained with the DNA-antibody fraction (lane 16), unfractionated serum of the AIDS patient (lane 14), and unfractionated serum from a healthy donor (lane 17).

2. A 35-kDa nuclear protein crossreacts with the antibodies;
3. Three HIV proteins, gp160, gp120, and p65, crossreact with the antibodies; and
4. DNA-hydrolyzing antibodies are found, in addition to patients with SLE, in leukemia and AIDS patients.

These observations represent the first step in identifying the natural targets of the antibodies in vivo and the original stimuli for their formation.

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DISCUSSION

G. Gololobov

Stollar: Are the antibodies in sera of patients with leukemia and other diseases IgM or IgG? Do they bind native DNA or denatured DNA?

Gololobov: We have analyzed mainly the IgG fractions. It is difficult to make good IgM preparations. Preliminary evidence suggests that IgM is also active. Our IgG preparations interact both with single-stranded DNA and double-stranded DNA.

Zouali: In the first part of your work, you have noted activity with T-cell clones and B-cell clones, but not with cells of normal individuals. If you activate these cells, what happens?

Gololobov: The activation of the T-lymphocytes results in appearance of the activity.

Zouali: How do you activate?

Gololobov: We used retinoic acid.

Zouali: Why did you not use a more conventional mitogen?

Gololobov: Phytohemagglutin-activated lymphocytes are also stained by the DNA antibodies.

Zouali: I would like to suggest a straightforward experiment, that is, to test monoclonal antibodies to gp120 and gp160 for DNA-hydrolyzing activity.

Gololobov: For this, we will need to use a panel of antibodies. An individual antibody may be inactive. Ideally, the immunization should be with the whole virus core in order to produce antibodies to all possible epitopes.

Zouali: How do you explain the hydrolytic activity that you observe?

Gololobov: In a simple sense, the hydrolytic activity may represent a crossreactivity reaction. The combining site of our antibodies may be specific partly for a protein and partly for DNA, and the DNA-recognizing site may possess the catalytic activity.

Paul: Tight docking between a ligand and an antibody raised against a structurally related antigen may not be possible. The conformational freedom afforded by this type of interaction may permit expression of catalysis.