Comparative Study of Catalytic (DNA-Hydrolyzing) and Cytotoxic Properties of Anti-DNA Autoantibodies

S. V. Suchkov

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 131, No. 4, pp. 420-423, April, 2001
Original article submitted February 21, 2001

DNA-hydrolyzing and cytotoxic properties of anti-DNA autoantibodies isolated from patients with systemic autoimmune diseases and from autoimmune MRL-lpr/lpr, SJL/J, and (NZB×NZW)F_1 mice were studied. Cytotoxic and catalytic properties of these antibodies correlated. A relationship between the stage of systemic lupus erythematosus and catalytic and cytotoxic properties of DNA abzymes was revealed. Of all studied cells, L929 cells were most sensitive were most sensitive to *in vitro* effect of antibodies. Treatment of target cells with anti-DNA autoantibodies with cytotoxic properties induced internucleosomal DNA fragmentation, which is characteristic of apoptosis.

Key Words: *DNA abzymes; anti-DNA autoantibodies; cytotoxicity; autoimmune mice; systemic lupus erythematosus*

Recent studies showed that DNA-binding autoantibodies (AAB) with catalytic activity trigger apoptosis in cell culture and exert a cytotoxic effect on lymphocytes *in vitro* [3,9,11,14].

We previously demonstrated catalytic and cytotoxic effects of anti-DNA AAB isolated from the blood of patients with chronic lympholeukemia. It was showed that catalytic and cytotoxic activities are determined by Fab fragment, while the cytotoxic effect can be blocked with DNA [4].

Here we compared the cytotoxic and catalytic (DNA-hydrolyzing) activities of anti-DNA AAB isolated from patients with various autoimmune diseases and from inbred autoimmune mice.

MATERIALS AND METHODS

We compared DNA-hydrolyzing activity of anti-DNA AAB IgG from 68 patients with systemic lupus erythematosus (SLE), 12 patients with rheumatoid arthritis (RA), 8 with systemic scleroderma (SSD), and 10 donors. Supercoiled plasmid DNA (scDNA) was used as the substrate.

Laboratory of Clinical Immunology, Moscow Regional Research and Clinical Institute, Moscow. ***Address for correspondence:*** grei@ibch.ru. Suchkov S. V.

Blood samples were obtained from the Institute of Rheumatology, Russian Academy of Medical Sciences, and Moscow Regional Research and Clinical Institute. The cytotoxic properties of anti-DNA AAB were studied on L929, HL-60, Raji, and K562 cells from American Type Culture Collection.

N-acetyl-YVAD-CHO was from Sigma and materials for cell culturing were from Life Technologies and Amersham Pharmacia.

DNA-hydrolyzing activities of anti-DNA AAB from inbred MRL-lpr/lpr and hybrid (NZB×NZW)F_1 and SJL/J mice [13] and control nonautoimmune BALB/c mice were compared.

Precipitation of IgG fraction from the serum with 40% ammonium sulfate and subsequent purification by immunoadsorption on protein A sepharose were described previously [4]. The purity of IgG fraction was confirmed by SDS-PAGE and immunoblotting analysis. DNA-hydrolyzing activity of serum IgG fraction towards plasmid DNA was analyzed and the studied blood samples were referred to certain groups depending on the depth of the substrate cleavage (Fig. 1).

Serum samples showing DNA-hydrolyzing activity were quantitatively analyzed by flow linear dichroism as described previously [5]. The duration of cleavage of 1 μg of plasmid DNA is 2 h, which allows

0007-4888/04/1314-0353$25.00

us to evaluate specific catalytic activity of anti-DNA AAB by the integral Michaelis—Mentene formula [7]. Activity needed for complete transformation of 1 μg scDNA into circular or linear forms during 10-h incubation with AAB was taken for a unit of DNA-hydrolyzing activity.

For evaluation of AAB-mediated cytotoxicity, target cells were cultured in RPMI-1640 medium supplemented with 0.002 M L-glutamine, 10 mg/ml gentamicin, and 10% inactivated fetal calf serum. AAB were dialyzed against Na phosphate buffer and added to target cells in a concentration of 10^{-7} M for 1-48 h depending on the purpose of the experiment. The number of dead cells after incubation with AAB was evaluated by trypan blue exclusion or MTT tests [10,12].

RESULTS

No DNA-hydrolyzing activity was detected in 10 donor sera. In 40 (59%) of 68 patients with SLE, anti-DNA AAB exhibited DNA-hydrolyzing activity and this activity depended on the stage of the disease. The sera from these patients showed also cytotoxic activity (Table 1). According to the kinetics of scDNA hydrolysis and linear dichroism data, the efficiency of catalysis (k_{CAT}/k_M) was 0.32 $nmol^{-1}$/min. In 12 (18%) of 68 SLE patients anti-DNA AAB exhibited neither DNA-hydrolyzing nor cytotoxic activity. In 2 SLE patients (3%) AAB showed catalytic, but not cytotoxic activity.

DNA-hydrolyzing activity was detected in only 3 (25%) of 12 RA patients, while in SSD patients no DNA-hydrolyzing activity was detected.

In SLE patients, DNA-hydrolyzing activity positively correlated with the stage of the disease, primarily with the severity of clinical symptoms.

AAB isolated from the blood of MRL-lpr/lpr mice exhibited the highest DNA-hydrolyzing activity and transformed plasmid scDNA not only into relaxation circular (24%), but also into linear forms (76%) (Fig. 2). The presence of DNA-hydrolyzing activity correlated with the presence of cytotoxicity in all cases (Table 1). Incubation of scDNA with AAB isolated from the blood of SJL/J and (NZB×NZW)F_1 mice led to the appearance of single-strand breaks. AAB from non-autoimmune BALB/c mice virtually did not hydrolyze the substrate DNA and had no cytotoxic properties (Table 1).

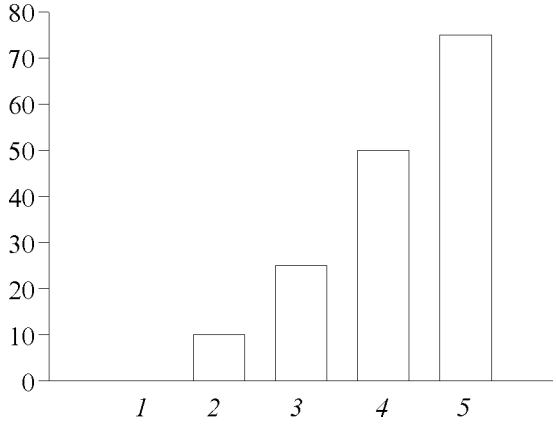

Fig. 1. Relationship between DNA-hydrolyzing activity and the depth of the substrate cleavage. *1*) plasmid DNA at the start (no activity); conversion of supercoiled into linear DNA: *2*) <10%; *3*) <25%; *4*) 25-50%; *5*) >50%.

Judging from the kinetics of scDNA hydrolysis by AAB from MRL-lpr/lpr mice, the efficiency of catalysis was 0.12 $nmol^{-1}$/min, which was comparable with that of polyclonal IgG antibodies from SLE patient. DNA-hydrolyzing activity positively correlated with the development of SLE symptoms, *i. e.* with evolution of the autoimmune process. Though autoimmune processes in MRL-lpr/lpr and (NZB×NZW)F_1 mice are similar and characterized by high content of DNA-binding AAB, high DNA-hydrolyzing activity was found only in MRL-lpr/lpr mice.

L929 target cells were the most sensitive to AAB.

Thus, comparative studies of cytotoxic and catalytic activities of DNA abzymes from SLE patients and autoimmune mice indicate direct participation of DNA abzymes in the pathogenesis and evolution of autoimmune states, the more so as the treatment of target cells with DNA abzymes from SLE patients induced DNA degradation (ladder formation) typical for apoptosis [4].

TABLE 1. Comparative Study of DNA-Hydrolyzing and Cytotoxic Properties of DNA-Binding AAB in Patients with SLE and Autoimmune MRL-lpr/lpr Mice with SLE-Like Syndrome

Parameter	Patients with SLE (*n*=68)	Donors (*n*=10)	Mice	
			MRL-lpr/lpr (*n*=15)	BALB/c (*n*=15)
Sera with DNA-binding AAB, %				
with DNA-hydrolyzing activity	59	0	51	0
with cytotoxic properties	59	0	49	0
Coincidence of catalytic and cytotoxic characteristics of AAB in a preparation, %	100	0	98	0

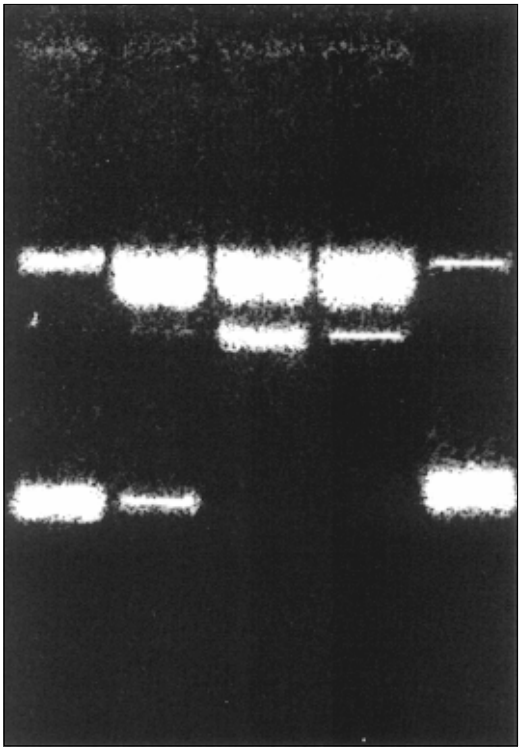

Fig. 2. DNA-hydrolyzing activity of antibodies isolated from BALB/c (*1*), (NZB×NZW)F_1 (*2*), MRL-lpr/lpr (*3*), and SJL/J mice (*4*). *5*) control plasmid incubated under similar conditions.

The results indicate the presence of anti-DNA AAB with catalytic and cytotoxic activities in autoimmune diseases. DNA abzymes differ by the mechanism of their action both in SLE patients and genetically different autoimmune mice. Of particular importance are strict correlation between catalytic and cytotoxic activities of AAB and positive correlation between these activities and the stage of the autoimmune process. The appearance of DNA abzymes and the dynamics of their content during SLE evolution are not always directly associated with autoimmune conflict, as is seen from the experiments on SJL/J mice, but are determined by immunogenetic factors in mice of this strain.

We investigated only catalytic and cytotoxic characteristics of DNA-binding AAB on clinical (SLE, RA, SSD) and experimental (SLE and RA) autoimmune models, which is explained by the presence of a variety of AAB in these diseases [2,8,9]. Involvement of DNA abzymes in the evolution of systemic autoimmune diseases was demonstrated on mouse model of SLE. Autoimmune MRL-lpr/lpr, SJL/J, and (NZB× NZW)F_1 mice are most perspective for further investigation of mechanisms of DNA abzyme cytotoxicity, their role in the regulation of apoptosis. Another object for further research is the function of DNA abzymes in SLE patients in the course of autoimmune process.

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