0273-2289/98/7501—0063$11.50

Catalytic DNA- and RNA-Hydrolyzing Antibodies from Milk of Healthy Human Mothers

VALENTINA N. BUNEVA,[1] TAT'YANA G. KANYSHKOVA,[1] ALEXANDER V. VLASSOV,[1] DMITRY V. SEMENOV,[1] DENIS YU. KHLIMANKOV,[1] LYUBOV' R. BREUSOVA,[2] AND GEORGY A. NEVINSKY*,[1]

[1]*Novosibirsk Institute of Bioorganic Chemistry, Siberian Division of Russian Academy of Sciences, Novosibirsk 630090, Lavrentieva Ave., 8, Russia, E-mail: Nev@niboch.nsc.ru;* [2]*Novosibirsk Specialized Hospital for Children, Novosibirsk, Russia*

Received December 8, 1997; Accepted June 22, 1998

ABSTRACT

Various catalytically active antibodies (Abs), or abzymes, have been detected recently in the sera of patients with autoimmune pathologies, in whom their presence is probably associated with autoimmunization. Normal humans are generally not considered to have abzymes, since no obvious immunizing factors are present. Here is shown by different methods that IgG from the milk of normal females possesses both DNase and RNase activities. The activities were also present in the IgG $F(ab')_2$ and Fab fragments.

Affinity modification of IgG by the chemically reactive derivative of an oligonucleotide led to preferential modification of the L chain of IgG. After separation of the subunits by sodium dodecyl sulfate electrophoresis in a gel containing DNA, an in-gel assay showed DNase activity in the L chain. The L chain separated by affinity chromatography on DNA-cellulose was catalytically active. These findings speak in favor of the generation of catalytic Abs by the immune system of healthy mothers. It is known that the treatment of adults with DNases and RNases offers protection from viral and bacterial diseases. Since breast milk protects the infants from infec-

*Author to whom all correspondence and reprint requests should be addressed.

tions until the immune system is developed, this raises the possibility that catalytic Abs like nucleases, may possess a protective role.

Index Entries: Human milk; DNase; RNase; IgG activities.

Nomenclature: Abs, antibodies; sIgA, secretory IgA; PAGE, polyacrylamide gel electrophoresis.

INTRODUCTION

The field of artificial and natural catalytically active antibodies (Abs), or abzymes, has been amply reviewed recently *(1–6)*. Patients with autoimmune diseases produce Abs to nucleoprotein complexes *(7)*, to DNA, and to enzymes that participate in nucleic acid metabolism *(8)*. In autoimmune diseases, there can be spontaneous induction of anti-idiotypic Abs, which are Abs elicited by a primary antigen (Ag). These anti-idiotypic Abs may have characteristics of the primary Ag, including catalytic activity.

Peptide- *(9)*, DNA- *(10)*, and RNA-hydrolyzing autoantibodies *(11)* were detected in the sera of patients with various autoimmune pathologies. In spite of no obvious immunizing factors that are found in normal humans, the authors have recently shown that the milk of normal human mothers contains secretory immunoglobulin A (sIgA) possessing protein kinase activity *(12)*.

It was clearly demonstrated that passive immunity in mice could be acquired both via the placenta before birth and through the milk after birth *(13)*. The immune status of the newborn varies from species to species, but all mammalian neonates are essentially agammaglobulinemic at their mucosal surfaces at birth. Milk contains a wide array of Abs to bacterial, viral, and protozoal Ags *(13–16)* with antimicrobial activity, which reach mucosal surfaces of intestinal and respiratory tract, and as a result protect the infants from infection and disease. The Abs are active at the mucosal surface both in dealing with the replication and colonization of pathogenic microorganisms and in limiting the access of environmental Ags. Passive immunity of the child may also be acquired as the result of mother's milk IgG after the Abs transfer across the epithelium of intestinal surfaces to the newborn circulatory system *(17)*.

Taking into account the above data, the authors hypothesized that healthy mothers may secrete catalytic Abs in breast milk. It is known that injection of nucleases into the circulatory system, as well as treatment of human respiratory mucosal surfaces with DNases and RNases, leads to protection against different viral and bacterial diseases *(18–21)*. Recently, an inverse correlation between mammary tumor incidence and the

amount of RNase activity in human milk was revealed (*22*). Like nucleases, RNA-hydrolyzing and DNA-hydrolyzing catalytic Abs, if present in breast milk, may contribute to the protective role of Abs through the hydrolysis of viral and bacterial nucleic acids. This article presents evidence that the IgG fraction from human milk is capable of catalyzing the hydrolysis of nucleic acids.

MATERIALS AND METHODS

Materials

All chemicals were of high-grade quality (from Merck [Darmstadt, Germany] or Euromedex [Souffelweyersherm, France]). Imidazole and bovine pancreatic ribonuclease (RNase A) were obtained from Boehringer Mannheim (Mannheim, Germany), RNase T1 from Worthington Biochemical (Freehold, NJ), radioisotopes (3000 Ci/mmol) were from Amersham (Little Chalfont, UK) and T4 polynucleotide kinase from Biolabs (Beverly, MA).

We also employed protein A-Sepharose, NTP, and dNTP (Sigma, Maidstone, UK), diethylaminoethyl (DEAE) cellulose DE-52 (Whatman [Maidstone, UK]), Sepharose 4B (Pharmacia, Uppsala, Sweden), Toyopearl HW-55 fine (Toyo Soda, Japan), Triton X-100 (Ferak, Berlin, Germany).

Antibody Purification

Samples of milk were taken from 35 healthy human mothers (19–35 yr old). The milk was collected within the period from 1 wk until 4 mo of lactation. After removing the lipid phase as described in ref. *12*, milk (100 mL) was loaded on a 7-mL protein A-Sepharose column equilibrated in TBS buffer (150 m*M* NaCl, 20 m*M* Tris-HCl, pH 7.4). Proteins adsorbed nonspecifically were eluted with this buffer containing 1% Triton X-100 and 0.3 *M* NaCl, followed by 0.1 *M* sodium citrate, pH 4.6. Antibodies were eluted in 50 m*M* glycine-HCl, pH 2.6. The column fractions were immediately neutralized and dialyzed against 10 m*M* Tris-HCl, pH 7.5. sIgA was removed from the IgG fraction on DEAE-cellulose (*12,23*), and the IgG was then further chromatographed on Toyopearl HW 55 (0.8 × 180 mm) in 20 m*M* Tris-HCl, pH 7.5, containing 0.2 *M* NaCl.

The amount of sIgA, IgG, and IgM Abs in bound and nonbound fractions from different adsorbents (and in different peaks under various chromatography conditions) was evaluated using the Ouchterlony double-immunodiffusion method against anti-IgA, anti-IgG, and anti-IgM Abs. At all steps of IgG purification, the different fractions of Abs were also analyzed using immunoblotting (*see* below).

Immunoblotting Analysis of Abs

Identification of IgG, IgA, and sIgA Abs, as well as the types of light (L) and heavy (H) chains of Abs, was carried out using specific antihuman Abs (Sigma), according to Towbin et al. *(24)*. Separation of proteins in SDS-polyacrylamide gels, and their subsequent transfer to a nitrocellulose membrane, was performed in a Trans-Blot apparatus (Bio-Rad, Hercules, CA), as described previously *(24)*. The nitrocellulose membrane containing the blotted proteins was then incubated with different conjugates of alkaline phosphatase with specific polyclonal Abs to IgA, IgM, and IgG κ and λ light chains for 2 h at 37°C. The membranes were then washed 5× with 50 m*M* sodium phosphate buffer, pH 7.5, containing 0.2 *M* NaCl. 1-Naphthyl phosphate and amido black were used for the staining of proteins.

DNA-Cellulose Chromatography

A glutaraldehyde-modified DNA-cellulose *(25)* column (8 mL) was equilibrated in 20 m*M* Tris-HCl, pH 7.5, and IgG applied to the adsorbent was eluted with a concentration gradient of NaCl (0–1 *M*) in the same buffer. In order to analyze the affinity of separated IgG L and H subunits for DNA, the IgG was dissociated by incubation at 0.7 mg IgG/mL in 50 m*M* Tris-HCl, pH 7.5, containing 0.3 *M* dithiothreitol (DTT) for 2 h at 25°C, addition of urea to a final concentration of 8 *M*, and incubation for a further 30 min. IgG subunits were then separated on the DNA-cellulose column using the above buffer (containing 0.3 *M* DTT and 5.0 *M* urea) and a NaCl gradient (0–1 *M*). The protein peaks were analyzed by SDS-PAGE and by immunoblotting.

DNA-Hydrolyzing Activity

Conditions for DNA and RNA hydrolysis were optimized by varying the concentrations of all reagents. The specific activity of IgG fractions in all cases was measured after dialysis of Abs. Reaction mixtures (20 μL) for analysis of specific DNA-hydrolyzing activity of IgG contained optimal concentrations of the standard components: 150 ng supercoiled pBR322 DNA, 5.0 m*M* $MgCl_2$, 1.0 m*M* EDTA, 20 m*M* 3-morpholinopropanesulfonic acid buffer, pH 7.5, and 1–3 μg of antibodies. Reactions were incubated for 2–6 h at 37°C. For screening of fractions during purification of IgG, 5 μL of each fraction was incubated in 20 μL standard reaction mixture. The cleavage products were analyzed by electrophoresis in 0.8% agarose gel. Ethidium bromide-stained gels were photographed and the films were scanned. The activities of IgG preparations were determined from the scanning data.

DNA- and RNA-Hydrolyzing Activity

For evaluation of the DNA- and RNA-hydrolyzing activities of Abs, various 5′-[^{32}P]ribo- and deoxyribooligonucleotides (20 pmol) were incubated with 50 μg/mL of IgG or its fragments in 25 μL reaction mixtures containing 20 m*M* Tris-HCl, pH 7.5, 5.0 m*M* $MgCl_2$ (or in the absence of $MgCl_2$), and 1.0 m*M* EDTA, for 2 h at 37°C. The products were analyzed in a 20% PAGE gel containing 8 *M* urea.

Transcript of human mitochondrial $tRNA^{Lys}$ with a point mutation C9A was prepared in vitro by transcription of the appropriate DNA species (i.e., the tRNA gene) by T7 RNA polymerase, according to Milligan et al. *(26)*, and labeled with [^{32}P], as in Vlassov et al. *(27)*. tRNA was then incubated in final volume of 20 μL with IgG (100 μg/mL), or with pancreatic RNase (7 μg/mL) in 50 m*M* Tris-HCl, pH 7.5, 0.1 m*M* EDTA, for 2 h at 37°C (in the presence or absence of 100 m*M* NaCl and 5 m*M* $MgCl_2$). tRNA cleavage products were resolved by electrophoresis in a 15% PAGE gel containing 8 *M* urea. The hydrolysis products were identified by autoradiography.

It is known that RNA hydrolysis by RNase T1 yields certain specific products *(26–28)*. Hydrolysis of RNA with imidazole, on the other hand, leads to cleavage of RNA at all possible sites with comparable efficiency and is known as statistical hydrolysis of RNA *(28)*. The authors used the product of partial hydrolysis of tRNA by RNase T1 and by imidazole to deduce the positions at which tRNA is cleaved by RNase A and IgG.

Partial hydrolysis of tRNA by *Aspergillus orysae* RNase T1 (50 μg/mL) was carried out according to Milligan et al. *(26)*. Partial hydrolysis of tRNA by imidazole was performed as in Vlassov et al. *(28)*.

Gel-Filtration of Antibodies After pH Shock

Abs (IgG purified on DNA-cellulose; 0.4–1.0 mg/mL, 0.2 mL) were incubated in 20 m*M* glycine-HCl, pH 2.6, containing 0.2 *M* NaCl, for 2 h at 25°C, and then chromatographed in this buffer on Toyopearl HW 55 (0.8 × 180 mm). The fractions were dialyzed against 20 m*M* Tris-HCl buffer, pH 7.5, and the nuclease activities were measured.

Affinity Labeling

Affinity labeling of the IgG was carried out in a 20-μL reaction mixture containing 5 μg IgG and 100 pmol of the [^{32}P]-labeled 5′-phospho-(4-dimethylaminopyridine) derivative of $d(pT)_{10}$ (or [^{32}P]$d(pT)_{10}$ as a control) in 20 m*M* 3-morpholinopropanesulfonic acid buffer, pH 7.5, 5 m*M* $MgCl_2$. After incubation for 30 min at 20°C, the reaction mixtures were analyzed by SDS-PAGE. The 5′-phospho-(4-dimethylaminopyridine) derivative of $d(pT)_{10}$ was synthesized as in Buneva et al. *(29)*.

In Situ Gel Assay of DNase Activity

DNase activity was detected in a 12% SDS-PAGE gel containing 20 μg/mL phage λ DNA, according to Rosenthal and Lacks *(30)*. SDS was removed by incubating the gel for 1 h at 37°C with 7 *M* urea. The gel was washed 5× with water. To allow protein renaturation, the gel was incubated for 16 h at 37°C in 20 m*M* Tris-HCl buffer, pH 7.5, containing 5.0 m*M* $MgCl_2$ and 1.0 m*M* EDTA. To reveal the products of the DNA hydrolysis, the gel was stained with ethidium bromide *(25)*. The positions of the IgG subunits on the gel were revealed by Coomassie blue staining.

Preparation of Antibody Fab and $F(ab')_2$ Fragments

The $F(ab')_2$ fragment was obtained by pepsin cleavage *(31)*, and was subsequently purified using gel filtration and DNA-cellulose, as described above. The Fab fragment was obtained by papain cleavage *(32)* and by purification on protein-A Sepharose, DEAE-cellulose, and Sepharose columns with immobilized anti-L chain of IgG Abs *(32)*. $F(ab')_2$ and Fab fragments were electrophoretically homogeneous.

SDS-PAGE Analysis

SDS-PAGE analysis of IgG fractions in nonreducing conditions was carried out in 7–16% gradient gel (0.1% SDS). SDS-electrophoresis for the polypeptide spectrum analysis of Abs was performed in a reducing 12% gel (in the presence of 0.1% SDS and 0.1% 2-mercaptoethanol). In all cases, the Ab samples were boiled for 5 min prior to the electrophoresis in 50 m*M* Tris-HCl buffer, pH 8.2, containing 2% SDS, in the presence or absence of 0.1% 2-mercaptoethanol, respectively.

Binding of Catalytic IgG to Antibodies to L Chain

Binding of catalytic Abs to Sepharose containing immobilized monoclonal Abs against κ or λ L chain of IgG was analyzed as follows. After purification of IgG using DNA-cellulose chromatography, 0.5 mg Abs was loaded on a 2-mL anti-L chain Sepharose column (anti-κ or anti-λ) equilibrated in TBS buffer. The IgG fraction adsorbed nonspecifically was eluted with this buffer containing 1% Triton X-100 and 0.3 *M* NaCl. Abs bound to the immobilized Abs against κ or λ chains were eluted in 50 m*M* glycine-HCl, pH 2.6. The column fractions were immediately neutralized and dialyzed against 10 m*M* Tris-HCl, pH 7.5, and the DNase and RNase activities of IgG were measured.

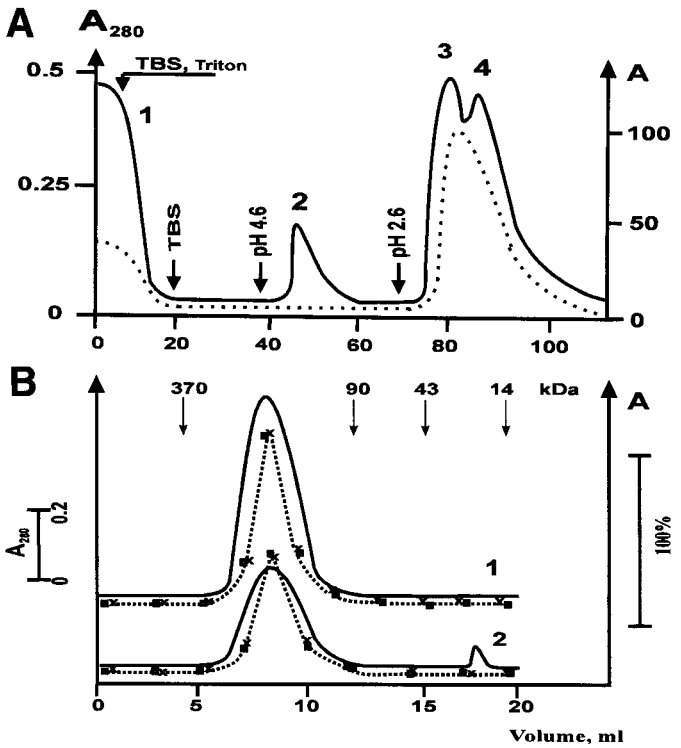

Fig. 1. Purification of IgG from human milk. **(A)** The milk was chromatographed on protein A-Sepharose. Fractions 3 and 4 were pooled, sIgA was removed on DEAE cellulose, and the protein was subjected to gel filtration **(B)** on Toyopearl HW 55 (profile 1). Profile 2 in B was observed using IgG purified on DNA-cellulose subjected to pH 2.4 shock. (—), Absorption at 280 nm; (....) and (■), DNA-cleaving activity using pBR-322 plasmid; (x), RNA-cleaving activity using 5′-[^{32}P](pA)$_{13}$ substrate (compared to the activity of the fraction having maximal activity [100%]).

RESULTS AND DISCUSSION

To search for nuclease Abs in the milk of healthy mothers, the IgG fraction was purified by chromatography on protein A-Sepharose under conditions designed to remove nonspecifically bound proteins (Fig. 1A), followed by DEAE-cellulose chromatography *(23)*, and by gel filtration *(10)* to remove secretory IgA. The purified IgG fraction contained 0.5–1.5% of the total milk Abs.

Catalytic Abs were bound by protein A-Sepharose more strongly, in comparison with IgG without catalytic activity. According to electrophoretic and immunoanalysis data, protein peak 2 eluted by acidic buffer, pH 4.6 (Fig. 1A), was composed of IgG, and did not possess RNase or DNase activity. Adsorbed Abs (4–10% of the milk Abs; 95–97% of the initial milk Ab nuclease activities) were eluted with glycine-HCl buffer, pH 2.6.

The authors have not found any detectable level of DNase activity in IgG from the sera of 50 normal human (men and women). It should be mentioned that the extent of DNase and RNase activities of IgG purified from milk (35 samples) were dependent very much on donors. Nevertheless, in contrast to IgG from the blood of normal humans, IgG of all 35 milk samples had detectable levels of nuclease activity. The specific DNase activities of milk IgG were 0.05–10% that of DNase II.

The IgG from maternal sera during pregnancy also possessed DNase activity (detailed data to be published elsewhere).

The purity of a typical IgG preparation was confirmed by SDS-PAGE *(33)*, which showed a single 150-kDa band in a nonreducing condition (Fig. 2D), and two bands corresponding to the H and L chains after reduction (Fig. 2E). Chromatography on DNA-cellulose showed that the IgG was composed of Ab fractions having different affinity for DNA: 40–70% of the IgG from 30 different donors bound to the column. The amount of protein recovered in peaks 1–3 (Fig. 2A) was variable in different milk donors.

Usually, strong noncovalent protein complexes dissociate under acidic conditions. The IgG was incubated at pH 2.6 to dissociate noncovalent interactions, and to ensure that other proteins were not tightly bound to it. A single protein peak was observed upon gel filtration (Fig. 1B). The IgG was found to possess DNase (and RNase, *see* below) activity at every step of purification (Fig. 3A). After the final step of IgG purification at pH 2.6 (Fig. 1B), it contained about 80% of the initial DNase activity loaded on the gel-filtration column.

DNase activity was also present in the homogeneous $F(ab')_2$ and Fab fragments (Fig. 2D) of milk IgG purified using several different chromatography procedures (Fig. 2B).

In an earlier example using creatine kinase, the authors developed a method to separate the enzyme subunits displaying different affinities for ATP by fractionation on ATP-Sepharose, after dissociation of the oligomer with urea *(34)*. Similar studies on IgG showed that the DNase activity resided in the L chain, as shown by purification on DNA-cellulose after dissociation of IgG oligomer with urea and DTT (Fig. 2C, peak 3). The L chain showed the correct mobility on SDS-PAGE (Fig. 2E) and reacted positively with specific anti-L-chain antibodies. About 90–95% of purified IgG (and its DNase and RNase activities) was adsorbed by Sepharose with immobilized monoclonal Abs against κ chains immobilized on Sepharose, and only 5–10% of the IgG was adsorbed by immobilized Abs to λ chains (data not shown).

Two further methods provided direct evidence that the L chain possessed DNase activity. First, after separation of the subunits by SDS-PAGE in a gel containing DNA, an in-gel assay showed DNase activity in the L chain (Fig. 3C). The nuclease activity was revealed after staining with

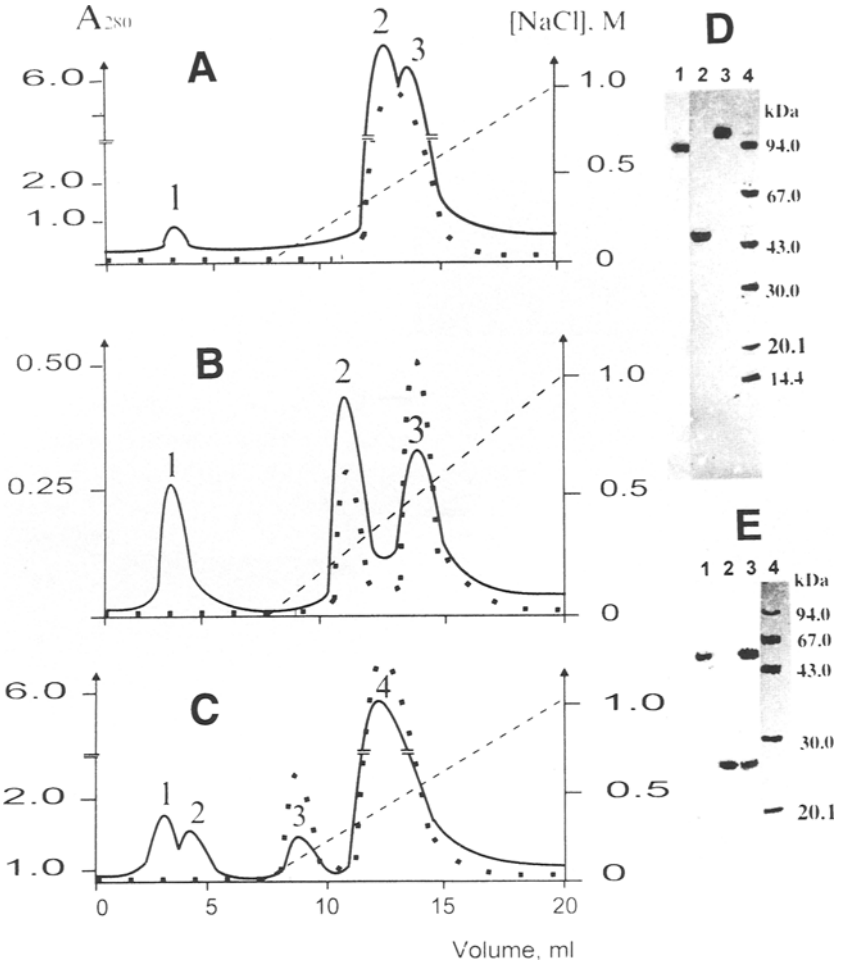

Fig. 2. Chromatography of IgG and its subfragments on DNA-cellulose. **(A)** IgG purified after gel filtration and DEAE-cellulose chromatography. **(B)** $F(ab)_2$ fragment; **(C)** IgG dissociated into subunits using 0.3 *M* DTT and 8 *M* urea (*see* Methods). **(D)** Silver-stained SDS-PAGE in a nonreducing 7–16% gradient gel of: $F(ab)_2$ fragment (lane 1); Fab fragment (lane 2); IgG after chromatography on DNA-cellulose (lane 3); molecular mass markers (lane 4). **(E)** SDS-PAGE of peak fractions from panel C in a reducing 12% gel: peak 2 (lane 1); peak 3 (lane 2); peak 4 (lane 3); molecular mass markers (lane 4).

ethidium bromide as a sharp dark band on a fluorescent background of DNA bound to ethidium bromide. Second, the L chain became labeled after incubation of IgG with a [^{32}P]oligonucleotide affinity probe for the active site of DNase, i.e., the 5′-phospho-(4-dimethylaminopyridine) derivative of 5′-[^{32}P]$d(pT)_{10}$ (Fig. 3B).

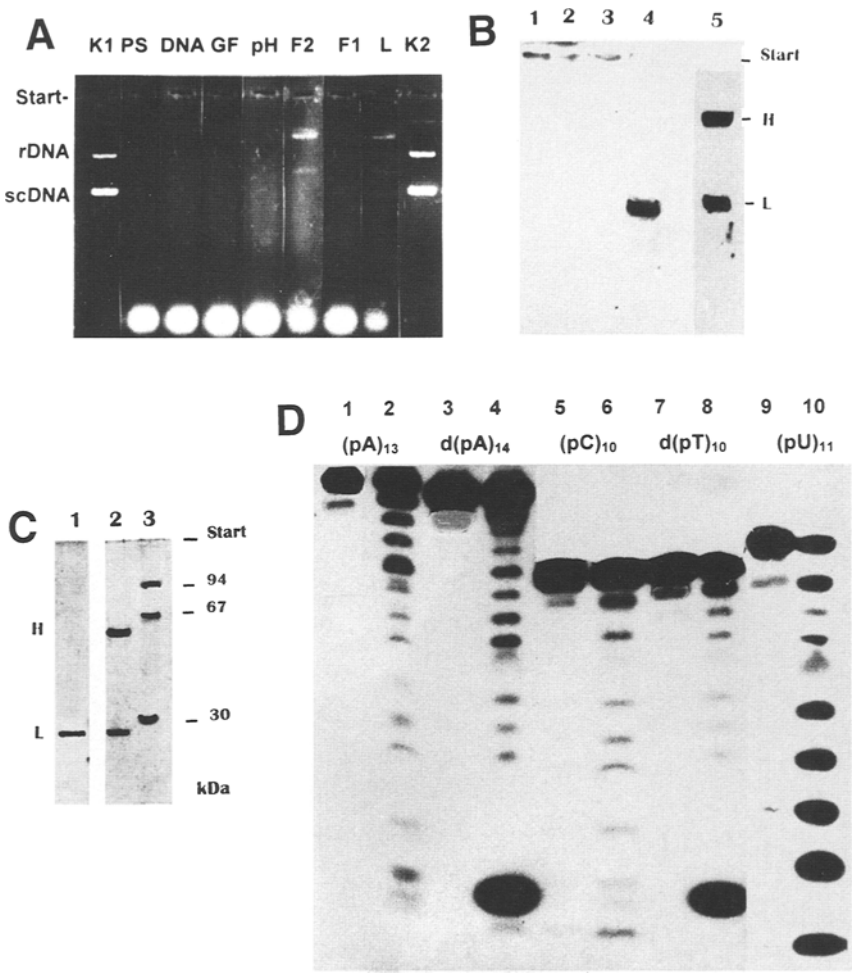

Fig. 3. DNase and RNase activities of IgG and its fragments. **(A)** cleavage of supercoiled (sc) and relaxed (r) pBR322 DNA by IgG (50 μg/mL) after different purification steps. PS, protein A-Sepharose; DNA, DNA-cellulose; GF, gel filtration; pH, incubated at pH 2.4; F2 and F1, $F(ab)_2$ and Fab fragments, respectively; L, purified L chain; K_1 and K_2, pBR322 DNA incubated without antibodies. **(B)** affinity labeling of the L chain after incubation of IgG with [^{32}P]-labeled 5′-phospho-(4-dimethylaminopyridine) derivative of $d(pT)_{10}$: autoradiographs of SDS-PAGE gel of IgG, respectively, before and after incubation with [^{32}P]$d(pT)_{10}$ (lanes 1 and 2, respectively); IgG before and after incubation with 5′-phospho-(4-dimethylaminopyridine) derivative of [^{32}P]$d(pT)_{10}$ (lanes 3 and 4, respectively); silver-stained IgG under reducing conditions (lane 5). **(C)** In gel DNase activity of the L chain after separation of IgG by SDS-PAGE in a gel containing DNA. DNase activity revealed as a sharp dark band on a fluorescent background by ethidium bromide staining (lane 1); Coomassie blue-stained IgG under reducing conditions (lane 2); molecular mass markers (lane 3). **(D)** Cleavage of 5′-[^{32}P]oligonucleotides by milk IgG. Odd- and even-numbered lanes show the products after incubation without and with IgG, respectively.

The properties of the DNase of milk IgG distinguished it from other known DNases. The IgG hydrolyzed DNA within the entire pH range tested (6.0–9.0), with an optimum at pH 7.0–7.2, a value markedly higher than that of human blood DNase II (pH optimum 5.2; *[18]*). Although DNase I is metal-dependent *(18)*, the DNase activity of milk IgG was only slightly activated by Mg^{2+} or Mn^{2+}, and cleavage of oligodeoxyribonucleotides was strongly stimulated by EDTA (Fig. 4A), or after passage through Chelex (Bio-Rad) (data not shown).

Milk IgG, as well as its $F(ab')_2$ and Fab fragments, was found to possess not only DNase, but also RNase activity at every step of purification. After pH shock of IgG, the gel filtration experiments showed only one protein peak containing about 80% of the initial IgG activity in hydrolysis of 5′-[^{32}P](pA)$_{13}$ (Fig. 1B).

Various oligoribo- and oligodeoxyribonucleotides were cleaved by IgG with comparable efficiencies (Figs. 3D and 4A). In contrast, the 80-kDa and 14-kDa RNases reported in human milk *(22)*, like pancreatic RNase A, cannot hydrolyze oligo(A). Unlike all known RNases, milk IgG was not capable of hydrolyzing cCMP (data not shown).

The IgG from serum of patients with systemic lupus erythematosus hydrolyzes homo-oligoribonucleotides 20–50× faster than oligodeoxyribonucleotides; the best substrates are d(pA)$_n$ and (pA)$_n$, and hydrolysis of d(pT)$_n$ and (pU)$_n$ is 10–15× slower *(11)*. In addition, the RNase activity of milk IgG could be distinguished from RNase A and RNase T1 by its $tRNA^{Lys}$ cleavage pattern: Cleavage was more pronounced in the region between nucleotides 52–56, although the major cleavage positions were similar (Fig. 4B and C). In order to identify the tRNA cleavage positions in RNase and catalytic Abs, the authors studied the partial statistical hydrolysis of tRNA by imidazole and specific hydrolysis by RNase T1 (Fig. 4B). Cleavage by IgG between nucleotides 52–53 and 55–56 was stimulated by NaCl and $MgCl_2$ (Fig 4B, lane 2); cleavage by RNase A was essentially completely inhibited (lane 4).

These data taken together show that the DNase and RNase activities of milk IgG are intrinsic properties of the L chain of the IgG, and are not caused by co-purifying nucleases. The specific activities of the DNase and RNase with polymer substrates were observed to vary from 0.05 to 10% in the IgG from 35 milk samples studied, compared to the activities of DNase II and RNase A. The K_m values for d(pT)$_{10}$ (0.5–1.5 μM) and (pU)$_{10}$ (0.1–0.5 μM) and k_{cat} values $3 - 14 \times 10^{-2}$/min and $2 - 10 \times 10^{-2}$/min, respectively, were estimated for three preparations of Fab fragment.

It is plausible that these two catalytic Ab activities reside in the same protein, because the authors have shown recently *(35)* that monoclonal lupus Abs, which recognized specific DNA sequences, show both activities,

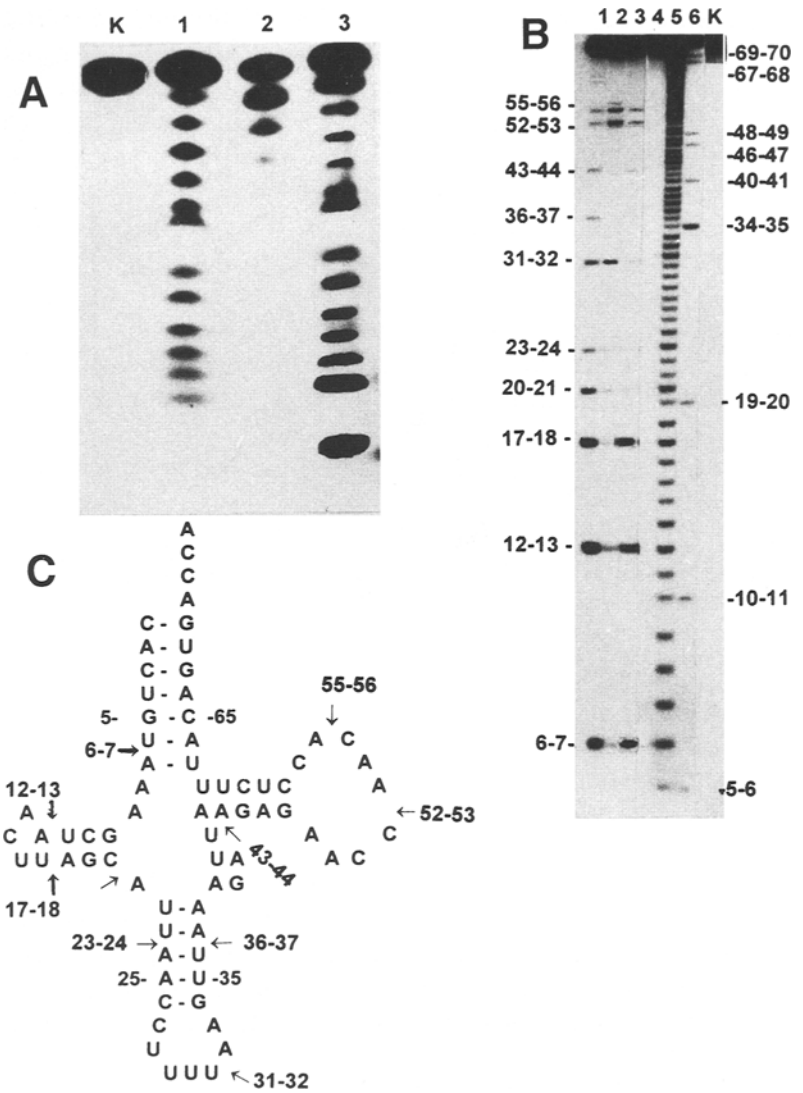

Fig. 4. Characteristics of the DNase and RNase activities of IgG and Fab fragment (50 μg/mL). **(A)** Hydrolysis of 5′-[^{32}P]d(pA)$_{14}$. Oligonucleotide incubated alone (lane K); Fab (lane 1); IgG (lanes 4 and 4); IgG + 20 m*M* EDTA (lane 3). **(B)** Partial hydrolysis of human 5′-[^{32}P]tRNA^{Lys} by milk IgG (100 μg/mL) (lanes 1 and 2) and RNase A (7 μg/mL) (lanes 3 and 4), in the absence (lanes 1 and 3) or in the presence (lanes 2 and 4) of 100 m*M* NaCl and 5 m*M* $MgCl_2$; imidazole (lane 5); RNase T1 (lane 6); tRNA incubated alone (lane K). **(C)** The cloverleaf structure of tRNA^{Lys} showing the sites of tRNA major cleavages by RNase A and by milk IgG.

with the RNase activity being 30–40× greater than the DNase activity. On the other hand, a mixture of different catalytic Abs which hydrolyze either DNA or RNA, may be present in milk IgGs, because these are polyclonal.

These observations demonstrate that DNA- and RNA-hydrolyzing catalytic Abs are intrinsic components of IgG derived from healthy human mother's milk. Since it is known that DNase and RNase therapy of patients leads to protection against different viral and bacterial diseases, the nuclease activities of Abs raise the possibility that these Abs may provide additional protective functions for the newborn.

ACKNOWLEDGMENTS

The research was made possible in part by a grant (95-04-12950) from the Russian Fund of Fundamental Researches and a grant from the Siberian Division of the Russian Academy of Sciences. The authors wish to thank R. Giege for tRNA preparations.

REFERENCES

1. Tramontano, A., Janda, K. D., and Lerner, R. A. (1986), *Science* **234,** 1566–1569.
2. Lerner, R. A., and Tramontano, A. (1981), *TIBS* **12,** 427–438.
3. Lerner, R. A., Benkovic, S. J., and Shultz, P. J. (1991), *Science* **252,** 659–667.
4. Benkovic, S. J. (1992), *Annu. Rev. Biochem.* **61,** 29–54.
5. Hilvert, D. (1992), *Pyre Appl. Chem.* **64,** 1103–1113.
6. Suzuki, H. (1994), *J. Biochem.* **115,** 623–628.
7. Reimer, C. Raska, I., Tan, E. M., and Sheer, U. (1987), *Virchows Arch.* **54,** 131–136.
8. Earnshow, W. C., and Rothfield, N. (1985), *Chromosoma* **91,** 313–320.
9. Paul, S., Volle, D. J., Beach, C. M., Johnson, D. R., Powell, M. J., and Massey, R. J. (1989), *Science* **244,** 1158–1161.
10. Shuster, A. M., Gololobov, G. V., Kvashuk, O. A., Bogomolova, A. E., Smirnov, I. V., and Gabibov, A. G. (1992), *Science* **256,** 665–667.
11. Buneva, V. N., Andrievskaya, O. A., Romannikova, I. V., Gololobov, G. V., Yadav, R. P., Yamkovoi, V. I., and Nevinsky, G. A. (1994), *Mol. Biol.* **28,** 483–486.
12. Kit, Yu. Ya., Semenov, D. V., and Nevinsky, G. A. (1995), *Mol. Biol.* **29,** 519–526.
13. Saif, L. J. and Bohl, E. H. (1979), in *Immunology of Breast Milk,* Ogra, P. L. and Dayton, D. H., ed., Raven, New York, pp. 237–255.
14. Redhead, K., Hill, T., and Mullroy, B. (1990), *FEMS Microbiol. Lett.* **70,** 269–274.
15. Gillin, F. D., Reiner, D. S., and Wang, C. S. (1983), *Science* **221,** 1290–1292.
16. Fiat, A.-M., and Jolles, P. (1989), *Mol. Cell. Biochem.* **87,** 5–9.
17. Brambell, F. W. (1970), in *The Transmission of Passive Immunity from Mother to Young.* Neuberger, A. and Tatum E. L., ed., North-Holland, Amsterdam and London.
18. Shapot, V. S. (1968), in *Nucleases,* Medicine Press, Moscow, pp. 7–125.
19. Glukhov, B. M., Ierusalimskii, R. P., and Salganik R. I. (1968) *J. Neurol. Psychiatr.* (Russian) **10,** 361–368.
20. Mashkovsky, M. D. (1984) in *Medical Preparations,* Medicine Press, Moscow, **2,** 53–54.
21. Lobzin, V. S., and Sichko, J. V. (1969) *Medical Approaches (Vrachebnoe delo),* **10,** 38–41.
22. Ramaswamy, H., Swamy, Ch. V. B., and Das, M. R. (1993), *J. Biol. Chem.* **268,** 4181–4187.
23. Friemel, F. (1984), in *Immunologische Arbeitsmethogen.* Veb Gustav Fisher Verlag Jena.
24. Towbin H., Staehein T., and Gordon J. (1979) *Proc. Natl. Acad. Sci. USA,* **76,** 4350–4354.
25. Gololobov, G. V., Chernova, E. A., Schourov, D. V., Smirnov, I. V., Kudelina, I. A., and Gabibov, A. G. (1995), *Proc. Natl. Acad. Sci. USA* **92,** 254–257.

26. Milligan, J. F., Groebe, D. R., Witherell, G. W., and Uhlenbeck, O. C. (1987), *Nucleic Acids Res.* **15,** 8783–8798.
27. Vlassov, V. V., Giege, R., and Ebel, J.-P. (1981) *Eur. J. Bioch.* **119,** 51–59.
28. Vlassov, V. V., Zuber, G., Felden, B., Behr, J.-P., and Giege, R. (1995), *Nucl. Acids Res.* **23,** 3161–3167.
29. Buneva, V. N., Godovikova, T. S., and Zarytova, V. F. (1986), *Bioorgan. Chemistry (Russian)* **12,** 906–910.
30. Rosenthal, A. L. and Lacks, S. A. (1977), *Anal. Biochem.* **80,** 76–82.
31. Harlow, E. and Lane, D. (1988), in *Antibodies: A Laboratory Manual.* Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, pp. 630–631.
32. Lee, J. S., Dombrovskii, D. F., and Mosmann, T. (1982) *Biochemistry* **21,** 4940–4947.
33. Cathou, R. E. (1978), in *Immunoglobulins,* Litman, K. W., and Good, R. A., ed., New York: Plenum, p. 100.
34. Nevinsky, G. A., Ankilova, V. N., Lavrik, O. I., Mkrtchyan, Z. S., Nersesova, L. S., and Akopyan J. I. (1982), *FEBS Lett.* **149,** 36–40.
35. Andrievskaya, O. A., Buneva, V. N., Yamkovoi, V. I., and Nevinskii, G. A. (1997), *Dokl. Ross. Akad. Nauk. (Russia)* **355,** 401–303.