DNA-hydrolyzing activity of the light chain of IgG antibodies from milk of healthy human mothers

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Abstract Various catalytically active antibodies or abzymes have been detected recently in the sera of patients with several autoimmune pathologies, where their presence is most probably associated with autoimmunization. Normal humans are generally considered to have no abzymes, since no obvious immunizing factors are present. Recently we have shown that IgG (its Fab and $F(ab)_2$ fragments) from the milk of normal humans possesses DNase activity. Here we demonstrate for the first time that the light chain of IgG catalyzes the reaction of DNA hydrolysis. These findings speak in favor of the generation of abzymes in the tissue of healthy mothers, and since a mother's breast milk protects her infant from infections until the immune system is developed, they raise the possibility that these abzymes may contribute to this protective role.

Key words: Human milk; DNase activity; Light chain; IgG antibody

1. Introduction

The field of catalytically active antibodies or abzymes has been amply reviewed recently [1–6]. Patients with autoimmune diseases produce antibodies (Abs) to nucleoprotein complex [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, including catalytic activity. Peptide- [9], protein- [10], DNA- [11–13] and RNA-hydrolyzing auto-Abs [14–16] were detected in the sera of patients with several autoimmune pathologies. Since no obvious immunizing factors are found in normal humans, they are considered to have no abzymes.

Human milk provides the breast-fed child not only with nutrients but also with a mucosal immune system. Milk contains Abs to bacterial, viral, and protozoal antigens [17–20], which reach mucosal surfaces in the respiratory and gastrointestinal tracts and as a result protect the infants from infections and diseases. Passive immunity of the child may be acquired also from IgG in the mother's milk after Abs transfer across the epithelium of the intestinal surface to the newborn circulatory system [21].

Taking into account the above data, we supposed that it is in principle possible that abzymes are present in breast milk [22–25]. First the ability of sIgA purified from the milk of healthy human mothers to phosphorylate milk proteins was shown to be a property of the Abs [24,25]. Recently we have shown that DNA- and RNA-hydrolyzing IgG is also present in human milk [22,23,26]. In the present article we demonstrate by different methods that active centers, catalyzing DNA hydrolysis, are located on light chains of the IgG antibodies.

2. Materials and methods

In this work we used DEAE-cellulose DE-52 (Whatman), Toyopearl HW-55 fine (Toyo Soda), pBR322 plasmid DNA and phage λ DNA (Sib-Enzyme, Novosibirsk), all other chemicals were from Sigma or Pharmacia.

The mixture (20 μl) for analysis of DNA-hydrolyzing activity of IgG containing 150 ng supercoiled pBR322 DNA, 5 mM $MgCl_2$, 1 mM EDTA, 20 mM MOPS buffer, pH 7.0, and 2–3 μg of Abs was incubated for 1–5 h at 37°C. The cleavage products were analyzed by electrophoresis in 0.8% agarose gel. Ethidium bromide-stained gels were photographed and the films were scanned.

Several steps of IgG purification from human milk (100 ml, see below) were done as in [26] and then using a DNA-cellulose column (8 ml), which was equilibrated in 20 mM Tris-HCl, pH 7.5. Abs were eluted with a concentration gradient of NaCl in the same buffer. The $F(ab)_2$ and F(ab) fragments were obtained and purified as described in [26].

IgG subunits were dissociated by incubation at 0.7 mg IgG/ml in 50 mM Tris-HCl, pH 7.6, containing 0.3 M DDT for 2 h at 30°C, addition of urea to 8 M, and incubation for a further 30 min. Then IgG subunits were separated on a DNA-cellulose column using the above buffer supplemented with 0.3 M dithiothreitol (DTT) and 5 M urea.

Affinity labeling of the light chain was carried out in a 20 μl reaction mixture containing 5 μg IgG and 100 pmol of 5′-phospho-(4-dimethylaminopyridine) derivative of 5′-[^{32}P]d$(pT)_{10}$ (or [^{32}P]d$(pT)_{10}$) in 20 mM MOPS, pH 7.0, 5 mM $MgCl_2$, incubated for 30 min at 20°C and analyzed by SDS-PAGE in reducing conditions.

DNase activity in the 12% SDS-PAGE gel containing 20 μg/ml phage λ DNA was detected as in [27]: SDS was removed by incubating the gel for 1 h at 37°C with 7 M urea, the gel was washed 5 times with H_2O, and then to allow protein renaturation it was incubated for 16 h at 37°C; 20 mM Tris-HCl buffer, pH 7.5, containing 5.0 mM $MgCl_2$ and 1.0 mM EDTA. For revealing DNA hydrolysis the gel was stained with ethidium bromide according to [27]. Then, the position of the IgG subunits on the gel was revealed by Coomassie staining.

3. Results and discussion

The pool of human milk Abs consists of 2–3, 1–2 and 93–96% of IgG, IgM and sIgA antibodies, respectively [28]. To search for abzymes in the milk of healthy mothers, the IgG fraction was purified by chromatography on protein A Sepharose in conditions to remove non-specifically bound proteins, followed by DEAE-cellulose and by gel filtration on Toyopearl HW 55 to remove sIgA [26]. The purity of the

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Abbreviations: Abzyme, catalytically active antibody; Ab, antibody; SDS-PAGE, SDS-polyacrylamide gel electrophoresis

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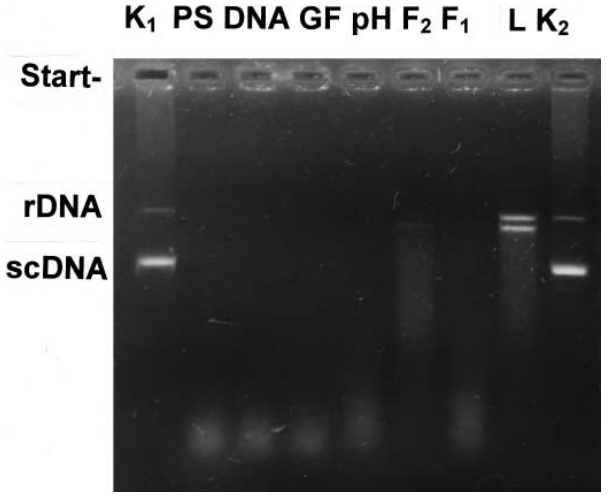

Fig. 1. DNase activities of IgG and its fragments: cleavage of supercoiled (sc) and relaxed (r) pBR322 DNA by IgG after different purification steps (see text): PS, protein A Sepharose; DNA, DNA-cellulose; GF, gel filtration; pH, abzymes incubated at pH 2.4 and then gel-filtered in the same buffer; F1 and F2, Fab and $F(ab)_2$ fragments of IgG, respectively; L, purified light chain (Fig. 2b); K_1, pBR322 DNA before incubation; K_2, DNA incubated alone (all mixtures were incubated for 4 h).

150 kDa IgG was confirmed by SDS-PAGE, which showed a single band in non-reducing conditions and two bands corresponding to the heavy and light chains after reduction. The IgG was found to possess DNase activity at every step of purification (Fig. 1) and after the final step of gel filtration at pH 2.4 (single peak in conditions of non-covalent protein complex dissociation) it contained about 80% of the DNase activity loaded on the column [26]. DNase activity was also present in homogeneous $F(ab)_2$ and Fab fragments of the milk IgG (Fig. 1).

The properties of the DNase of the milk IgG distinguished it from other known DNases. Its pH optimum was 7.0–7.2, a value markedly higher than that (5.2 [28]) of human blood DNase II, and whereas DNase I is metal-dependent [28] the DNAse activity of the milk IgG was only slightly activated by Mg^{2+} or Mn^{2+} and cleavage of oligodeoxyribonucleotides was strongly stimulated by EDTA or after passage through Chelex [26]. These data taken together showed that the DNase activity of milk IgG is an intrinsic property of the IgG, and is not due to copurifying DNases [25,26,28].

In order to analyze affinity of purified (as described above) abzymes to DNA, IgG was chromatographed on DNA-cellulose. Fig. 2 demonstrates that the IgG possesses a heterogeneous affinity for DNA: 40–70% of the IgG from 30 different

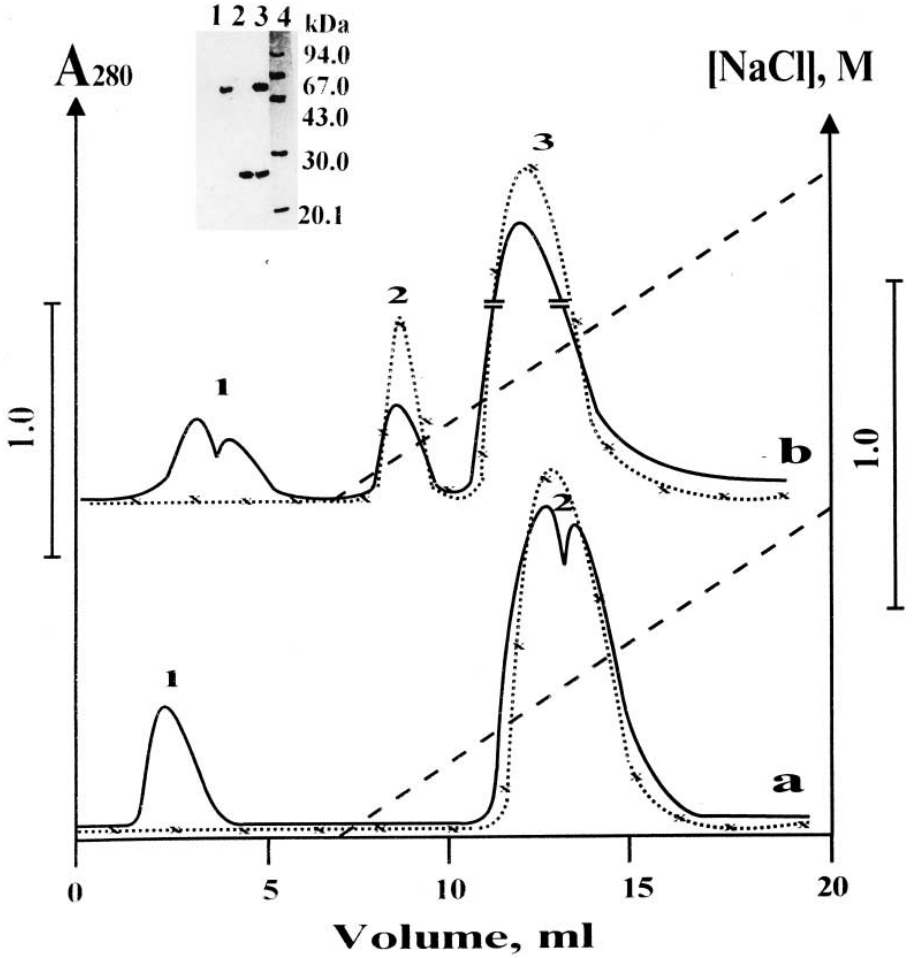

Fig. 2. Chromatography of the abzymes and their subfragments on DNA-cellulose. a: IgG after purification by gel filtration. b: DNA-purified IgG after dissociation into subunits. Inset: SDS-PAGE of peak fractions from (b) in the reducing 12% gel: lanes 1–3 correspond to peaks 1–3, (b); 4, molecular mass markers.

donors bound to the column, the relative amounts in peaks 1 and 2 varying between donors. The relative activity of abzymes in hydrolysis of DNA after DNA-cellulose was comparable to that at the previous steps of IgG purification (Figs. 1 and 2).

The DNase activity resided in the light chain of the IgG, as shown by purification on DNA-cellulose after dissociation of IgG with urea and DTT (Fig. 2, curve b, peak 2). The light chain showed the correct mobility on SDS-PAGE (Fig. 2; inset) and reacted with anti-light chain Abs (not shown). The relative specific activity of preparations of separated light chain was usually lower than that for initial IgG (Fig. 1, line L and Fig. 2, peak 2).

Two further methods provided direct evidence that the light 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 light chain (Fig. 3).

Second, the light chain became specifically labeled after incubation of IgG with the phosphorylating affinity reagent 5′-phospho-(4-dimethylaminopyridine) derivative of 5′-[^{32}P]d(pT)$_{10}$ (Fig. 4).

It should be mentioned that 90–95% of purified IgG (and its DNase activity) was adsorbed by Sepharose with immobilized monoclonal Abs against χ- and only 5–10% against λ-light chain of human IgG. The data speak in favor of the fact that the milk DNA-hydrolyzing abzymes contain χ-type IgG light chains.

These observations demonstrate that DNA-hydrolyzing abzymes are intrinsic components of IgG derived from healthy human mother's milk.

It should be mentioned that specific DNase activities of the IgG were 0.5–20% (means of 35 milk samples; only in individual milk samples they were higher) of that of DNase I, and varied very much for different donors.

We have not found any detectable level of DNase activity in IgG from the blood of normal humans (men and women). IgG from mother's sera possessed DNase activity, which was lower, comparable or higher than that for IgG from mother's milk depending on the donor (detailed data will be published separately).

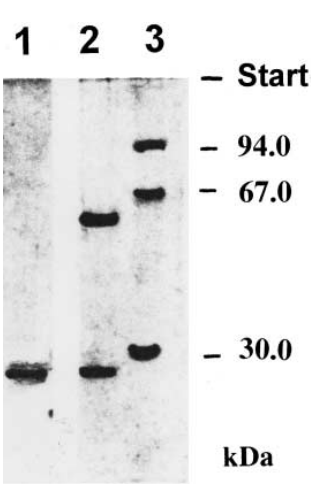

Fig. 3. DNase activity of the light chain after separation by SDS-PAGE in a gel containing DNA; 1, DNase activity revealed as a sharp dark band on a fluorescent background after ethidium bromide staining; 2, gel stained with Coomassie; 3, molecular mass markers.

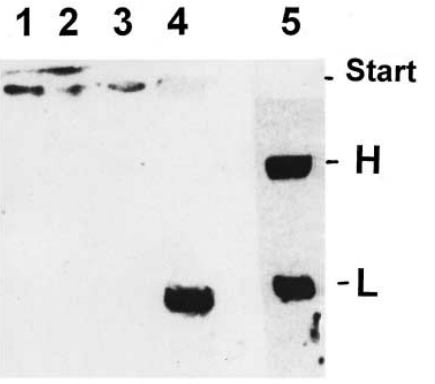

Fig. 4. Affinity labeling of the light chain at incubation of IgG with ^{32}P-labeled 5′-phospho-(4-dimethylaminopyridine) derivative of d(pT)$_{10}$: 1 and 2, autoradiographs of a SDS-PAGE gel of IgG before and after incubation with [^{32}P]d(pT)$_{10}$; 3 and 4, the same before and after incubation of IgG with the probe; 5, gel stained with silver.

So far an important question remains unanswered: what is the biological role of the abzymes in the blood of patients with various autoimmune diseases? Is it a function or a dysfunction of autoimmune Abs?

It is known that an increased amount of DNases and RNases in human blood or nuclease therapy of patients leads to protection against different viral and bacterial diseases [29–31]. Recently, an inverse correlation between mammary tumor incidence and amount of RNase activity in the milk plasma was revealed [32]. One can suppose that the milk DNA- and RNA-hydrolyzing abzymes are capable not only of neutralizing viral and bacterial nucleic acids due to complex formation with them, but also of hydrolyzing them. The DNA-hydrolyzing activity of Abs raises the possibility that these abzymes may provide protective functions for the newborn through the hydrolysis of viral and bacterial nucleic acids. Thus, the DNA-hydrolyzing activity of milk abzymes probably may have a positive function.

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