Biochimie 91 (2009) 1081–1086

Contents lists available at ScienceDirect

Biochimie

journal homepage: www.elsevier.com/locate/biochi

Research paper

HIV-1 integrase-hydrolyzing antibodies from sera of HIV-infected patients

Svetlana V. Baranova [a], Valentina N. Buneva [a,b], Marina A. Kharitonova [c], Ludmila P. Sizyakina [c], Christina Calmels [d], Marie-Line Andreola [d], Vincent Parissi [d], Georgy A. Nevinsky [a,b,*]

[a] Institute of Chemical Biology and Fundamental Medicine, Siberian Division of Russian Academy of Sciences, Novosibirsk, Russia
[b] Novosibirsk State University, Novosibirsk, Russia
[c] Scientific Research Institute of Clinical immunology of Rostov-na-Donu State Medical University, Rostov-na-Donu, Russia
[d] UMR 5097 CNRS-Université Victor Segalen Bordeaux 2, 146 rue Léo Saignat, 33076 Bordeaux cedex, France and IFR 66 Bordeaux, France

ARTICLE INFO

Article history:
Received 20 March 2009
Accepted 29 June 2009
Available online 4 July 2009

Kewords:
Human blood antibodies
HIV-infected patients
Catalytic IgGs
Hydrolysis of HIV integrase

ABSTRACT

Autoantibodies with enzymic activities (abzymes) are a distinctive feature of autoimmune diseases. It was interesting whether Abs from patients with viral diseases can hydrolyze viral proteins. Electrophoretically and immunologically homogeneous IgGs were isolated from sera of AIDS patients by chromatography on several affinity sorbents. We present evidence showing that 89.5% IgGs purified from the sera of HIV-infected patients using several affinity resins including Sepharose with immobilized integrase specifically hydrolyze only HIV integrase (IN) but not many other tested proteins. Several rigid criteria have been applied to show that the IN-hydrolyzing activity is an intrinsic property of AIDS IgGs but not from healthy donors. Similar to autoimmune proteolytic abzymes, IN-hydrolyzing IgGs from some patients were inhibited by specific inhibitors of serine and metal-dependent proteases but a significant inhibition of the activity by specific inhibitors of acidic- and thiol-like proteases was observed for the first time. Although HIV infection leads to formation of Abs to many viral and human antigens, no possible biological role for most of them is known. Since anti-IN IgG can efficiently hydrolyze IN, a positive role of abzymes in counteracting the infection cannot be excluded. In addition, detection of IN-hydrolyzing activity can be useful for diagnostic purposes and for estimation of the immune status in AIDS patients.

1. Introduction

Catalytically active artificial antibodies (Abs) or abzymes (Abzs) against transition chemical states of different reactions have been studied intensively [1]. During last two decades it has become clear that auto-Abs from the sera of patients with different autoimmune (AI) diseases can possess enzymic activities [1–6]. Natural Abzs hydrolyzing DNA, RNA, polysaccharides [2–7], oligopeptides, and proteins [8–11] are described from the sera of patients with several AI diseases. Some healthy humans produce Abzs with low proteolytic [8,9] and polysaccharide-hydrolyzing activities [7] but usually healthy volunteers and patients with many diseases with insignificant autoimmune reactions lack Abzs [3–6].

Similarly to artificial Abzs against analogs of transition states of catalytic reactions[1], naturally occurring Abzs may be Abs raised directly against the enzyme substrates acting as haptens and mimicking transition states of catalytic reactions [2–11]. On the other hand, antiidiotypic Abs can be induced in AI diseases by a primary antigen and may show some of its features including the catalytic activity [12,13].

According to the current point of view, Abzs may play a significant role in forming specific pathogenic patterns and clinical settings in different AI conditions through their broadened autoantibody properties [3–6]. Anti-VIP Abzs can have an important effect on pathogenesis due to a decrease in the concentration of VIP [14]. The protease activity of target-specific Abzs can attack myelin basic protein of the myelin-proteolipid shell of axons and play an important role in pathogenesis of multiple sclerosis [10,11,15]. DNase Abzs cause nuclear DNA fragmentation and induce cell death by apoptosis [5,16,17]. A decrease in the RAs of DNase Abzs from patients with Hashimoto thyroiditis correlates with normalization

Abbreviations: Ab, antibody; Abz, abzyme; AI, autoimmune; GL, generalized lymphoadenopathy; IN, HIV-1 integrase; pIgG, polyclonal IgG; RT, HIV-1 reverse transcriptase; SDS-PAGE, SDS-polyacrylamide gel electrophoresis; SLE, systemic lupus erythematosus; VIP, intestinal vasoactive peptide; RA, relative activity.

* Corresponding author. Institute of Chemical Biology and Fundamental Medicine, Siberian Division of Russian Academy of Sciences, Lavrentieva 8, Novosibirsk 630090, Russia. Tel.: +7 383 335 62 26; fax: +7 383 333 36 77.
E-mail address: nevinsky@niboch.nsc.ru (G.A. Nevinsky).

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doi:10.1016/j.biochi.2009.06.018

of the concentration of thyroid hormones and improvement of the patients' clinical status [18]. Thyroglobulin-hydrolyzing IgGs of patients with Hashimoto's thyroiditis [19] and proteolytic IgGs from patients with sepsis [20] may play a positive role in recovery from these diseases.

HIV-1 is the etiologic agent of an extremely dangerous human disease, AIDS [21]. HIV-1 integrase catalyzes integration of a DNA copy of the viral genome into the host genome. Therefore IN, together with reverse transcriptase (RT), is the main important target of anti-HIV drugs.

A pronounced immune response to the virus components is the most important factor slowing the transition of HIV infection to the stage of AIDS characterized by a progressive decrease in the number of T-helpers and their functional insufficiency [22]. HIV-dependent activation of B lymphocytes leads to the production of Abs to viral proteins, autoantibodies to components of human cells and various immune complexes. Recently we have shown that pIgGs from the sera of AIDS patients are active in hydrolysis of DNA [23]. pIgGs from AIDS patients hydrolyzing HIV-1 RT, human sera albumin (HSA) and human casein were the first examples of proteolytic Abzs appearing in humans due to a viral infection [24]. At the same time, a possibility of production of Abs specifically hydrolyzing HIV IN has not yet been analyzed.

In this report we use several methods to provide the first evidence that IgGs from HIV-infected patients can specifically hydrolyze HIV-1 IN.

2. Materials and methods

2.1. Chemicals, donors, and patients

All chemicals were from Sigma or Pharmacia. Homogeneous HIV-1 IN was obtained as in [25]. IN-Sepharose was prepared using BrCN-activated Sepharose according to the standard manufacturer's protocol.

Sera of 10 healthy volunteers and 19 HIV-infected patients (18–40 yr old; men and women) including 13 at the stage of pre-AIDS and 6 at the stage of generalized lymphoadenopathy according to the classification of the Center of Disease Control and Prevention were used to study proteolytic Abzs. The blood sampling protocol conformed to the local hospital human ethics committee guidelines.

2.2. Antibody purification

Electrophoretically and immunologically homogeneous pIgGs were obtained from healthy donors and AIDS patients by sequential affinity chromatography of the serum proteins on protein A–Sepharose and FPLC gel filtration on a Superdex 200 HR 10/30 column as in [10,11,18,23,24]. The blood sera were prepared by addition of 4% sodium citrate to blood samples (5 ml, 1/4 of the blood volume) and removal of cells by centrifugation (2000 rpm, 10 min). The solution was loaded on a protein A–Sepharose column (5 ml) equilibrated in TBS buffer (0.15 M NaCl, 20 mM Tris–HCl, pH 7.5) and the column was washed with TBS to zero optical density. Proteins bound non-specifically were eluted with the same buffer supplemented with 1% Triton X-100 and 0.3 M NaCl. The total IgG + IgM + IgA fraction was eluted with 40 mM glycine–HCl (pH 2.6), the fractions were collected to cooled tubes containing 50 μl of 0.5 M Tris–HCl (pH 9.0) and finally each fraction was additionally neutralized with this buffer and dialyzed against 10 mM Tris–HCl (pH 7.5) containing 0.1 M NaCl. The protein corresponding to the central part of the IgG peak was concentrated and purified further.

pIgGs were separated from IgAs and IgMs by FPLC gel filtration of the total Ab fraction on a Superdex 200 HR 10/30 column (GE Healthcare, New York, NY) equilibrated with TBS as described previously [10,11,18,23]. Before gel filtration, the Ig samples were incubated in TBS containing 2.5 M $MgCl_2$ for 30 min at 20 °C. TBS containing 3 M $MgCl_2$ (3 ml, "salt cushion") was applied to the column before the samples. The Abs were eluted with TBS. The type of Abs (IgA, IgG or IgM) in the fractions during different chromatographies was determined by Western blotting on a nitrocellulose membrane as described previously [10,11,18,23]. In order to protect the Ab preparations from bacterial contamination they were filtered through a Millex filter (pore size 0.2 μm). After one week of storage at 4 °C for refolding the Abs were used in activity assays as described below. To exclude possible artefacts due to hypothetical traces of contaminating enzymes, the IgG was analyzed using an in-gel assay (see below).

For gel filtration of Abs after the acid shock, IgGs were incubated in 50 mM glycine–HCl (pH 2.6) containing 0.2 M NaCl for 20 min at 25 °C. Separation of the IgGs under "acid shock" conditions was done by FPLC gel filtration on a Superdex 200 HR 10/30 column (GE Healthcare) using 10 mM glycine–HCl (pH 2.6) as described previously [10,11,18,23]. The column fractions were collected to cooled tubes containing 50 μl of 0.5 M Tris–HCl (pH 9.0) and finally each fraction was additionally neutralized with this buffer and dialyzed against 10 mM Tris–HCl (pH 7.5) containing 0.1 M NaCl and filtered through a 0.2 μm Millex syringe-driven filter. After a 3–6-day storage at 4 °C the fractions were used in the standard analysis of protease activity.

The IgGs purified by protein A–Sepharose and FPLC gel filtration were chromatographed on IN-Sepharose (1 ml) equilibrated with 50 mM Tris–HCl, pH 7.5, containing 0.1 M NaCl, the column was washed with this buffer to zero optical density; IgGs were eluted with 3 M $MgCl_2$ and dialyzed against 50 mM Tris–HCl, pH 7.5.

Chromatography of purified IgGs on Sepharose bearing mouse IgGs against light chains of human IgGs was performed similarly to the chromatography on Protein A–Sepharose.

After each purification step, protein concentration in the final fractions was measured using a standard Bradford assay calibrated with bovine serum albumin.

2.3. Ab proteolytic activity assay

The reaction mixture (10–60 μl) containing 50 mM Tris–HCl, pH 7.5, 30 mM NaCl, 0.2–0.3 mg/ml IN (31 kDa) or one of control proteins and 0.01–0.2 mg/ml of pIgGs was incubated for 2–24 h at 37 °C. To quantitatively estimate the protease activity, we have found a special concentration for each IgG preparation corresponding to the reaction of the pseudo first order (linear part of the dependence of the hydrolysis rate upon [IgGs]); ([Abzs] << [S]) where IN is cleaved during incubation within the linear part of the time course.

The concentration of IN was determined using a standard Bradford assay calibrated with bovine serum albumin.

In some cases IgGs (0.5–1 mg/ml) were preincubated for 30 min at 25 °C with one of specific inhibitors of different proteases: iodoacetamide (4 mM), pepstatin A (0.7 мМ), leupeptin (50 μM), AEBSF (0.15 mM), and EDTA (0.1 M), and then aliquots of these mixtures were added to standard reaction mixture [10,11]. SDS-PAGE analysis of Abs for homogeneity and for products of different proteins cleavage was performed in 5–18% gradient gels (0.1% SDS) and the polypeptides were revealed by silver staining and by Western blotting to a nitrocellulose membrane as in [10,11,18,23,24]. The gels were imaged by scanning and quantified using GelPro v3.1 software. All measurements were taken within the linear regions of the time courses and Ab concentration curves in the condition of the reaction of the pseudo first order.