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Diversity of Integrase-Hydrolyzing IgGs and IgMs from Sera of HIV-Infected Patients

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Abstract—It was previously shown that small fractions of IgGs and IgMs from the sera of AIDS patients specifically hydrolyze only HIV integrase (IN) but not many other tested proteins. Here we present evidence showing that these IgGs and IgMs are extreme catalytically heterogeneous. Affinity chromatography on IN-Sepharose using elution of IgGs (or IgMs) with different concentration of NaCl and acidic buffer separated catalytic antibodies (ABs) into many AB subfractions demonstrating different values of K_m for IN and k_{cat}. Nonfractionated IgGs and IgMs possess serine-, thiol-, acidic-like, and metal-dependent proteolytic activity. Metal-dependent activity of abzymes increases in the presence of ions of different metals. In contrast to canonical proteases having one pH optimum, initial nonfractionated IgGs and IgMs demonstrate several optima at pH from 3 to 10. The data obtained show that IN-hydrolyzing polyclonal IgG and IgM of HIV-infected patients are cocktails of anti-IN ABs with different structure of the active centers possessing various affinity to IN, pH optima, and relative rates of the specific substrate hydrolysis.

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Natural abzymes with different catalytic activities are characteristic of various autoimmune diseases [1-5]. During last 20 years it became clear that autoantibodies from blood of patients with autoimmune diseases could possess different enzymatic activities [1-5]. Natural IgG and/or IgM and IgA from blood of patients with various autoimmune and some viral diseases were shown to hydrolyze DNA, RNA, and polysaccharides [2-7]. In autoimmune and some other diseases abzymes were found capable of hydrolyzing various peptides and proteins: vasoactive intestinal peptide (VIP) in asthma [8], thyroglobulin in Hashimoto's thyroiditis and rheumatoid arthritis [9], prothrombin in multiple myeloma [10], protein factor VIII in hemophilia [11], myelin basic protein (MBP) in multiple sclerosis [12-15]. Moreover, IgG and sIgA of women's milk hydrolyze human casein [16]. In some healthy donors abzymes possessing low VIP-, thyroglobulin- [8, 9], and polysaccharide-hydrolyzing [7] activities were revealed [7], but as a rule no abzymes could be detected in sera of healthy donors and of patients with many diseases that were not accompanied by strong autoimmune reactions [2-5].

Note that total preparations of homogenous pIgG with different catalytic activities have lower values of relative catalytic activities than pIgM and sIgA abzymes [1-5, 14, 17-21], but data on such abzymes are scarce. Thus, pIgM from blood of patients with systemic lupus erythematosus (SLE) are more active in hydrolyzing DNA and RNA than IgG from blood of the same patients [17]. The

Abbreviations: AB, antibodies; AB_{appl}, AB_{NaCl}, and AB_{acid}, fractions of antibodies eluted from IN-Sepharose on application, with NaCl gradient concentration, and with acidic buffer, respectively; AIDS, acquired immunodeficiency syndrome; HIV, human immunodeficiency virus; IN, HIV-integrase; HSA, human serum albumin; MBP, myelin basic protein; pIgG, pIgM, and pIgA, polyclonal IgG, IgM, and IgA; SLE, systemic lupus erythematosus; VIP, vasoactive intestinal peptide.

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proteolytic activities of pIgM and pIgA specifically hydrolyzing MBP [14] and the amylolytic activity of IgM [18] from blood of patients with multiple sclerosis were also significantly higher than the corresponding activities of IgG. Values of kinetic parameters of the IgM-catalyzed hydrolysis of the protein gp120 from the HIV envelope varied over wide limits, whereas IgG did not manifest a noticeable activity in hydrolyzing this protein [19, 20]. IgM purified from blood of patients with Alzheimer's disease and monoclonal IgM hydrolyzed the amyloid β-peptide [21].

Similarly to artificial abzymes to analogs of transition states of catalytic reactions [1], natural abzymes under study can be represented by antibodies produced directly to enzyme substrates, which act as haptens and imitate the transitory state of chemical reactions [2-5]. Thus, abzymes, which hydrolyze VIP [8], thyroglobulin [9], and MBP [12-15] from blood of patients with bronchial asthma, Hashimoto's thyroiditis, and multiple sclerosis, respectively, are antibodies against these proteins, similarly to casein-hydrolyzing abzymes from human milk antibodies against casein [16]. On the other hand, antiidiotypic antibodies can be induced in autoimmune diseases by a primary antigen and possess its features including catalytic activity [21, 22]. If the active center of the enzyme acts as a primary antigen of the antiidiotypic chain, secondary antiidiotypic antibodies can have a structure part of which is an "inner image" or a "pattern" of the enzyme active center, and, consequently, the antibodies can possess catalytic properties of this enzyme [21, 22].

According to current concepts, abzymes can play both positive and negative roles in development of specific pathogenetic processes and the clinical course of diseases determined by different autoimmune reactions. Anti-VIP abzymes in patients with asthma play a negative role: they decrease the concentration of VIP [23]. The protease activities of highly specific abzymes hydrolyzing MBP from the myelin–phospholipid envelope of axons play an important role in pathogenesis of multiple sclerosis [12-15]. Abzymes with the DNase activity are cytotoxic – they fragment DNA of chromatin and induce apoptosis of tumor cells [24, 25]. The decrease in the relative DNase activity of abzymes in patients with Hashimoto's thyroiditis correlated with normalization of the thyroid hormone concentration and with improvement of clinical conditions of the patients [26]. Thyroglobulin-hydrolyzing IgG of patients with Hashimoto's thyroiditis [27] and proteolytic IgG of patients with sepsis [28] can play a positive role during the recovery of these patients.

HIV-1 is an etiological agent of an extremely dangerous human disease – the acquired immunodeficiency syndrome (AIDS) [29]. The replication cycle of HIV includes the reverse transcription of genomic RNA into DNA and integration of the latter into the host cell genome. The reverse transcription is catalyzed by HIV reverse transcriptase [30-32]. HIV-1 integrase integrates the DNA-copy of the viral genome into the human genome [33]. Integrase and reverse transcriptase are the most important targets for pharmaceutic preparations aimed for inhibition of HIV reproduction.

HIV-dependent activation of B-lymphocytes leads to production of antibodies against reverse transcriptase, HIV IN, nucleocapsid proteins, etc. Moreover, HIV infection stimulates the development of autoimmune reactions. In HIV-infected patients autoantibodies were found to human cell components and to various immune complexes, including antibodies to cardiolipin, β2 GPI, DNA, small nuclear ribonucleoproteins, thyroglobulin, thyroid peroxidase, myosin, and erythropoietin [34].

As in some patients with autoimmune diseases, in 89 and 96% of HIV-infected patients at the generalized lymphadenopathy and pre-AIDS stages, respectively, DNA-hydrolyzing abzymes are produced [35]. The first proteolytic abzymes found in HIV-infected patients were pIgG, which hydrolyze HIV reverse transcriptase [36]. Concurrently with production of abzymes against the viral reverse transcriptase, IgG were produced which specifically hydrolyze human serum albumin (HSA) and human casein [36]. IgG and IgM antibodies against HIV IN isolated from blood of 90-92% of HIV-infected patients were recently shown to specifically hydrolyze only HIV IN but not other proteins tested [37, 38]. In most cases, proteolytic abzymes in autoimmune diseases were serine-like proteases with activities inhibited by specific inhibitors PMSF and AEBSF [1-5, 8-12, 15, 16, 27]. However, some antibody preparations (from blood of patients with multiple sclerosis) capable of hydrolyzing MBP displayed features of metal-dependent proteases [13, 14, 16]. Only 20% of IgG and IgM preparations from HIV-infected patients capable of hydrolyzing HIV IN decreased their activities under the influence of serine protease inhibitors and 50-56% decreased activities under the influence of inhibitors of metal-dependent proteases [37, 38]. A significant inhibition of the proteolytic activities of IgG and IgM by specific inhibitors of acidic (20%) and thiol (100%) proteases was detected for the first time. These data confirmed the hypothesis that the pool of IgG and IgM from HIV-infected patients should contain IN-hydrolyzing abzymes of four types, which are like thiol, serine, acidic, and metal-dependent proteases, whereas their ratios could be individual for each HIV-infected patient.

Theoretically, the immune system can produce up to 10^6 different types of antibodies in response to a single antigen. But it is evident that in most cases only a small number of these theoretically possible variants of the response are realized. Immunization of autoimmune mice results in a sharp enlargement of the diversity of abzymes with various affinities for the antigens used and with different catalytic characteristics as compared to normal mice [39, 40]. Consequently, the abzyme reper-

toire in autoimmune diseases including HIV infection can also be greatly enlarged. Moreover, proteins can have several antigenic determinants and, respectively, the number of antibodies to the same protein can be significantly higher. Thus, sites of MBP hydrolysis by IgG abzymes from patients with multiple sclerosis are located in four different immune-dominant regions of MBP [15].

Studies of IgG and/or IgM possessing DNase and RNase activities from blood of patients with multiple sclerosis and SLE or of mice of the MRL-lpr/lpr strain prone to autoimmune diseases revealed their extreme heterogeneity in affinities for substrate and kinetic characteristics [17, 41-43]. Different patients were shown to have either a relatively small or a very large pool of polyclonal abzymes containing κ- and λ-type light chains demonstrating the maximal activities at different pH optimums and activated or non-activated by different metal ions and possessing different substrate specificities. IgG of all four subclasses (IgG1-IgG4) from blood of patients with multiple sclerosis have recently been shown to hydrolyze DNA [44] and MBP [45].

The present work has shown that IgG and IgM from blood of HIV-infected patients, which could specifically hydrolyze HIV IN, are different in the structure of active centers, affinities for IN, and in kinetic and other characteristics.

MATERIALS AND METHODS

Antibody purification. Taking into account the literature data [6, 8, 9] we earlier elaborated an approach [12-14, 17, 18, 26] for preparing electrophoretically and immunologically homogenous preparations of IgGs and IgMs from blood of patients with autoimmune diseases. This approach included affinity chromatography of blood plasma proteins on protein A-Sepharose and a high performance gel filtration on a Superdex-200 HR 10/30 column. Antibodies from blood of HIV-infected patients were prepared using the same approach as described in [37, 38].

Blood plasma was prepared by it mixing with 4% sodium citrate at the citrate/blood ratio of 1 : 4. The resulting mixture was maintained for 3-5 h, and cells were removed by centrifugation at 2000 rpm for 10 min. The supernatant was applied on a column with protein A-Sepharose (5 ml) equilibrated with TBS buffer (20 mM Tris-HCl (pH 7.5), 150 mM NaCl). Proteins not interacting with the sorbent were eluted with the same buffer until the full disappearance of absorption at 280 nm. Nonspecifically absorbed proteins and lipids were eluted with the same buffer containing 1% Triton X-100 and 0.3 M NaCl, and the column was washed with 50 mM Tris-HCl (pH 7.5) containing 0.3 M NaCl. The total antibody fraction containing IgG, IgA, and IgM was eluted with 40 mM glycine-HCl buffer (pH 2.6), and antibody fractions immediately after the column were neutralized with 1.5 M Tris-HCl (pH 8.0). The antibodies were dialyzed against 10 mM Tris-HCl (pH 7.5) supplemented with 0.1 M NaCl.

IgMs were separated from IgGs and IgAs by a high performance gel filtration on a column with Superdex-200 HR 10/30 (100 × 300 mm) (GE Healthcare, USA) equilibrated with 50 mM Tris-HCl (pH 7.5) containing 0.3 M NaCl as described in [37, 38]. To dissociate possible complexes, the antibody preparation previously to the high performance gel filtration was incubated for 30 min at 20°C in TBS buffer containing 2.5 M $MgCl_2$. Before application the specimen onto the column, 3 ml of TBS buffer containing 3 M $MgCl_2$ were applied onto the column as a "salt pillow", and then the antibody solution was applied. The elution was performed with TBS buffer. The immunological homogeneity of IgG, IgA, and IgM was confirmed by Western blotting on nitrocellulose membranes as described in [37, 38].

Protein fractions of the central parts of the IgG and IgM peaks were concentrated and used for the further purification and studies of the catalytic activity.

To prevent bacterial contaminations, the antibody preparations were filtered on Millex filters (with the pore size of 0.2 μm). The antibody preparations were stored at 4°C during a week to recover the protein native conformation, and afterwards the activities were studied as described below [37, 38].

Chromatography on IN-Sepharose. Homogenous preparations of HIV IN were prepared as described in [46]. IN-Sepharose was prepared from BrCN-activated Sepharose according to the standard protocol of the producer.

Upon protein A-Sepharose and high performance gel filtration, the IgG and IgM preparations were subjected to chromatography on IN-Sepharose (1 ml) equilibrated with 50 mM Tris-HCl (pH 7.5) buffer supplemented with 100 mM NaCl, then the column was washed until the optical absorption disappeared. IgGs and IgMs were eluted from IN-Sepharose with NaCl gradient (0.1-3.0 M) and then with 2.0 and 3.0 M $MgCl_2$ in the same buffer, and finally with 0.1 M glycine-HCl buffer (pH 2.6). The resulting fractions were neutralized with 1.5 M Tris-HCl (pH 9.0), dialyzed against 100-fold excess of 50 mM Tris-HCl (pH 7.5), and their relative integrase-hydrolyzing activities were assessed. To determine the relative activities, the dialyzed antibody fractions (5 μl) were added to the standard reaction mixture (final volume of 10 μl) containing 0.3 mg/ml integrase, and the mixture was incubated for 16 h as described below.

Determination of proteolytic activities of antibodies. The reaction mixture (10-60 μl) containing 20 mM Tris-HCl (pH 7.5), 30 mM NaCl, 0.3 mg/ml integrase (32 kDa) or one of the control proteins, and 0.01-0.2 mg/ml IgGs (or IgMs) was incubated for 2-24 h at

37°C. To quantitatively determine the protease activity, the concentration of IgG (IgM) preparations was found which corresponded to conditions of pseudo-first order reaction (linear dependence of the reaction rate on the antibody concentration [AB] << [S]). The conditions were chosen so that fragmentation of no more than 30-40% of HIV IN occurred. Products of cleavage of IN (and other proteins) were analyzed by SDS-PAGE in 5-18% polyacrylamide gel (0.1% SDS). Polypeptides were stained with $AgNO_3$ or with Coomassie G-250 [37, 38]. The gels were scanned and processed using the Gel-Pro Analyzer v.3.1 computer program. The relative activity was determined by a decrease in the protein amount in the initial band of HIV IN (or of other protein analyzed) as compared to the control, and in this case IN was incubated without antibodies.

In some cases IgG (IgM) (0.5-1.0 mg/ml) was preincubated for 30 min at 25°C with a specific protease inhibitor: iodoacetamide (4 mM), pepstatin A (1.0 mM), leupeptin (50 μM), AEBSF (0.15 mM), or EDTA (0.1 M); the inhibitors were added to the standard reaction mixture as described in [37, 38].

The pH optimum of HIV IN hydrolysis by immunoglobulins was determined using different buffers (50 mM): citrate-Na_2HPO_4 (pH 3.0-5.0), MES-NaOH (pH 5.4-6.6), MOPS-KOH (pH 7.0-7.8), Tris-HCl (pH 8.2-8.8), glycine-NaOH (pH 9.0-10.0), and Na_2HPO_4-NaOH (pH 11.0-12.0).

To analyze the influence of different metal ions, $MgCl_2$, $MnCl_2$, $CuCl_2$, $CoCl_2$ (20 mM), and antibody preparations were used before and after hydrolysis against 20 mM Tris-HCl (pH 7.5) containing 0.1 M EDTA, and then three washings with water (prior to use water and buffer solutions were passed through a column with Chelex-100).

Determination of kinetic parameters. Values of K_m and V_{max} were calculated based on kinetic data by a nonlinear regression approach using the MicroCal Origin v.5.0 programs, and the data were presented as a linear plot of double inverse Lineweaver–Burk coordinates [47]. The error on determination of the values was no more than 15-20%.

Reagents, preparations, and patients. All reagents were from Sigma (USA) and Pharmacia (Sweden). Ten HIV-infected patients (18-40 years old, men and women) consisted of five patients in the stage of generalized lymphadenopathy and five patients in the pre-AIDS stage, according to classification of the Center of Disease Control and Prevention (USA).

RESULTS

Antibody purification. The affinity and catalytic heterogeneity of IN-hydrolyzing abzymes from blood of HIV-infected patients were studied using electrophoretically and immunologically homogenous pIgGs and pIgMs. Protein A-Sepharose binds not only IgG, but also IgA and IgM [48, 49]. In the present work electrophoretically and immunologically homogenous preparations of pIgG and pIgM were obtained by chromatography of serum proteins on protein A-Sepharose under conditions of removal of nonspecifically bound proteins and subsequent high performance gel filtration under conditions where immune complexes are dissociated as described in [37, 38]. By analogy with works [37, 38], the homogeneity of pIgG and pIgM was confirmed by staining of proteins after SDS-electrophoresis with silver, and also by testing standard rigid parameters, which demonstrated the absence of any canonic protease traces in the antibody preparations. The pIgG (pIgM) fraction upon chromatography on IN-Sepharose was shown to specifically hydrolyze only HIV IN but not other proteins.

Chromatography of antibodies on IN-Sepharose. Antibodies possessing the nuclease and protease activities prepared from blood of patients with autoimmune diseases and of autoimmune mice are heterogenous in their affinities for specific substrates and therefore can be separated into many fractions by affinity chromatography on a sorbent with an immobilized substrate [2-5, 17, 41-43]. The affinities of pIgGs and pIgMs from HIV-infected patients for IN were analyzed by chromatography on IN-Sepharose of an equimolar mixture of pIgG (and pIgM) preparations from five patients (Fig. 1). About 15 ± 3% of the total amount of IgG (Fig. 1a) and 17 ± 3% of the total amount of IgM (Fig. 1b) were bound with IN-Sepharose.

As discriminated from canonical proteases, antibody preparations purified on IN-Sepharose hydrolyzed only HIV IN (see below).

IgG (and IgM) fractions corresponding to the first peak (~83-85% of the protein applied onto IN-Sepharose) with a low affinity for HIV IN had a high IN-hydrolyzing activity (Fig. 1, peak 1). As it was shown before, pIgGs and pIgMs from blood of HIV-infected patients before their separation on specific affinity sorbents contained antibodies specifically hydrolyzing not only HIV IN [37, 38] but also reverse transcriptase of HIV and HSA and human casein [36]. The pIgG and pIgM fractions corresponding to the first peak (Fig. 1) also hydrolyzed reverse transcriptase, HSA, and casein.

In the present work pIgG and pIgM fractions possessing a high affinity for IN-Sepharose and eluted from it with different concentrations of NaCl and $MgCl_2$ and also with the acidic buffer (Fig. 1, protein peaks 2-4, activity peaks II-V) could hydrolyze only HIV IN. These findings suggest that IgG and IgM capable of hydrolyzing some other proteins do not have affinity for IN-Sepharose; however, some IgG are likely to be able to nonspecifically bind with immobilized HIV IN.

The IN-hydrolyzing activities of pIgG or of pIgM were distributed throughout the whole profile of the affinity chromatogram (Fig. 1). It should be emphasized that

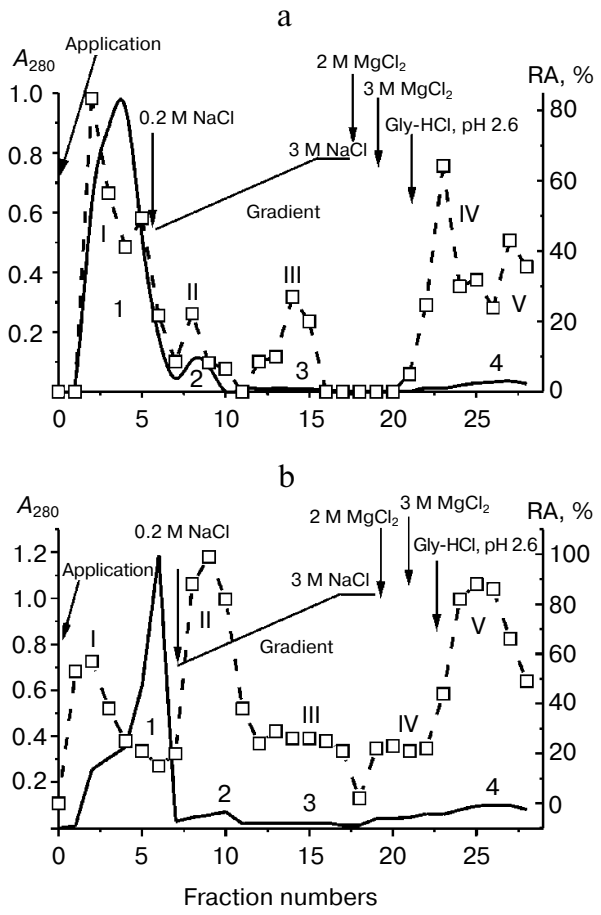

Fig. 1. Affinity chromatography of equimolar mixture of five pIgG (a) and pIgM preparations (b) on IN-Sepharose: (–) absorptions at 280 nm; RA, the relative activity of IgG and IgM. The complete hydrolysis of 0.3 mg/ml HIV IN during incubation for 16 h is taken for 100% of RA. Numbers of peaks of protein density are indicated by Arabian ciphers, numbers of activity peaks are shown by Roman numerals. The mean error of the initial rate determination did not exceed 10%.

even the pIgG or pIgM fractions with a low optical density (e.g. the fractions 11-16) possessed rather high relative activity in hydrolyzing HIV IN (Fig. 1). The findings demonstrate an extreme heterogeneity of pIgG- and pIgM-hydrolyzing HIV IN in their affinity for IN.

Affinity and catalytic activity of antibodies. Differences in catalytic properties of individual fractions of the total pool of polyclonal antibodies (and of their variations in individual patients) can be demonstrated using kinetic parameters characterizing these fractions. If polyclonal antibodies contain only one type of monoclonal abzymes, the dependence of the reaction initial rate on the substrate concentration is described by the Michaelis–Menten equation; and only one pair of K_m and V_{max} values corresponds to these antibodies [2-5, 47]. If polyclonal antibodies contain a number of monoclonal abzymes, these dependences cannot be described by the Michaelis–Menten kinetic equation but will be represented by a sum of a number of hyperbolic curves. The contribution of some of them to the total dependence can be great, whereas the contribution of others can be small: some pairs of the K_m and V_{max} values can be revealed only at comparable values of k_{cat} and significant differences in the abzyme affinities for the substrate. Therefore, in some cases only those values of K_m and V_{max} can be determined which characterize the major abzymes possessing the high activities [2-5, 47].

At first, the K_m and V_{max} values were estimated that characterized the HIV IN hydrolysis by two preparations of pIgG and pIgM that had not been fractionated on IN-Sepharose. The resulting dependences of the initial rate of IN hydrolysis by these antibodies on the IN concentration could not be described by the Michaelis–Menten

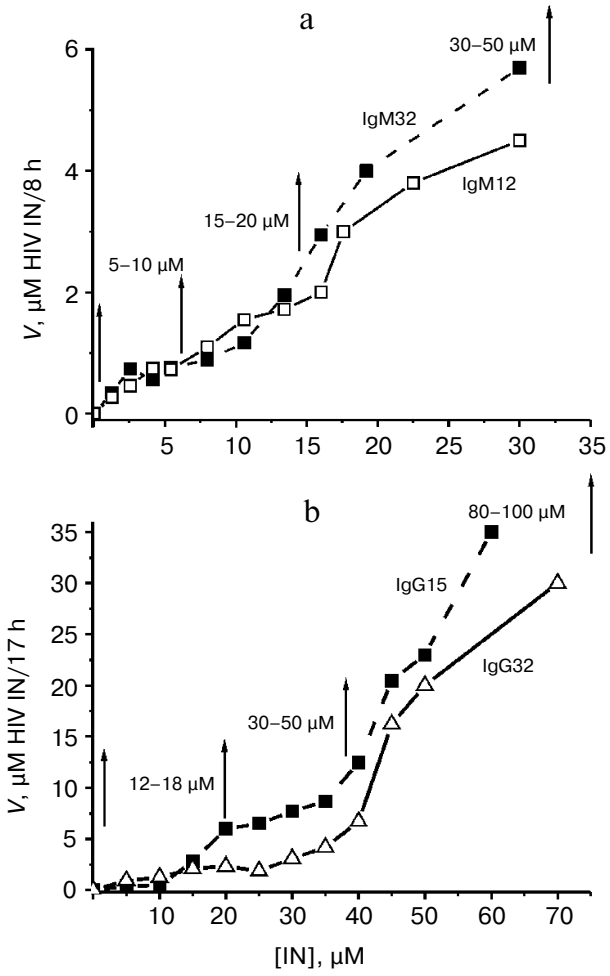

Fig. 2. Determination of initial rates of HIV IN hydrolysis catalyzed by individual preparations of IgM12 and IgM32 (0.2 μM) not separated by affinity chromatography (a), IgG15 and IgG32 (0.7 μM) (b) in V/[S] coordinates. The arrows indicate the regions corresponding to hyperbolic dependences constituting these complex curves. By data of three independent experiments, the error on determination of relative rates at every concentrations of HIV IN did not exceed 5-10%.

equation but presented a superposition of at least three or four hyperbolic curves corresponding to several K_m values in the limits of 5-10, 15-20, 30-50, and above 70-100 μM (Fig. 2). On one hand, these findings are in agreement but, on the other hand they lower the estimation of an extremely high heterogeneity of IgG and IgM revealed by affinity chromatography of antibodies on IN-Sepharose (Fig. 1).

To analyze in detail the K_m and k_{cat} values characterizing different antibody fractions in the general pool of antibodies, a number of antibody fractions eluted from IN-Sepharose were analyzed (Fig. 1). At first, the K_m and k_{cat} values were determined for the HIV IN hydrolysis catalyzed by IgG and IgM antibodies that corresponded to the second fraction eluted after applying the antibody onto IN-Sepharose (Fig. 1). The V/[S] (Fig. 3a, hyperbolic curves) and $1/V$ dependences on 1/[S] (Fig. 3b) for these fractions of abzymes nearly completely correspond to the Michaelis–Menten equation. Table 1 presents the calculated K_m and k_{cat} values for IgG (156 ± 40 μM; 0.3 ± 0.1 min^{-1}) and IgM (130 ± 30 μM; 2.0 ± 0.4 min^{-1}). The V/[S] and $1/V$ dependences on 1/[S] for each fraction of pIgG and pIgM eluted from IN-Sepharose with NaCl concentration gradient (IgG_{NaCl} and IgM_{NaCl}) and

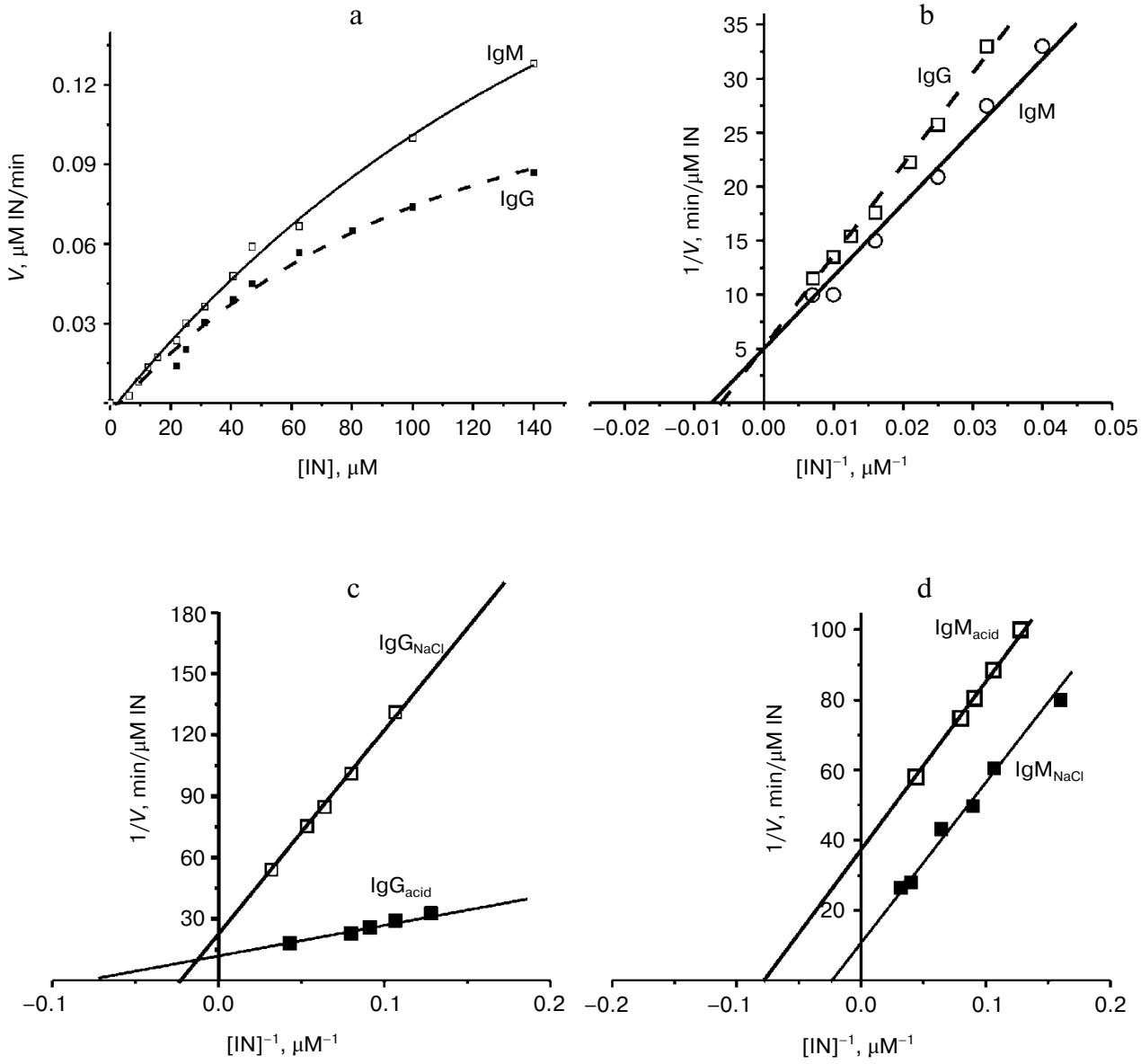

Fig. 3. Dependences of initial rates of HIV IN hydrolysis catalyzed by IgG and IgM individual fractions eluted from IN-Sepharose on application (IgG_{appl} and IgM_{appl}; Fig. 1 (a and b), fractions 1) in the V/[S] coordinates (a) and in Lineweaver–Burk coordinates (b). Data for the fractions of IgG_{NaCl} (16.7 nM) and of IgG_{acid} (32.5 nM) (c) (fractions 8 and 23 in Fig. 1a) and also for the fractions of IgM_{NaCl} (15 nM) and IgM_{acid} (5 nM) (d) (fractions 10 and 24 in Fig. 1b) eluted from the sorbent, respectively, with NaCl and acidic buffer are presented in Lineweaver–Burk coordinates.

with acidic buffer (IgG_{acid} and IgM_{acid}) also correspond to classic Michaelis–Menten kinetics (Fig. 3, c and d). The pIgG affinity for HIV IN (in terms of K_m values) increases with increase in their affinity for IN-Sepharose. Thus, affinities of IgG_{NaCl} fraction (Fig. 1a, fraction 8) (K_m = 44 μM) and of IgG_{acid} fraction (Fig. 1a, fraction 24) (K_m = 14 μM) were, respectively, 3.5 and 11 times higher than the affinity of the IgG_{appl} fraction eluted on application (Table 1). A similar situation was observed for the separated fractions of pIgMs (Fig. 1b). Affinities of the IgM_{NaCl} (fraction 10) and of IgM_{acid} (fraction 24) were, respectively, 3 and 10 times higher than the affinity of the IgM_{appl} (Table 1). It should be noted that the K_m values for IgG_{appl} (IgM_{appl}), IgG_{NaCl} (IgM_{NaCl}), and IgG_{acid} (IgM_{acid}) fractions are in agreement with the values for non-fractionated pIgG and pIgM preparations (K_m = 15-20, 30-50, and more than 80-100 μM) (Fig. 2). Overall, the findings demonstrate an extremely wide range of the IgG and IgM affinities for integrase in HIV infection and also of relative values of k_{cat}, which characterize a specific hydrolysis of protein by anti-IN abzymes (Figs. 1-3 and Table 1).

Table 1. The K_m and k_{cat} values that characterize the interaction of HIV IN with different subfractions of pIgGs and pIgMs separated by chromatography on IN-Sepharose (Fig. 1)

Preparation	Fraction number	K_m, μM	k_{cat}, min^{-1}
Fractions of pIgG separated by chromatography on IN-Sepharose*			
IgG_{appl}	2	156.0 ± 40.0**	0.3 ± 0.1
IgG_{NaCl}	8	44.0 ± 9.0	2.6 ± 0.5
IgG_{acid}	23	14.0 ± 3.0	2.9 ± 0.6
Fractions of pIgM separated by chromatography on IN-Sepharose*			
IgM_{appl}	2	130.0 ± 30.0	2.0 ± 0.4
IgM_{NaCl}	10	43.0 ± 8.0	6.4 ± 1.0
IgM_{acid}	24	12.8 ± 2.5	5.4 ± 1.0

* IgG $(IgM)_{appl}$, IgG $(IgM)_{NaCl}$, and IgG $(IgM)_{acid}$ are Ig fractions eluted on application, with NaCl concentration gradient, and with acidic buffer, respectively.
** Averaged from three independent determinations.

Table 2. Inhibition of proteolytic activity of individual preparations of IgG and IgM from blood of HIV-infected patients by specific inhibitors of different canonic proteases

Preparation	Inhibition, %*					
	AEBSF	Leupeptin	Pepstatin A	Iodoacetamide	100 mM EDTA	Total effect***
IgG1**	54 ± 5	0	0	10 ± 4	0	64
IgG2	0	0	48 ± 5	75 ± 7	94 ± 9	217
IgG3	45 ± 5	76 ± 8	34 ± 4	93 ± 9	0	248
IgM1	0	0	0	85 ± 6	0	85
IgM2	0	7 ± 2	0	56 ± 5	98 ± 8	161
IgM3	0	70 ± 6	56 ± 6	71 ± 8	97 ± 8	294
IgM4	47 ± 5	0	89 ± 9	90 ± 10	65 ± 4	291

* Complete hydrolysis of HIV IN in the absence of inhibitors is taken as 100%. Deviations are calculated based on data of three measurements.
** Numbers of IgG and IgM preparations correspond to numbers designating the individual patient.
*** Total effect of all five specific inhibitors of proteolytic activity.

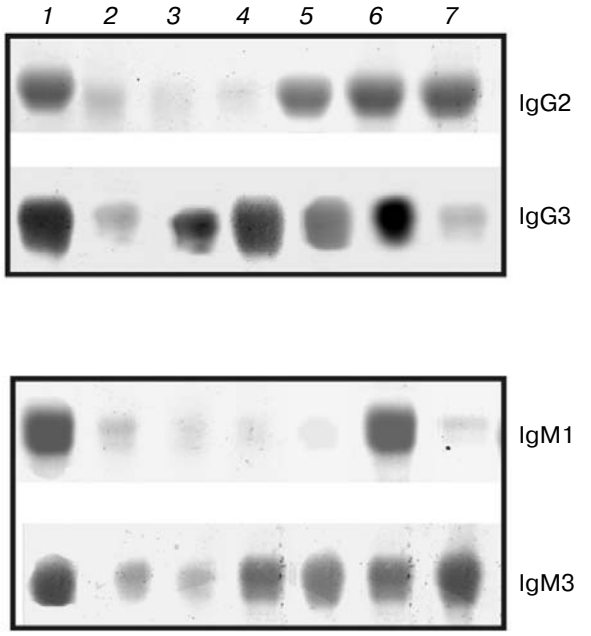

Fig. 4. Electrophoretic analysis of hydrolysis of HIV IN by pIgG (a) and pIgM (b) preparations from blood of HIV-infected patients in the presence of specific inhibitors of different proteases. IN (0.3 mg/ml) was incubated in the absence of antibodies (lane *1*); in the presence of pIgG (0.1-0.2 mg/ml) or pIgM (0.05-0.1 mg/ml) preparations without inhibitors (lane *2*), and also with different inhibitors: AEBSF (lane *3*), leupeptin (lane *4*), pepstatin A (lane *5*), iodoacetamide (lane *6*), and EDTA (lane *7*). The hydrolysis effect was assessed by the decrease in the amount of intact HIV IN.

Diversity of active centers of the abzymes. Abzymes in autoimmune diseases are, as a rule, serine-like proteases [1-5, 8-12, 15, 16, 27].

In the present work we have analyzed the type of hydrolyzing activity of HIV IN and pH dependences for three preparations of IgG and four preparations of IgM. The effect of specific inhibitors of different proteases on the relative activity of antibodies was determined by degree of IN hydrolysis (Fig. 4). The full transition of intact IN into its hydrolyzed forms in the absence of inhibitors was taken as 100%. The data on inhibition of IN hydrolysis for all of the antibodies are summarized in Table 2.

Leupeptin, which is an inhibitor of different proteases, significantly inhibited (70-76%) the proteolytic activity only of IgG3 and IgM3 (Table 2). The specific inhibitor of acidic proteases pepstatin A significantly inhibited the IN-hydrolyzing activity of IgG2 and IgG3 (34-48%) and of IgM3 and IgM4 (56-89%). AEBSF significantly inhibited the serine-like activity only of IgG1, IgG3, and IgM4 (45-54%). EDTA inhibited the proteolytic activity only of one of three IgGs, whereas this chelating agent decreased by ~65-98% the relative activity of three of four IgMs preparations, and the IgM1 preparation displayed metal-independent activity (Table 2). Incubation of pIgGs and pIgMs with iodoacetamide (a specific inhibitor of thiol proteases) usually does not have a noticeable effect on the proteolytic activity of antibodies in autoimmune diseases described in the literature [1-5, 8-12, 15, 16, 27]. However, it was recently shown for the first time that the IN-hydrolyzing activity of all ten IgG preparations and of nine IgM preparations from blood of HIV-infected patients was inhibited by iodoacetamide by 12-99% [37, 38]. Similar results were obtained in the present work: iodoacetamide inhibited all IgG and IgM preparations by 10-93% (Table 2).

Because some preparations of abzymes contained metal-dependent fractions, it was interesting to determine which metal ions could stimulate the antibody-dependent hydrolysis of HIV IN. To obtain an averaged picture of a possible dependence of the proteolytic activity on different metal ions, we used equimolar mixtures of pIgG (and pIgM) preparations from five patients (see above). Figure 5 shows that an intensive dialysis of antibodies against an EDTA-containing buffer results in a strong decrease in the relative proteolytic activity of the abzyme initial preparations containing already bound metal ions that were not completely removed on their isolation. Note that salts of all four metal ions (Mg^{2+}, Mn^{2+}, Cu^{2+}, and Ni^{2+}) are effective activators of the abzyme proteolytic activity. However, Mn^{2+} and Cu^{2+} are the optimal cofactors, respectively, for pIgGs and for pIgMs.

The pH dependence of HIV IN hydrolysis. Depending on their biological functions, classic proteases of mammals, bacteria, and plants are known to have pH optimums in the range from acidic (2.0) to neutral and alkaline (8-10) values [50, 51]. The heterogeneity of proteo-

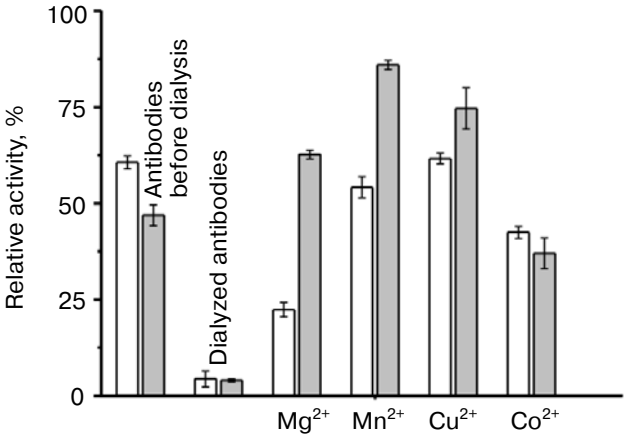

Fig. 5. Effects of different metal ions on the relative rate of HIV IN hydrolysis by preparations of equimolar mixtures of pIgM (white columns) and IgG (gray columns) from five HIV-infected patients after dialysis of these preparations against an EDTA-containing buffer. A decrease in the activity of antibodies is also shown upon the removal of metal ions initially bound with antibodies by dialysis of the preparations against EDTA.

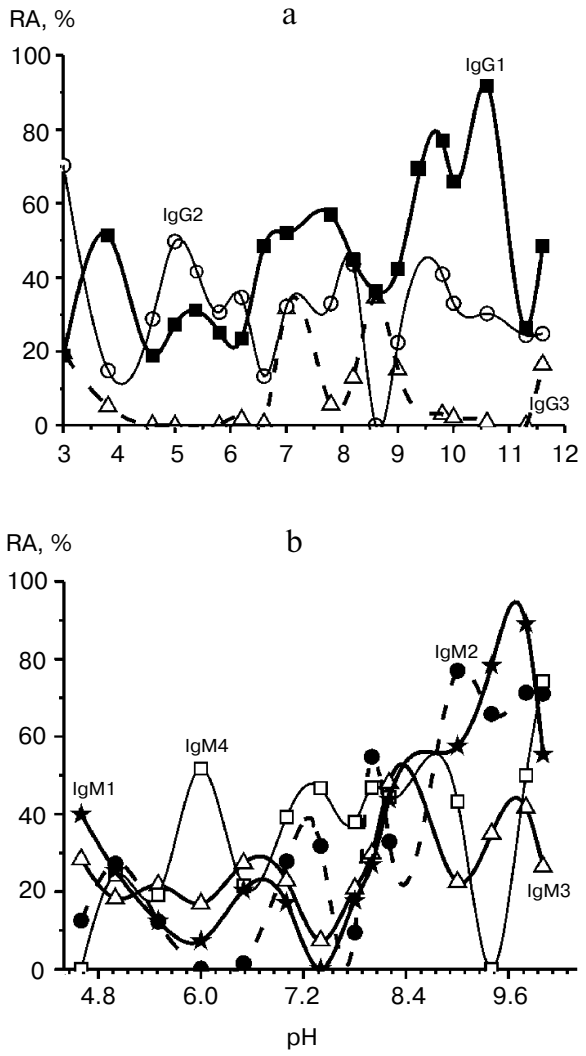

Fig. 6. Dependence on pH of the relative IN-hydrolyzing activity (RA) of three pIgG preparations (a) and of four pIgM preparations (b). The complete transition of 0.3 mg/ml HIV IN into shorter hydrolysis products during 8-10 h of incubation in the presence of 0.03-0.1 mg/ml pIgGs or IgMs is taken as 100% RA.

lytic activity of IN-hydrolyzing pIgG and pIgM seems to indicate a possibility of hydrolysis at different pH optimums, especially in the case of abzymes with different active center structure.

We have determined the relative proteolytic activity of pIgG and pIgM preparations at pH values from 3.0 to 11.0. The pH dependence profile occurs to be unique for each IgG preparation (Fig. 6a). As differentiated from classic human proteases, which have a single clear pH optimum, catalytically active IgG display high specific IN-hydrolyzing activities over a wide range of pH values, from 3 to 11. It should be noted that the IgG preparation possessing only the serine-like activity (Table 2) demonstrated at least four noticeable pH optimums: 3.9, 5.0, 7.8, and 10.6 (Fig. 6a) associated with different rates of the substrate hydrolysis. However, in the case of IgG3 possessing serine-like, acidic, and thiol proteolytic activities, three pronounced pH optimums were detected: 3.0, 7.0, and 8.7. The IgG2 preparation possessing an acidic, thiol, and metal-dependent protease activities (Table 2) demonstrated several pH optimum peaks in the range of pH values from 3 to 11, and the peaks at pH values of 3.0, 5.0, 8.2, and 9.8 were the most pronounced (Fig. 6a). These findings indicate that the total pIgG preparations from each of the HIV-infected patients can be strongly different in the enzymatic properties and catalyze the IN proteolysis at different pH values. Taking into account the effective hydrolysis of IN at pH 3.0, it was suggested that the human immune system could be capable of producing IgGs with a proteolytic activity similar to that of acidic stomach proteases.

Relative proteolytic activities of four IgM preparations from HIV-infected patients were studied at physiological pH values from 4.6 to 10.0 (Fig. 6b). Like IgG preparations, all IgMs displayed either a high or a noticeable activity over the wide range of pH values from 4.5 to 10.0 with pronounced activity peaks at pH values of: 4.6, 6.8, 8.4, and 9.7 (IgM1); 5.0, 7.3, 8.0, 9.0, and 9.9 (IgM2); 4.6, 5.3, 6.8, 8.4, and 9.7 (IgM3); 5.0, 6.0, 7.3, 8.7, and 10.0 (IgM4) (Fig. 6b). Note that the IgM1 preparation possessing only thiol proteolytic activity and the IgM2-IgM4 preparations possessing two, three, and four activity types had several pH optima. Relative proteolytic activities and their ratios at different pH optima were specific for each IgM preparation.

DISCUSSION

It has been shown earlier that pIgGs, sIgAs, and IgMs isolated from blood of patients with various autoimmune and viral diseases and also from women's milk which are capable of hydrolyzing different peptides and proteins are serine-like proteases, and their activities significantly decrease after preincubation with specific inhibitors of serine proteases: PMSF and/or AEBSF [1-5, 8-12, 15, 16, 27]. Small fractions of pIgGs and pIgMs in multiple sclerosis and sIgAs of women's milk possess a Me^{2+}-dependent protease activity [13, 14, 16].

In the present work the IN-hydrolyzing IgGs and IgMs from blood of HIV-infected patients are shown to possess not only serine-like and metal-dependent activities, but also acidic and thiol-like proteolytic activities, and the ratio of these activities is specific for each patient (Table 2). Note that all IgG and IgM preparations with different ratios of the above-mentioned proteolytic activities have individual profiles of pH dependences (Fig. 6).

There is a question why immunization of HIV-infected patients with integrase can induce production of IgG- and IgM-abzymes with very different affinities for HIV IN and different types of proteolytic activities. Note

that HIV IN is a hydrophobic and poorly soluble protein. In solution IN is in a dynamic equilibrium as monomers, dimers, tetramers, and also higher order oligomers, which can be a result of specific interactions and nonspecific association of different oligomeric forms of IN [52, 53]. Conformations of monomers and oligomers of IN are highly sensitive to DNA sequences [53]. Nonspecific DNAs stimulate generation of catalytically inactive dimers and tetramers of the first type, whereas in the presence of specific DNAs catalytically active dimers and inactive tetramers with more compact second type structure are produced [53]. Catalytically active tetramers of IN are produced on the interaction of the dimers of the first and second type [53].

The human immune system can produce up to 10^6 different antibody variants per antigen. In the human organism HIV IN can be present in a free form or in complexes with specific and nonspecific DNAs inducing generation of different oligomeric forms of the enzyme. Note that conformation of IN monomers and oligomers depends on the presence of Mg^{2+} and Mn^{2+} and also on the presence in the enzyme of the zinc finger [29, 53]. Moreover, at different stages of HIV infection and development of AIDS, integrase can interact in the cytosol and nucleus with a great number of viral proteins (reverse transcriptase, NCp7, Vpr, NLS-2, NLS-1) and of host cellular proteins (HSP60, BAF, LAP, MAN, LEDGF/p75, etc.) [54-57], which can change the state of antigenic determinants and as a result influence the immune response to immunogenic determinants of HIV IN.

An immense diversity of IN forms suggests a possible multiplicity of immune responses to antigenic determinants in different complexes of HIV IN with DNA, RNA, and various proteins. Consequently, a great number of structurally different antigenic determinants of HIV IN can exist. Perhaps this can be the main reason for the existence of unusual IN-hydrolyzing abzymes possessing serine-like, acidic, thiol, and metal-dependent proteolytic activities with different catalytic characteristics.

It has been shown earlier that the contents of specific fractions of abzymes with different catalytic activities in autoimmune diseases is usually no more than 0.1-5% of the total amount of Ig [2-5]; therefore, the finding that IN-Sepharose binds 15-17% of the total amount of pIgGs or pIgMs occurs to be rather unexpected. However, IN is also known to be a hydrophobic protein, which can both specifically and nonspecifically interact with various hydrophobic compounds including the above-mentioned proteins. With this in mind, we supposed that an immobilized HIV IN could not only specifically bind anti-IN IgG and IgM but also nonspecifically interact with IgG and IgM to other antigens. In the case of IN complexes with other proteins antibodies can be produced against new antigenic determinants, which include structural elements of both these proteins and HIV IN. This can increase the amount of antibodies interacting with HIV IN.

On one hand, it can be supposed that the pool of polyclonal IgGs and IgMs from HIV-infected patients could be a "cocktail" of abzyme molecules each of which has only one type (serine-like, acidic, thiol, metal-dependent) of proteolytic activity. But the total effect of two, three, and four inhibitors of the different type protease activity is not always lower or equal 100%. This sum is lower and close to 100% (64-85%) only for IgG1 and IgM1 preparations, whereas for other five preparations of antibodies, the proteolytic activity of which was inhibited by specific inhibitors of serine-like, acidic, thiol, and metal-dependent proteases, this value varies from 161 to 294% (Table 2). Thus, it seems that the immune system of HIV-infected patients can produce antibodies against HIV IN with a combined structure of the active centers, which include amino acid residues specific for active centers of different proteases. For example, the pool of IgG2, IgG3, and IgM2-IgM4 for which inhibition reaches 161-294% can include antibody molecules with a chimerical structure of the active center containing elements of thiol and metal-dependent proteases, and in some cases also structural elements of acidic and serine-like proteases. These data demonstrate the possibility of extreme structural diversity of IgG- and IgM-abzymes hydrolyzing integrase under conditions of HIV infection.

HIV IN is hydrolyzed by IgG and IgM preparations with different rates at pH values from 3-4 to 9-10 for each antibody preparation (Fig. 6). These results clearly indicate that pIgGs and pIgMs from HIV-infected patients can be different in contents of the antibody subfractions with very different enzymatic properties. Thus, the abzyme-dependent hydrolysis of proteins, DNA, and RNA by isolated light chains is more effective than hydrolysis by intact immunoglobulins [2-5, 58]. However, the catalytic center of the recombinant variable fragment (scFv) of IgG with the DNase activity prepared from mice of the autoimmune strain MRL-lpr/lpr can be located in the region of contact of the light and heavy chains, because upon separation of these chains they both can hydrolyze DNA [59]. Moreover, the light and heavy chains of pIgG isolated from the strain MRL-lpr/lpr mice can hydrolyze different NTP, NDP, AMP, and dAMP [60]. The activity of separate H- and L-chains of IgGs and IgMs in HIV-infection in hydrolyzing HIV IN was also shown [37, 38]. Therefore, active centers with different type of proteolytic activity with different pH optima are likely to be located on both heavy and light chains of different IgG and IgM molecules.

The dependence of the initial reaction rate on the IN concentration during hydrolysis catalyzed by IgG and IgM fractions eluted from IN-Sepharose with NaCl, or with acidic buffer is described by the Michaelis–Menten equation (Fig. 3). The affinity of IN (K_m = 12.8-156 μM; Table 1) to IgGs and IgMs from HIV-infected patients (purified on IN-Sepharose) is comparable with the affinity (K_m = 0.038-7.3 μM) for other abzymes hydrolyzing various proteins [2-5, 8-9, 16].

Catalysis by artificial abzymes against analogs of the transient state is usually characterized by relatively low reaction rates: the k_{cat} values are 10^2-10^6 times lower than the values for canonic enzymes [1]. The k_{cat} values for natural abzymes in autoimmune diseases vary in the limits of 0.0001-40 min^{-1} [1-5, 16, 17, 35, 42, 61]. The apparent k_{cat} values (0.3-6.4 min^{-1}) for IgG and IgM from HIV-infected patients purified on IN-Sepharose in hydrolysis of HIV IN (Table 1) are comparable with those for the known abzymes.

Even a partial purification of IgGs and IgMs on IN-Sepharose results in a significant increase in the k_{cat} values in hydrolysis of HIV IN. Because the k_{cat} values were calculated on consideration of the total concentration of purified IgGs and IgMs and the affinity chromatography on IN-Sepharose did not allow us to separate catalytically active antibodies against HIV IN from inactive antibodies and some contaminating Igs, the specific activity of individual monoclonal subfractions in the IgG and IgM pool could be significantly higher as compared with nonfractionated or partially fractionated antibodies.

Thus, IN-hydrolyzing IgGs and IgMs from blood of HIV-infected patients are extremely heterogenous in their affinity for HIV IN, demonstrate different K_m and k_{cat} values, and can hydrolyze the substrate over a wide range of pH values, from 3.0 to 10.0. Moreover, in contrast to other proteolytic abzymes they can also have a serine-like, acidic, thiol, and metal-dependent proteolytic activities.

The immune response to viral components is an important factor responsible for a deceleration of HIV-infection developing to the AIDS stage [62]. Consequently, IgGs and IgMs capable of hydrolyzing reverse transcriptase and HIV IN can cooperatively protect HIV-infected patients against the development of AIDS. Because each HIV-infected patient is characterized by their own specific set of abzymes, protective functions of these abzymes can also be individual.

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REFERENCES

1. Keinan, E. (ed.) (2005) *Catalytic Antibodies*, Wiley-VCH Verlag GmbH and Co. KgaA.
2. Nevinsky, G. A., and Buneva, V. N. (2002) *J. Immunol. Methods*, **269**, 235-249.
3. Nevinsky, G. A., Favorova, O. O., and Buneva, V. N. (2002) in *Protein–Protein Interactions. A Molecular Cloning Manual* (Golemis, E., ed.) Cold Spring Harbor Laboratory Press, N. Y., pp. 523-534.
4. Nevinsky, G. A., and Buneva, V. N. (2003) *J. Cell Mol. Med.*, **7**, 265-276.
5. Nevinsky, G. A., and Buneva, V. N. (2005) in *Catalytic Antibodies* (Keinan, E., ed.) Wiley-VCH Verlag GmbH and Co. KgaA, pp. 503-567.
6. Shuster, A. M., Gololobov, G. V., Kvashuk, O. A., Bogomolova, I. V., Smirnov, I. V., and Gabibov, A. G. (1992) *Science*, **256**, 665-667.
7. Savel'ev, A. N., Eneyskaya, E. V., Shabalin, K. A., Filatov, M. V., and Neustroev, K. N. (1999) *Protein Peptide Lett.*, **6**, 179-184.
8. Paul, S., Volle, D. J., Beach, C. M., Johnson, D. R., Powell, M. J., and Massey, R. J. (1989) *Science*, **244**, 1158-1162.
9. Kalaga, R., Li, L., O'Dell, J. R., and Paul, S. (1995) *J. Immunol.*, **155**, 2695-2702.
10. Thiagarajan, P., Dannenbring, R., Matsuura, K., Tramontano, A., Gololobov, G., and Paul, S. (2000) *Biochemistry*, **39**, 6459-6465.
11. Lacroix-Desmazes, S., Moreau, A., Sooryanarayana, K., Bonnemain, C., Stieltjes, N., Pashov, A., Sultan, Y., Hoebeke, J., Kazatchkine, M. D., and Kaveri, S. V. (1999) *Nat. Med.*, **5**, 1044-1047.
12. Polosukhina, D. I., Kanyshkova, T. G., Doronin, B. M., Tyshkevich, O. B., Buneva, V. N., Boiko, A. N., Gusev, E. I., Favorova, O. O., and Nevinsky, G. A. (2004) *J. Cell. Mol. Med.*, **8**, 359-368.
13. Polosukhina, D. I., Buneva, V. N., Doronin, B. M., Tyshkevich, O. B., Boiko, A. N., Gusev, E. I., Favorova, O. O., and Nevinsky, G. A. (2006) *Immunol. Lett.*, **103**, 75-81.
14. Polosukhina, D. I., Buneva, V. N., Doronin, B. M., Tyshkevich, O. B., Boiko, A. N., Gusev, E. I., Favorova, O. O., and Nevinsky, G. A. (2005) *Med. Sci. Monit.*, **11**, BR266-BR272.
15. Ponomarenko, N. A., Durova, O. M., Vorobiev, I. I., Belogurov, A. A., Kurkova, I. N., Petrenko, A. G., Telegin, G. B., Suchkov, S. V., Kiselev, S. L., Lagarkova, M. A., Govorun, V. M., Serebryakova, M. V., Avalle, B., Tornatore, P., Karavanov, A., Thomas, D., Friboulet, A., and Gabibov, A. G. (2006) *Proc. Natl. Acad. Sci. USA*, **103**, 281-286.
16. Odintsova, E. S., Buneva, V. N., and Nevinsky, G. A. (2005) *J. Mol. Recognit.*, **18**, 413-421.
17. Andrievskaya, O. A., Buneva, V. N., Naumov, V. A., and Nevinsky, G. A. (2000) *Med. Sci. Monit.*, **6**, 460-470.
18. Savel'iev, A. N., Ivanen, D. R., Kulminskaya, A. A., Ershova, N. A., Kanyshkova, T. G., Buneva, V. N., Mogelnitskii, A. S., Doronin, B. M., Favorova, O. O., Nevinsky, G. A., and Neustroev, K. (2003) *Immunol. Lett.*, **86**, 291-297.
19. Paul, S., Karle, S., Planque, S., Taguchi, H., Salas, M., Nishiyama, Y., Handy, B., Hunter, R., Edmundson, A., and Hanson, C. (2004) *J. Biol. Chem.*, **279**, 39611-39619.
20. Taguchi, H., Planque, S., Nishiyama, Y., Symersky, J., Boivin, S., Szabo, P., Friedland, R. P., Ramsland, P. A., Edmundson, A. B., Weksler, M. E., and Paul, S. (2008) *J. Biol. Chem.*, **283**, 4714-4722.

21. Izadyar, L. A., Friboulet, M. H., Remy, A., Roseto, A., and Thomas, D. (1990) *Proc. Natl. Acad. Sci. USA*, **90**, 8876-8880.
22. Kolesnikov, A. V., Kozyr, A. V., Alexandrova, E. S., Koralewski, F., Demin, A. V., Titov, M. I., Avalle, B., Tramontano, A., Paul, S., Thomas, D., Gabibov, A. G., and Friboulet, A. (2000) *Proc. Natl. Acad. Sci. USA*, **97**, 13526-13531.
23. Paul, S. (1998) *Appl. Biochem. Biotechnol.*, **75**, 13-23.
24. Kozyr, A. V., Kolesnikov, A. V., Aleksandrova, E. S., Sashchenko, L. P., Gnuchev, N. V., Favorov, P. V., Kotelnikov, M. A., Iakhnina, E. I., Astsaturov, I. A., Prokaeva, T. B., Alekberova, Z. S., Suchkov, S. V., and Gabibov, A. G. (1998) *Appl. Biochem. Biotechnol.*, **75**, 45-61.
25. Sinohara, H., and Matsuura, K. (2000) *Appl. Biochem. Biotechnol.*, **83**, 85-94.
26. Nevinsky, G. A., Breusov, A. A., Baranovskii, A. G., Prints, A. V., Kanyshkova, T. G., Galvita, A. V., Naumov, V. A., and Buneva, V. N. (2001) *Med. Sci. Monit.*, **7**, 201-211.
27. Li, L., Paul, S., Tyutyulkova, S., Kazatchkine, M. D., and Kaveri, S. (1995) *J. Immunol.*, **154**, 3328-3332.
28. Lacroix-Desmazes, S., Bayry, J., Kaveri, S. V., Hayon-Sonsino, D., Thorenoor, N., Charpentier, J., Luyt, C. E., Mira, J. P., Nagaraja, V., Kazatchkine, M. D., Dhainaut, J. F., and Mallet, V. O. (2005) *Proc. Natl. Acad. Sci. USA*, **102**, 4109-4113.
29. Katz, A. R., and Skalka, A. M. (1994) *Ann. Rev. Biochem.*, **63**, 133-173.
30. Skalka, A. M., and Goff, S. P. (eds.) (1993) *Reverse Transcriptase*, Cold Spring Harbor Laboratory Press, N. Y.
31. Litvak, S. (1996) in *Molecular Biology Intelligence Unit Series* (Landes, R., ed.) Chapman and Hall, New York/Springer Verlag, Heidelberg, pp. 1-206.
32. Arts, E. J., and Wainberg, M. A. (1996) *Adv. Virus Res.*, **46**, 97-163.
33. Asante-Appiah, E., and Skalka, A. M. (1999) *Adv. Virus Res.*, **52**, 351-369.
34. Zandman-Goddard, G., and Shoenfeld, Y. (2002) *Autoimmun. Rev.*, **1**, 329-337.
35. Odintsova, E. S., Kharitonova, M. A., Baranovsky, A. G., Sizyakina, L. P., Buneva, V. N., and Nevinsky, G. A. (2006) *Mol. Biol.*, **40**, 857-864.
36. Odintsova, E. S., Kharitonova, M. A., Baranovsky, A. G., Sizyakina, L. P., Buneva, V. N., and Nevinsky, G. A. (2006) *Biochemistry (Moscow)*, **71**, 251-261.
37. Baranova, S. V., Buneva, V. N., Kharitonova, M. A., Sizyakina, L. P., Calmels, C., Andreola, M. L., Parissi, V., and Nevinsky, G. A. (2009) *Biochimie*, **91**, 1081-1086.
38. Baranova, S. V., Odintsova, E. S., Buneva, V. N., Kharitonova, M. A., Sizyakina, L. P., Calmels, C., Andreola, M.-L., Parissi, V., Zakharova, O. D., and Nevinsky, G. A. (2010) *Int. Immunol.*, **22**, 671-680.
39. Nishi, Y. (2002) *J. Immunol. Methods*, **269**, 213-233.
40. Tawfik, D. S., Chap, R., Green, B. S., Sela, M., and Eshhar, Z. (2002) *Proc. Natl. Acad. Sci. USA*, **92**, 2145-2149.
41. Baranovskii, A. G., Ershova, N. A., Buneva, V. N., Kanyshkova, T. G., Mogelnitskii, A. S., Doronin, B. M., Boiko, A. N., Gusev, E. I., Favorova, O. O., and Nevinsky, G. A. (2001) *Immunol. Lett.*, **76**, 163-167.
42. Andrievskaya, O. A., Buneva, V. N., Baranovskii, A. G., Gal'vita, A. V., Benzo, E. S., Naumov, V. A., and Nevinsky, G. A. (2002) *Immunol. Lett.*, **81**, 191-198.
43. Kuznetsova, I., Orlovskaya, I. A., Buneva, V. N., and Nevinsky, G. A. (2007) *Biochim. Biophys. Acta*, **1774**, 884-896.
44. Parkhomenko, T. A., Legostaeva, G. A., Doronin, B. M., Buneva, V. N., and Nevinsky, G. A. (2010) *J. Mol. Recognit.*, **23**, 486-494.
45. Legostaeva, G. A., Polosukhina, D. I., Bezuglova, A. M., Doronin, B. M., Buneva, V. N., and Nevinsky, G. A. (2010) *J. Cell Mol. Med.*, **14**, 699-709.
46. Caumont, A., Jamieson, G., de Soultrait, V., Parissi, V., Fournier, M., Zakharova, O. D., Bayandin, R., Litvak, S., Tarrago-Litvak, L., and Nevinsky, G. A. (1999) *FEBS Lett.*, **455**, 154-158.
47. Fersht, A. (1985) *Enzyme Structure and Mechanism*, W. H. Freeman, Co, N.Y.
48. Balint, J. R., and Ikeda, Y., Jr. (1981) *Immun. Commun.*, **10**, 533-540.
49. Hakoda, M., Hayashimoto, S., Yamanaka, H., Terai, C., Kamatani, N., and Kashiwazaki, S. (1994) *Clin. Immunol. Immunopathol.*, **72**, 394-401.
50. Horl, W. H., Wanner, C., and Schollmer, P. J. (1987) *Parenteral Nutr.*, **11**, 98S-103S.
51. Rao, M. B., Tanksale, A. M., Ghatge, M. S., and Deshhange, V. V. (1998) *Microbiol. Mol. Biol. Rev.*, **62**, 597-635.
52. Deprez, E., Tauc, P., Leh, H., Mouscadet, J. F., Auclair, C., and Brochon, J. C. (2000) *Biochemistry*, **39**, 9275-9284.
53. Baranova, S., Tuzikov, F. V., Zakharova, O. D., Tuzikova, N. A., Calmels, C., Litvak, S., Tarrago-Litvak, L., Parissi, V., and Nevinsky, G. A. (2007) *Nucleic Acids Res.*, **35**, 975-987.
54. Van Maele, B., Busschots, K., Vandekerckhove, L., Christ, F., and Debyser, Z. (2006) *Trends Biochem. Sci.*, **31**, 98-105.
55. Engelman, A., and Cherepanov, P. (2008) *PLoS Pathog.*, **4**, e1000046.
56. Thomas, J. A., and Gorelick, R. J. (2008) *Virus Res.*, **134**, 39-63.
57. Piller, S. C., Caly, L., and Jans, D. A. (2003) *Curr. Drug Targets*, **4**, 409-429.
58. Mei, S., Mody, B., Eklund, S. H., and Paul, S. (1991) *J. Biol. Chem.*, **266**, 15571-15574.
59. Kim, J. S., Lee, S. Y., Lee, W. R., Sohn, J. N., Chung, Y. C., Shim, H. K., Lee, S. C., Kwon, M. H., and Kim, Y. S. (2006) *J. Biol. Chem.*, **281**, 15287-15295.
60. Andryushkova, A. A., Kuznetsova, I. A., Orlovskaya, I. A., Buneva, V. N., and Nevinsky, G. A. (2009) *Int. Immunol.*, **21**, 935-945.
61. 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.
62. Fauci, A. S., Braunwald, E., Kasper, D. L., Hauser, S. L., Longo, D. L., Jameson, J. L., and Loscalzo, J. (2008) *Harrison's Principles of Internal Medicine*, McGraw-Hill Professional.