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RESEARCH PAPER

Surface plasmon resonance-based methodology for anti-adalimumab antibody identification and kinetic characterization

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Abstract Adalimumab (ADA) is a TNF-α blocker drug antibody fully humanized and thus indistinguishable in structure and function from natural human IgG1, used in the juvenile idiopathic arthritis (JIA) treatment. Immunogenicity against the drug has been frequently detected in treated patients, and the presence of anti-ADA antibodies is correlated to treatment failure or lower clinical remission. Herein, we measured by surface plasmon resonance (SPR) both the binding and the affinity of anti-ADA antibodies to the ADA-immobilized biosensor. The binding of anti-ADA antibodies was evaluated by testing sera from ADA-treated patients (n=30), untreated patients (n=9), and healthy donors (n=20) in the SPR biosensor. The optimal cut-off point was defined using the receiver operating characteristic curve (ROC-curve) analysis with 79 % (60.28 to 92.01 %, 95 % CI) sensitivity, 99 % (88.06 to 100.0 %, 95 % CI) specificity, and a positive likelihood ratio of 23. The area under the curve was 0.9298 (p<0.0001). The apparent affinity of anti-ADA antibodies from pediatric patients' sera was measured, analyzing the interaction of anti-drug antibodies using whole sera, enriched IgG fractions, and isolated anti-ADA antibodies. The immobilized drug ADA interacted with purified antibodies at low affinities (10^{-6} M>K_D>10^{-9} M).

Keywords Adalimumab · Surface plasmon resonance · Anti-drug antibodies · Affinity

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Introduction

Several chronic inflammatory diseases are mediated by up-regulation of the cytokine tumor necrosis factor-alpha (TNF-α) [1, 2]. The TNF-α antagonists are biologic medications increasingly used to treat autoimmune disorders by suppressing inflammatory response [2]. Monoclonal antibodies acting as TNF-α blockers are commonly prescribed in juvenile idiopathic arthritis (JIA), the most common chronic rheumatic disease of childhood [3]. The causes and pathogenesis of JIA are still largely unknown, and the term includes a heterogeneous group of inflammatory arthropathies. Landmark trials and clinical studies demonstrated that monoclonal antibodies against TNF-α effectively induce and maintain symptoms remission in patients with JIA [4–7].

Adalimumab (ADA) is a TNF-α blocker antibody indistinguishable in structure and function from natural human IgG1, genetically engineered through phage display technology [8]. Unfortunately, despite ADA is fully humanized, immunogenicity against the drug has been frequently detected in treated patients suffering from inflammatory disorders [9–12]. A comprehensive review by Vincent et al. [13] analyzes the literature related to TNF-specific therapies from 1994 to 2012, reporting that the frequency of detection of anti-ADA antibodies varies from 0.04 to 87 %. Similar large variability is

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observed in patients treated with other TNF inhibitors. Interestingly, anti-drug antibodies incidence appears to vary not only among diseases and therapies but also depending on the method employed for detection, making difficult a comparison among different studies. More recently, Bloem et al. compared the measurement of anti-ADA antibodies in ADA-treated rheumatoid arthritis patients using five different assays, finding that the percentage of anti-ADA positive patients ranges from 51 to 66 % [14]. These authors also observed that these methods possibly break down at the lower end of the detectable range and therefore if a more sensitive assay could be developed, one would probably find additional positive samples. In any case, a general consensus associating the presence of anti-ADA antibodies to treatment failure or lower clinical remission was reached [15]. In fact, anti-ADA antibodies not only increase drug clearance but also appear to be directed mainly, if not exclusively, to the TNF binding site, thus efficiently neutralizing the drug [16, 17].

The development of anti-ADA antibodies detection assays poses several technical problems, due to the lack of proper secondary reagents discriminating between the drug and the immune-response antibodies, which are both human immunoglobulins, and to the presence of drug-antibody complexes and/or other serum factors. The heterogeneity of these assays and the lack of standardization may cause difficulties in results interpretation, thus hampering the comparison among different studies and, most importantly, a correct evaluation of anti-ADA antibodies relevance [18]. The classical ELISA methodology solved these problems by the use of indirect and bridging techniques, including acid treatments to remove immune-complexes interference [19–21]. These tests are highly specific but more prone to inhibition by ADA present in the sample, hence may underestimate the quantification [18]. In contrast, despite inherent limitations due to the use of radioactivity, radioimmunoassay (RIA) is less susceptible to drug inhibition. Yet, RIA is less sensitive to drug interference than the ELISA. The homogeneous mobility shift assay, as alternative methodology not incorporated in routine measurements, is described overcoming many limitations encountered in ELISA and RIA methods [22].

The affinity of autoantibodies for their target is considered a relevant parameter in determining their pathogenicity. Surface plasmon resonance (SPR)-based optical biosensors have been successfully employed in the evaluation of both the binding and the affinity and/or kinetics of a large number of biomolecular interactions, including those of anti-drug antibodies [23–25]. SPR-based optical biosensors detect in real-time mass changes on the chip surface, registering the interaction between the immobilized ligand and the analyte flowing in solution. SPR-based biosensors have been applied in a comparative study of affinities of ADA and other biologic drugs to the target TNF-α [26], or the aggregation tendencies of these therapeutic drugs when mixed with human plasma and serum [27]. However, a direct evaluation of anti-ADA antibodies and their affinity by means of SPR-based biosensors is to our knowledge not yet available.

We have previously demonstrated the presence of anti-infliximab (a monoclonal chimeric TNF inhibitor) antibodies in sera of pediatric patients receiving infliximab for juvenile idiopathic arthritis and other pediatric rheumatic diseases, measured by an in-house ELISA [28]. Since infliximab has now been largely replaced by ADA in the treatment of JIA, the aim of the present study is to identify anti-ADA antibodies in pediatric patients' sera treated with ADA and to evaluate their affinity by analyzing the direct binding of purified antibodies to ADA immobilized on the biosensor.

Materials and methods

All experiments were performed using a Biacore® T100 instrument from GE Healthcare (Uppsala, Sweden). All solutions and buffers were prepared with Milli-Q water obtained by the Sartorius system (Arium® 611 VF) and filtered daily with a Millipore Express™ PLUS 0.22 μm system. Sensor chips CM5-type, amine coupling kit, and running buffer HBS-EP+ 10× (0.1 mol/L HEPES, 1.5 mol/L NaCl, 30 mmol/L EDTA, 0.5 % *v*/*v* p20) were purchased by GE Healthcare (Uppsala, Sweden). Running buffer was diluted ten times with Milli-Q water at pH 7.4 and filtered. Sodium acetate was purchased by Carlo Erba (Milano). Sodium hydroxide was provided by Honeywell-Riedel deHaen (Seelze, Germany). All binding analyses were performed at +25 °C using HBS-EP+ as running buffer. Commercial ADA was obtained in a 50 mg/mL solution prepared for clinical use (Humira® lot 30404XD11; AbbVie Ltd., Maidenhead, United Kingdom). Soluble fraction (aa 77–233) of recombinant active human tumor necrosis factor alpha (TNF-α) was purchased from Abcam (Cambridge, UK). CNBr-activated Sepharose resin and Protein G Sepharose®, Fast Flow were purchased from Sigma-Aldrich (St. Louis, MO).

Serum collection

Recruitment of patients was performed in 2014 in the pediatric rheumatology clinic of Meyer Children Hospital in Florence, Italy. The study group consisted of 30 patients affected by oligoarticular JIA ($n=8$), polyarticular JIA ($n=12$), enthesitis-related arthritis JIA ($n=2$), uveitis ($n=5$), and Behçet's disease ($n=3$). Nine were males, 21 were females; mean age at the time of sampling was 10.6 years (range 2.75–19.41) and the mean duration of ADA treatment was 19.86 months (range 2.96–86.26). ADA was always administered at the standard dose of 24 mg/m^2/every 2 weeks.

Blood samples were obtained by venipuncture during routine follow-up. A group of 9 untreated JIA patients and 20

healthy age-matched subjects was used as control groups. Each sample was centrifuged, and supernatant aliquoted and stored at −20 °C until assayed. Patients or guardians gave their informed consent.

Adalimumab immobilization

Experiments were conducted following the previously described SPR protocol [29] properly optimized for the present study. ADA was immobilized on the sensor chip CM5-type surface. ADA was covalently linked according to the amine coupling strategy. Immobilization buffer was selected separately using the pH scouting procedure, as described in the instrument protocol, using the following buffers: D-PBS buffer pH 7.2, sodium acetate buffer 10 mM pH 4.0 and 5.0, sodium acetate buffer 5 mM pH 4.0 and 6.0, and sodium acetate buffer 1 mM pH 4.0, 5.0, and 6.0. ADA was solubilized in each buffer at a final concentration of 10 μg/mL. The immobilization buffer selected was 10 mM sodium acetate pH 4.0.

For immobilization, sensor chip surface was activated with two injections of *N*-hydroxysuccinimide (NHS 0.1 M) and 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC 0.4 M) 50:50 for 420 s at a flow rate of 10 μL/min. ADA was solubilized in the previously selected immobilization buffer 1 mM acetate pH 4.0 (10 μg/mL) and injected for 420 s at a flow rate of 10 μL/min. Non-reacted carboxylic groups on sensor chip surface were blocked injecting ethanolamine-HCl 1M pH 8.5 for 420 s at a flow rate of 10 μL/min. The immobilized ADA raised a final immobilization level of 3000±300 resonance units (RUs). Reference channel activated by injecting NHS/EDC 50:50 was directly blocked with ethanolamine-HCl and used as blank to remove the non-specific signal depending on interactions between molecules present in the biological samples and gold on sensor chip surface.

SPR binding experiments

Human serum samples were thawed to ambient temperature and then diluted 1:100 in running buffer. The diluted serum samples were injected in triplicate at flow rate of 30 μL/min over the immobilized ADA during 120 s. Dissociation was monitored for 30 s by injecting the running buffer suddenly after samples at a flow rate of 30 μL/min. Interactions were recorded as separate sensorgrams and measurements registered 15 s after the end of each sample injection. Responses were measured in RUs as the difference between active and reference channel. After each sample injection, surface was regenerated with a 30 s pulse of 50 mM glycine-HCl pH 2.5 followed by a 30 s pulse of a solution 1 mM NaOH at a flow rate of 30 μL/min allowing the complete removal of specifically and non-specifically bound biological material from the surface. The 10 μg/mL soluble TNF-α solution was used as system positive control, and the 10 μg/mL ADA solution was used as negative control, both repeated each 15 measurements verifying the stability of the probe upon a large number of cycles. Following this protocol, all further experiments were performed not over and above 100 measurements per channel.

Antibody purification

Treated patients' sera containing anti-ADA antibodies were identified and selected according to the SPR method herein described. In order to isolate anti-ADA antibodies, ADA was conjugated to CNBr-activated Sepharose resin, washed with 12 mL of ice cold HCl (1 mM). This washing step facilitated the removal of lactose, used for stabilization of the resin during freeze-drying cycle. The resin was hence washed with Milli-Q water (12 mL) and a coupling buffer composed of $NaHCO_3$ (0.1 M) and NaCl (0.5 M) pH 8.3 (10 mL). ADA solution (2 mg/mL) was diluted in 1 mL coupling buffer ($NaHCO_3$ 0.1 M, NaCl 0.5 M, pH 8.3). ADA solution was thereafter added to the resin and shaken overnight at room temperature. The functionalized resin was hence transferred to a solid phase extraction column closed at one end with a frit. The resin was allowed to settle and was subsequently washed with the coupling buffer. Unreacted groups were blocked using a glycine solution (0.2 M, pH 8.0). In order to remove the washing solution, the functionalized resin was washed with the alkaline coupling buffer (10 mL), followed by washing with an acetate buffer (0.1 M, pH 4.3, 0.5 M NaCl) (10 mL). Serum samples diluted 1:10 were applied to the column, and the flow-through was collected for subsequent analysis. The column was extensively washed with 20 mM Na_2HPO_4, 150 mM NaCl (pH 7.2), and the antibodies bound to the column were eluted by 100 mM glycine buffer (pH 2.8), immediately neutralized with 50 μL Tris 1M (pH 8.0), concentrated and recovered in D-PBS using the AMICON® Ultra centrifugal filters 50 K MWCO (Merk Millipore, Billerica, MA, USA). The anti-drug antibody concentration in each fraction was tested using the standard methodology by ultraviolet absorbance at 280 nm [30].

Immunoglobulin G (IgG) fractions from ADA-treated patients and controls were obtained from sera using Protein G Sepharose®, Fast Flow, according to the manufacturer's instructions.

Kinetic experiments

Purified-enriched IgG fractions and purified anti-ADA antibody fractions at different initial calculated concentrations were diluted in running buffer to final concentration of 200, 150, 100, 50, 20, 10, and 5 μg/mL. Kinetic experiments were conducted following the previously described SPR protocol [29] optimizing the dissociation phase times and regeneration solutions for the present study. Diluted samples were injected

in duplicate over immobilized ADA for 120 s at a flow rate of 30 μL/min. Running buffer was then flushed for 600 s during the dissociation phase at a flow rate of 30 μL/min and finally the chip surface was regenerated by injecting a glycine solution 50 mM pH 2.5 for 30 s and a sodium hydroxide solution 1 mM for 30 s both at a flow rate of 30 μL/min. Control samples were employed to monitor the sensor chip surface stability, demonstrating the reproducibility throughout the duration of the experiments.

Statistical analysis

The serum antibody levels obtained in the binding experiments were expressed as mean values in resonance units (RUs) and elaborated using the statistical software GraphPad Prism version 6.01. Kinetic experiments were elaborated with Biacore Evaluation Software 2.0 and summarized with Biacore T100 Kinetic Summary.

Results and discussion

SPR binding method

In the present study, we identified and characterized specific anti-ADA antibodies in ADA-treated patients' sera using an ADA-based SPR biosensor. For this purpose, ADA was covalently immobilized onto the gold sensor chip surface following the amine coupling immobilization strategy. All samples were flowed separately over the immobilized ADA in individual cycle of analysis with an initial association phase (sample injection), a second dissociation phase (washing step with running buffer), and finally a chip surface regeneration phase. Background levels due to non-specific serum interactions were monitored on the reference channel of the chip and subtracted from sample signals. A TNF-α solution was used as positive system control, while a solution of ADA was used as negative control. Initial attempts to capture and measure the direct binding of TNF-α to immobilized ADA proved that the biosensor was able to detect TNF-α, which therefore was used as positive control, while ADA in solution did not interact with immobilized ADA and thus was used as negative control (Fig. 1). Then, we evaluated the ability of the biosensor chip to capture specific anti-ADA antibodies directly from sera of treated patients, untreated patients, and controls. A representative example of the binding levels in patients' and healthy control sera is summarized in Fig. 1.

Sample sera screening

The specificity of anti-ADA antibodies was evaluated by testing sera from ADA-treated patients (n=30), untreated patients (n=9), and healthy donors (n=20) in the SPR biosensor.

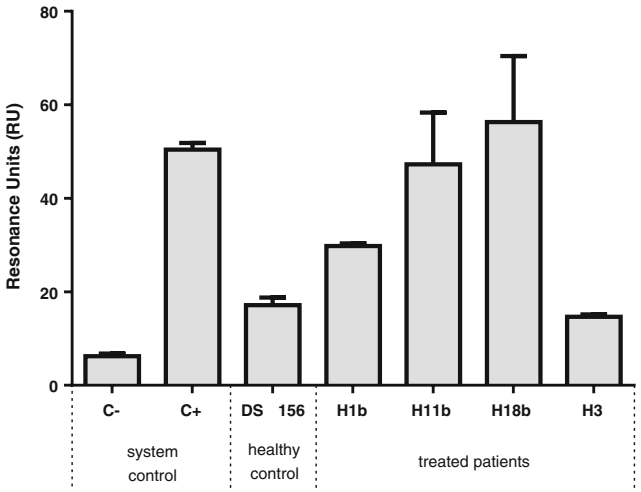

Fig. 1 Binding levels to immobilized adalimumab (ADA) of four representative treated patients and one healthy control sera diluted 1:100. The positive and negative controls are TNF-α and ADA (10 μg/mL solution), respectively

Serum anti-ADA antibody levels were significantly elevated in treated patients compared to both untreated patients and controls (Fig. 2). The differences between the groups were evaluated with the unpaired non-parametric Mann–Whitney test. The obtained p value (<0.0001) confirmed statistically significant differences between the groups.

The diagnostic power of anti-ADA antibody binding levels was investigated by the receiver operator curve (ROC) analysis comparing different values as sensitivity, specificity, and likelihood ratios [31]. ROC-curve for anti-ADA activity was constructed based on 30 treated cases versus 29 untreated patients and healthy controls (Fig. 3). The optimal cut-off

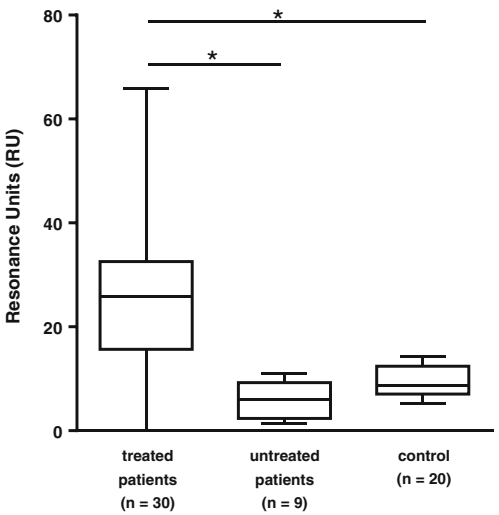

Fig. 2 Anti-ADA serum antibody titers measured by the biosensor assay: comparison between treated patients (n=30), untreated patients (n=9), and controls (n=20). *P value <0.0001 were calculated with the unpaired non-parametric Mann–Whitney analysis, α=0.05. Data are expressed as box and whiskers plot with medians and inter-quartile range values

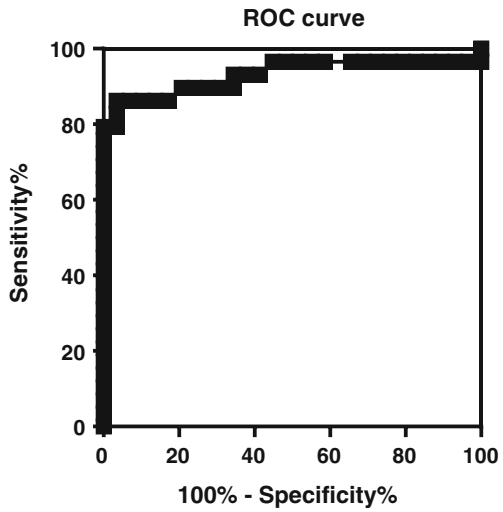

Fig. 3 ROC curve analysis of anti-ADA antibodies in treated patients versus untreated patients and controls determined by SPR. The area under the curve is 0.9298 ($p<0.0001$). Cut-off was set at 15 RU with a sensitivity of 79 % (60.28 to 92.01 %, 95 % CI), a specificity of 99 % (88.06 to 100.0 %, 95 % CI), and a positive likelihood ratio of 23

point was 15 RU with 79 % (60.28 to 92.01 %, 95 % CI) sensitivity, 99 % (88.06 to 100.0 %, 95 % CI) specificity, and a positive likelihood ratio of 23. The area under the curve was 0.9298 ($p<0.0001$). Using the cut-off value defined trough the ROC analysis, we found that 23 out of 30 (79 %) treated patients and none of the untreated and healthy controls are positive to the anti-ADA assay. This data appears to be in line with those reported by the literature [13, 14], which, however, are highly variable (0.04 to 87 %), depending upon the method employed for detection. It should be noted that, to the best of our knowledge, very few data have been reported in the literature about anti-drug antibody in pediatric rheumatology patients. We previously reported the presence of anti-drug antibodies in 43 % of pediatric patients receiving infliximab for juvenile idiopathic arthritis and other pediatric rheumatic diseases, measured by an in-house ELISA [28]. Two recent studies reported the measurement of anti-drug antibody levels in pediatric rheumatology patients treated with ADA: Imagawa et al. [32] analyzed 25 Japanese pediatric patients with JIA treated with ADA, finding a 24 % frequency of anti-ADA antibodies using a double-antigen ELISA, while Murias et al. [33] observed 32 % anti-ADA antibody positivity on the same number of children, using a two-site bridging ELISA. In the latter study, anti-ADA antibodies correlated with disease activity.

From a methodological point of view, several studies indicate that anti-drug antibody detection is hampered in the majority of the assays by the concomitant presence of the drug in the analyzed sera. In fact, several methodologies employ acid treatment and/or addition of chaotrope to avoid the formation of immune complexes [10, 34]. Despite that the protocol herein described does not provide any kind of sample pre-treatment, the differences observed between treated patients and control groups were significant. Moreover, several experiments in the presence of acid/chaotrope/thermal treatments were performed obtaining inconclusive results (data not shown). For this reason, we decided to proceed without additional sample treatments.

We also compared the performances of our new SPR assay with those of a commercially available kit (LISA-TRACKER, Theradiag, Marne la Vallée, France). To this aim, each serum sample was tested with the kit, following the manufacturer instructions, obtaining 4 positive samples out of 30 (13 %) treated patients and none among the control groups. Indeed, the correlation between the two assays is low ($R=0.319$). These results evidenced the differences in terms of specificity and sensitivity between ELISA- and SPR-based assays, already observed in the case of other anti-drug antibodies [25]. We are currently evaluating our method against different available ELISA kits in a larger cohort sample.

Anti-ADA antibodies isolation and kinetic analysis

A deep study of anti-ADA antibody apparent affinity was performed isolating the relevant antibodies from three sera samples, selected among those showing the highest anti-ADA titer by SPR and ELISA. For this purpose, the target ADA was immobilized on a Sepharose column and specific antibodies were purified. Surprisingly, the immuno-affinity purification procedure from one of the three selected patients

Table 1 Kinetic parameters k_a and k_d and affinity constant K_D calculated with the 1:1 binding model for interactions between purified antibodies fractions and the immobilized drug ADA

Sample		k_{on} (1/Ms)	k_{off} (1/s)	K_D (M)
H1b	a) anti-ADA antibodies	1.816×10^{4}	1.59×10^{-3}	8.74×10^{-8}
	b) Protein G-isolated antibodies	6.359×10^{3}	1.115×10^{-3}	1.75×10^{-7}
H11b	a) anti-ADA antibodies	3.500×10^{4}	5.77×10^{-4}	1.65×10^{-8}
	b) Protein G-isolated antibodies	1.200×10^{4}	5.19×10^{-4}	4.31×10^{-8}
H18b	a) anti-ADA antibodies	Not enough purified antibodies obtained		
	b) Protein G-isolated antibodies	994.1	1.012×10^{-3}	1.02×10^{-6}
DS156	a) anti-ADA antibodies	No purified antibodies		
	b) Protein G-isolated antibodies	No binding		

(sample H18b) did not allow reaching a sufficient antibody concentration to perform the kinetic study. Then, IgG-enriched fractions were obtained from the three previously selected treated patients' sera using protein G affinity columns. A control IgG antibody fraction was also obtained by protein G chromatography from the control sample DS156, while, on the other hand, the same control serum did not yield anti-ADA antibodies by specific immune-affinity purification, as expected.

The kinetic study was performed testing different concentrations of isolated antibodies and IgG fractions in SPR against immobilized ADA. Binding results were elaborated independently for each sample, fitting the experimental values to theoretical kinetic models and identifying the model most suitable to the experimental data [35]. High-quality fittings were obtained both with the binding 1:1 kinetic model and the bivalent analyte one. According to this latter model, after analyte-ligand interaction at the first site, a sort of rearrangement of the bound analyte occurs and the engagement of the second site does not contribute to SPR response. Then, the use of complex models to calculate the k_{on} and k_{off} constants corresponding to the single binding sites contribution can be approximated to a global apparent K_D calculated with a simple binding 1:1 interaction. Consequently, the binding model 1:1 was employed and validated by three statistical parameters: the residual values between the experimental points and the theoretical ones closely distributed along the zero, the Chi^2 value, and the standard errors less than 10 % of the referred parameter value.

Thus, the affinity constants K_D (k_d/k_a) were calculated describing the interactions between each purified antibody fraction and the immobilized drug ADA (Table 1). The kinetic of interaction between IgG enriched antibodies from H18b patient and immobilized adalimumab is given as representative example of a high K_D (1.02×10^{-6} M) in Fig. 4a. The lack of binding between the enriched-IgG fraction of control sample DS156 and the adalimumab did not allow calculation of kinetic parameters (Fig. 4b). Kinetic and affinity results are summarized in the k_a/k_d plot (Fig. 5). The graph supplies an overview of kinetic properties for the different interactions studied by plotting the association rate parameter (k_a) against the dissociation rate parameter (k_d). The affinity constants K_D (calculated as k_d/k_a ratio) are displayed on diagonal lines. Thus, interactions having the same affinity but different kinetic are indicated by points lying on the same diagonal line.

The immobilized drug ADA interacted with purified antibodies at an apparent affinity of 10^{-6} M$>K_D>10^{-9}$ M (Fig. 5). On the other hand, the lack of interaction between IgG-enriched antibodies of healthy control DS156 did not allow calculation of kinetic parameters.

On the whole, the data reported in Table 1 indicate that the two antibody fractions, obtained by affinity chromatography with immobilized ADA (samples H1b/ADA and H11b/ADA), are characterized by higher affinity, while the IgG fractions obtained from the same patients' sera by G protein chromatography, containing also non-specific antibodies (samples H1b/protG and H11b/protG), display slightly lower affinity. The interaction between IgG-enriched antibodies from H18b patient and immobilized ADA is characterized by a slower antigen/antibody dissociation. Finally, the

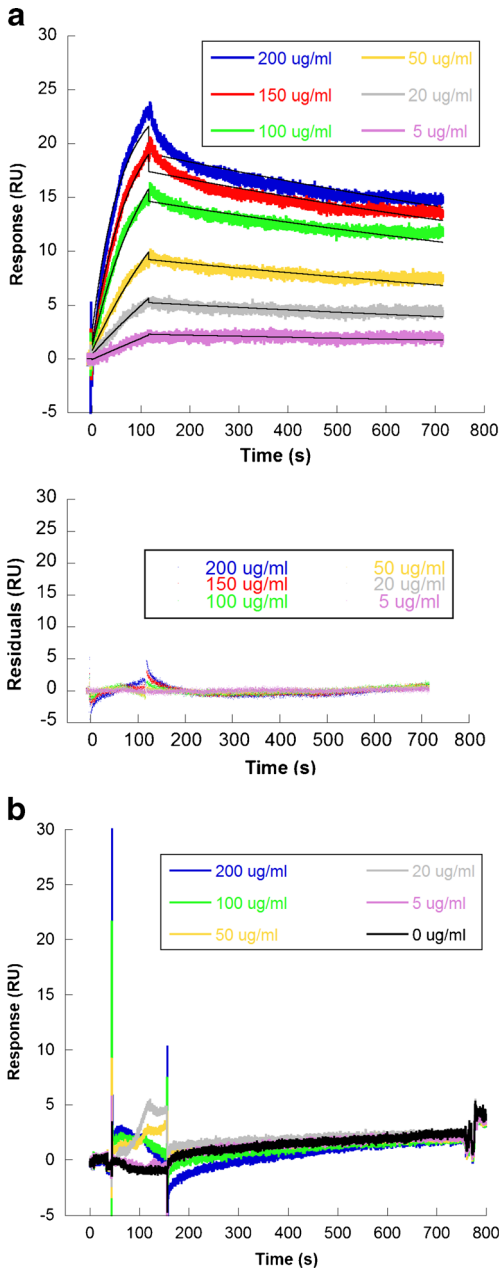

Fig. 4 Sensorgrams of interaction between anti-ADA antibodies purified from sample H18b and the immobilized adalimumab at different concentrations (**a**) comprising the theoretical curves for a 1:1 binding model (*black*). Residuals from the nonlinear regression analysis are displayed in the lower graph. Sensorgrams of the enriched-IgG fraction of control sample DS156 and the adalimumab did not allow calculation of kinetic parameters (**b**)

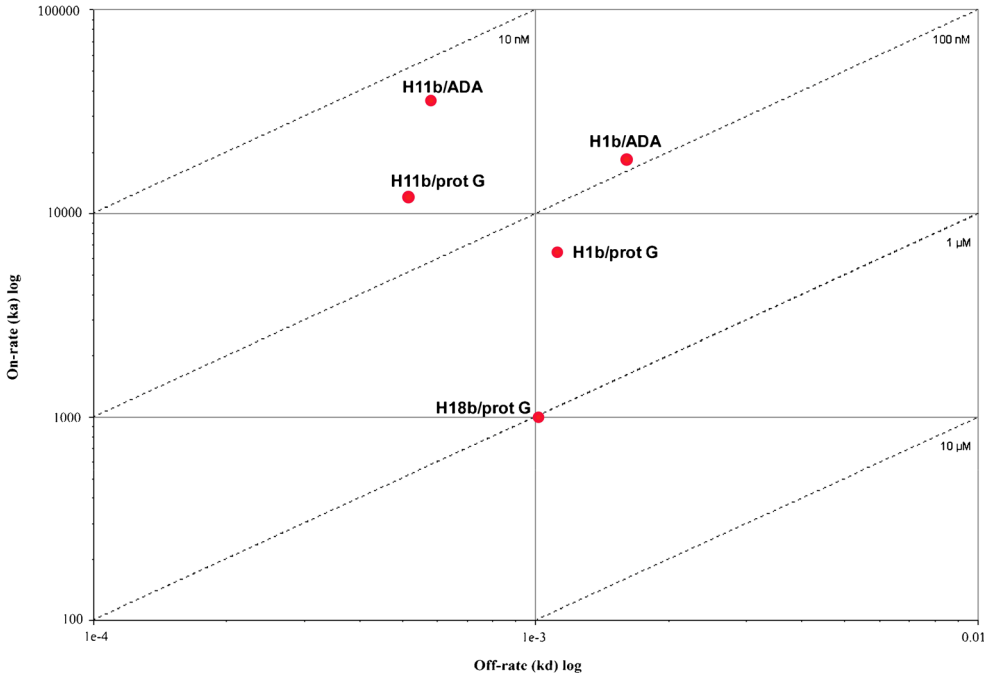

Fig. 5 k_a/k_d plot for the 1:1 binding model summarizing interactions between purified antibody fractions and the immobilized drug ADA. Affinity constants K_D (calculated as k_d/k_a ratio) are displayed on *diagonal lines*

background values, measured using control sera from healthy individuals, were negligible.

These results are relevant in the context of the discussion on anti-ADA antibody neutralizing capacity [16]. In fact, a recent study reporting affinity and specificity of patient-derived monoclonal anti-ADA antibodies hypothesized that although antibodies compete for binding to TNF, the response is clonally diverse and involves multiple epitopes on the drug ADA [36]. The screening method employed in this study may introduce a bias toward high affinities, lacking in low affinity antibody evaluation. Our method, based on direct antibody detection, probably reveals not only IgG antibodies, which commonly present high affinity and therefore are more potent drug inhibitors, but also IgM antibodies, which typically present lower affinities and are less effective in neutralizing the drug [17].

Conclusions

In the present study, we measured by surface plasmon resonance both the binding and the affinity of anti-ADA antibodies from pediatric patients' sera, analyzing the interaction of anti-drug antibodies using whole sera, enriched IgG fractions, and isolated anti-ADA antibodies. After immobilizing ADA on the chip surface by a covalent approach, we registered the binding signals flowing directly on the chip untreated diluted patients' sera or the isolated antibody fractions. In contrast to ELISA, in which information is provided by the use of a specific secondary antibody, the SPR assay yields data directly, as the antibody binds to the immobilized ADA. In this way, we developed a label-free method for the screening of ADA-specific antibodies, developing a protocol not only endowed with good sensitivity and high specificity, but also able to produce a response within a few minutes.

Since anti-drug antibodies might interfere with clinical response, an easy and reliable detection method would be of help to the clinician, especially in case of treatment failures who might benefit from a drug switch.

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Conflict of interest The authors declare that they have no conflict of interest in this article.

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