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Research paper

A novel method for the detection of antibodies to adalimumab in the presence of drug reveals "hidden" immunogenicity in rheumatoid arthritis patients

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ABSTRACT

Production of anti drug antibodies (ADA) in adalimumab treated RA patients is associated with reduced serum adalimumab levels and less clinical response. However, most current assays to measure ADA are unable to detect ADA in complex with adalimumab. Thus, ADA is only measured if antibody production exceeds drug levels in the serum, meaning that ADA formation is underestimated. The aim of this study is to develop a method to detect ADA in the presence of drug. A pH-shift-anti-idiotype Antigen binding test (PIA) was used to enable ADA measurement in the presence of adalimumab. ADA–adalimumab complexes were dissociated by acid treatment and addition of excess rabbit anti-idiotype-F(ab) before neutralization. Rabbit anti-idiotype-F(ab) blocks reformation of ADA–drug complexes by competing with patient ADA for adalimumab binding. Released ADA are measured by an antigen binding test (ABT). The PIA enabled detection of ADA in the presence of large excess of adalimumab and was used to measure ADA in 30 adalimumab treated rheumatoid arthritis (RA) patients during the first 28 weeks of treatment. It revealed ADA in 21 out of 30 tested patients, while the ABT detected ADA in only 5 patients. Indicating that an immunogenic reaction towards adalimumab is present in the majority of adalimumab treated patients.

1. Introduction

The introduction of therapeutic monoclonal antibodies has given a major boost to the treatment of several diseases such as rheumatoid arthritis (RA), multiple sclerosis and Bechterew disease (Elliott et al., 1994; Maini et al., 1998; Moreland et al., 1997; Polman et al., 2006; Weinblatt et al., 2003). Therapeutic antibodies are known to induce an immune response in part of the treated patients leading to formation of anti drug antibodies (ADA). The production of ADA has been described for several monoclonal antibodies available for the treatment of RA (adalimumab and infliximab), Crohn's disease (infliximab), multiple sclerosis (natalizumab) and plaque psoriasis (adalimumab) (Baert et al., 2003; Bartelds et al., 2007; Calabresi et al., 2007; Lecluse et al., 2010; Wolbink et al., 2006).

ADA have been detected in 17% of 121 adalimumab treated RA patients within the first 28 weeks of treatment. Production of ADA is associated with low drug levels and reduced clinical response (Bartelds et al., 2007). However, in currently used assays ADA detection is only possible if the production of ADA exceeds the amount of drug present in patients' serum due to the formation of ADA–adalimumab complexes (Wolbink et al., 2009). Hence, drug interference will lead to an underestimation of the number of patients producing ADA. Therefore the ability to measure ADA in the presence of adalimumab will provide a better insight in the immune response against adalimumab.

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Antibodies against adalimumab have been described to become undetectable upon continuation of treatment (Bartelds et al., 2007). This could be the result of drug interference, but it is tempting to speculate that prolonged exposure to the drug might induce tolerance. To discriminate between these two an assay which is able to detect ADA in the presence of adalimumab is needed. Furthermore, such an assay might allow preclinical testing of new monoclonal antibodies without the need of long wash-out periods.

Various groups have reported methods to overcome drug interference. Most of these assays are based on acid dissociation of ADA–drug complexes followed by neutralization in the presence of solid phase bound antigen (Bourdage et al., 2007; Lofgren et al., 2006; Lofgren et al., 2007; Patton et al., 2005; Schmidt et al., 2009; Sickert et al., 2008). Here we present a pH-shift-anti-idiotype (PIA) method where re-association of complexes is prevented by the addition of excess fluid phase F(ab) fragments of rabbit anti-idiotype antibodies that compete with patient antibodies for binding to adalimumab. The result is an assay which is able to detect ADA present in the circulation in the form of immune complexes. Using this assay we observed that the majority of RA patients treated with adalimumab develop ADA.

2. Materials and methods

2.1. Patient material

Sera were obtained in the first 28 weeks of treatment from the first thirty consecutive patients of a prospective observational cohort as previously described by Bartelds et al. (2007). All patients had a disease activity score in 28 joints (DAS28) of $\geq$3.2 and fulfilled the American College of Rheumatology 1997 revised criteria for RA. Despite earlier treatment with two disease-modifying anti-rheumatic drugs (DMARDs), including methotrexate, all patients had active disease at the start of adalimumab treatment. This was according to the Dutch consensus statement on the initiation and continuation of TNF blocking therapy in RA (Smolen et al., 2005). All patients used 40 mg adalimumab every other week by subcutaneous injections. In patients with an inadequate response, dose was increased to 40 mg every week. The study was approved by the ethics committee of the BovenIJ Hospital, the Academic Medical Center/University of Amsterdam, Slotervaart Hospital and the JBI.

2.2. Production of adalimumab F(ab)$_2$

To produce adalimumab F(ab)$_2$ fragments, 10 μg/ml pepsin (Boehringer, Mannheim, Germany) was incubated with 1 mg/ml adalimumab overnight at 37 °C in 0.1 M sodium citrate pH 3.5. Afterwards the F(ab)$_2$ fragments were dialyzed against phosphate buffered saline (PBS). Undigested adalimumab was removed by incubation with sepharose-immobilized protein A (GE healthcare, Chalfont St. Giles, UK).

2.3. Generation of adalimumab specific polyclonal rabbit anti-idiotype antibodies

Rabbits were injected intramuscularly every four weeks with 1 ml adalimumab F(ab)$_2$ (0.1 mg/ml in PBS) using montanide as adjuvans. After four boosts the rabbits were bled and serum was collected. Antibodies were purified from the serum using sepharose-immobilized protein A (GE healthcare). To remove antibodies to F(ab)$_2$ framework determinants the purified antibodies were passed three times over a human IgG-sepharose column (50 mg Nanogam (Sanquin, Amsterdam, the Netherlands) coupled to 2.5 g sepharose).

2.4. Production of rabbit anti-idiotype-F(ab) fragments

Purified rabbit anti-idiotype antibodies were treated with pepsin as described previously for adalimumab. Twenty mg of rabbit-anti-idiotype (0.55 mg/ml) was incubated with pepsin (5 μg/ml) (Boehringer). To make monovalent F(ab) fragments, the F(ab)$_2$ was reduced by incubation with 10 mM dithioerythritol for 30 min. Subsequently free thiol groups were blocked with 20 mM N-ethyl-maleinimide and F(ab) fragments were purified using size exclusion chromatography.

2.5. Biotinylation

Antibodies were biotinylated by incubation of 10 mg antibody with 6.3 mg Sulfo-NHS-LC-Biotin (Thermo scientific, Rockford, USA) in 0.1 M NaHCO3. After 2 h of incubation at room temperature (RT) biotinylated antibodies were dialyzed against PBS.

2.6. Measurement of adalimumab concentration

To measure adalimumab levels maxisorp ELISA plates were coated overnight (o/n) at RT with 2 μg/ml monoclonal anti-TNF-7 (Sanquin) in PBS. After five times washing with PBS/0.02% Tween (PT), plates were incubated for 1 h at RT with recombinant TNFα (0.01 μg/ml) (Strathmann Biotech HmbH, Hannover, Germany) diluted in high performance ELISA buffer (HPE, Business Unit reagents, Sanquin). Next, the plates were washed and incubated for 1 h with patient serum which was serially diluted in HPE. Subsequently, the plates were washed with PT and incubated for 1 h with biotinylated adalimumab specific rabbit anti-idiotype (0.25 μg/ml in HPE). After washing streptavidin-poly-HRP (Sanquin) (1/25000, in HPE) was added for 1 h at 37 °C. After washing the ELISA was developed with 100 μg/ml tetramethylbenzidine in 0.11 M sodium acetate (pH 5.5) containing 0.003% (v/v) H_2O_2. The reaction was stopped with 2 M H_2SO_4. Absorption at 450 nm was measured with a multiscan (Multiskan; Titertek, Elfab Oy, Finland). Results were related to a titration curve of adalimumab in each plate. The lowest level of detection was 0.002 mg/l.

2.7. Measuring ADA by antigen binding test (ABT)

The test was essentially carried out as described before (Witteman et al., 1996). One microliter of serum diluted in PBS/0.3% bovine serum albumin (BSA) (PA buffer) was incubated o/n with 1 mg Sepharose-immobilized protein A (GE healthcare, Chalfont St. Giles, UK) in a final volume of 800 μl. Subsequently the samples were washed with PBS 0.005% Tween and specific ADA binding was detected by o/n incubation with 20,000 dpm (approximately 1 ng) ^{125}I labeled F(ab)$_2$ adalimumab diluted in Freeze buffer (Sanquin). Unbound