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Short communication

Using monoclonal antibodies as an international standard for the measurement of anti-adalimumab antibodies

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ABSTRACT

Comparing studies investigating anti-drug antibody (ADA) formation is hampered by the lack of comparability between study protocols, assay formats, and standardized reference materials. In this respect, the use of an international standard would mean a major step forward. Here we compared 11 fully human monoclonal antibodies against adalimumab in two assays commonly used for ADA measurement; the bridging ELISA and the antigen binding test (ABT). Our results show non-parallel titration of the monoclonal antibodies in both assays, which we also find for polyclonal ADA sources. Moreover, we observed that the output of the bridging ELISA depends to a large degree on the affinity of the monoclonal antibody. For the ABT, results reflect a combination of affinity and avidity. This suggests that rather than reporting ADA values in nanogram per milliliter, arbitrary units may be more appropriate. Together our data highlight the difficulty of ADA standardization by identifying several pitfalls that should be taken into account when selecting a standard for ADA testing.

1. Introduction

In recent years, the number of biological agents has increased tremendously; many new drugs become available each year and an increased number of diseases are being targeted. In most cases these treatments are very beneficial for patients. However, in patients treated long-term with biologicals, part of the patients develop an immune response against the therapeutic resulting in the formation of anti drug antibodies (ADA) [1]. ADA production has been linked to lower serum drug levels and reduced clinical response [1,2]. Therefore, the development of an assay measuring ADA is essential in both drug development and in monitoring of patient ADA levels during treatment.

During the development of new biological drugs, the development of an ADA assay requires a standard or positive control at a time when clinical samples of ADA+ subjects will not yet be available and polyclonal or monoclonal antibodies raised in e.g., mouse or rabbit are often used as surrogate. After the introduction of a new biological treatment, there are many labs measuring ADA formation in diagnostic as well as research settings. However, differences in assay formats and output units (nanogram per mililiter; arbitrary units) make data comparison difficult [2,3,10]. Currently studies are being performed to compare antibody measurements between different assays and different labs [4,5]. However, using an international standard would allow for an easier way to compare ADA levels between labs.

One of the biologicals of which immunogenicity is a well documented phenomenon is adalimumab, an anti-TNF agent used for the treatment of different auto-immune diseases. Polyclonal ADA from patient sera are currently the most often used standard for ADA measurements in adalimumab treated patients. These suffer from limited availability and are a finite source. To circumvent this, a recent study by Gils et al. proposes a mouse monoclonal anti-adalimumab antibody as an international standard [6]. We have recently described the production of 11 patient derived fully human monoclonal antibodies (mAb's) against adalimumab [7]. We have shown that they have a wide range of affinities and that they are all directed against different, but overlapping epitopes in the TNF binding region of adalimumab [7,8]. Their human origin might make them a preferred choice as a standard. We tested our antibodies in two commonly used assays for the measurement of ADA. In this manuscript we will describe complications that were encountered,

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which should be taken into account in the further development of an ADA standard.

2. Materials and methods

2.1. Bridging ELISA for the measurement of ADA

Measurement of ADA in the bridging ELISA was essentially carried out as described before [8]. Briefly, samples of the different monoclonal antibodies [7,8] were serially diluted in microtiter plates coated with adalimumab, and bound antibody was revealed using biotinylated adalimumab/streptavidin-horse radish peroxidase.

2.2. Antigen binding test

The ABT was carried out as described before [9]. Briefly, serially diluted samples of the different monoclonal antibodies were incubated with protein A Sepharose beads, followed by incubation with radiolabelled F(ab') 2 or Fab fragments of adalimumab. Results are expressed as percentage radioactivity bound relative to radioactivity added.

3. Results

3.1. Measurement of ADA in different assays

Previously, a panel of 11 patient-derived monoclonal anti-adalimumab antibodies was generated [7]. The antibodies are all recombinantly expressed as human IgG1, bind specifically to adalimumab, thereby interfering with TNF binding thus neutralizing the drug [7]. We evaluated the dose-response relationship of these antibodies in two commonly used assays for the detection of ADA, the bridging ELISA and the antigen binding test (ABT). In the bridging ELISA the therapeutic is used as coat and conjugate and the ADA will form a bridge between them. In the ABT samples containing ADA are added to protein A sepharose beads and ADA are detected using labelled adalimumab F(ab') 2.

In both assays all eleven mAb's are measured in three repetitive experiments. Fig. 1 shows dose response curves of five very diversely reacting mAb's measured in the ELISA (A) and ABT (B) in a representative experiment. In both assays there is a large variation in the dose response curves of the different mAb's, demonstrating that the signals produced by both assays are not only the result of the net amount of antibody being measured. In both assays, dose response curves do not necessarily run parallel, and in the ELISA, dose response curves reach plateaus at different extinctions. Comparing dose response curves of Fig. 1A and B also shows that antibodies that respond similarly in the bridging ELISA (1.2 and 2.12; 2.6 and 2.9) can behave differently in the ABT. This indicates that the characteristics of the mAb's influence their measurement in both assays, but also that this effect is different between both assays.

3.2. The difference between dose response curves is partly explained by affinity

We hypothesized that the large differences between dose response curves in Fig. 1A and B could be partly explained by the large variation in affinity between the mAb's, as was measured previously (range 0.66–50,000 pM) [7]. Therefore, we investigated the correlation between the affinity of the mAb's and their EC50 values found in both assays (Fig. 2). Fig. 2A shows that there is a significant correlation between the EC50 value in the bridging ELISA and the affinity of the mAb's ($r = 0.88$; $p < 0.001$). This implies that the

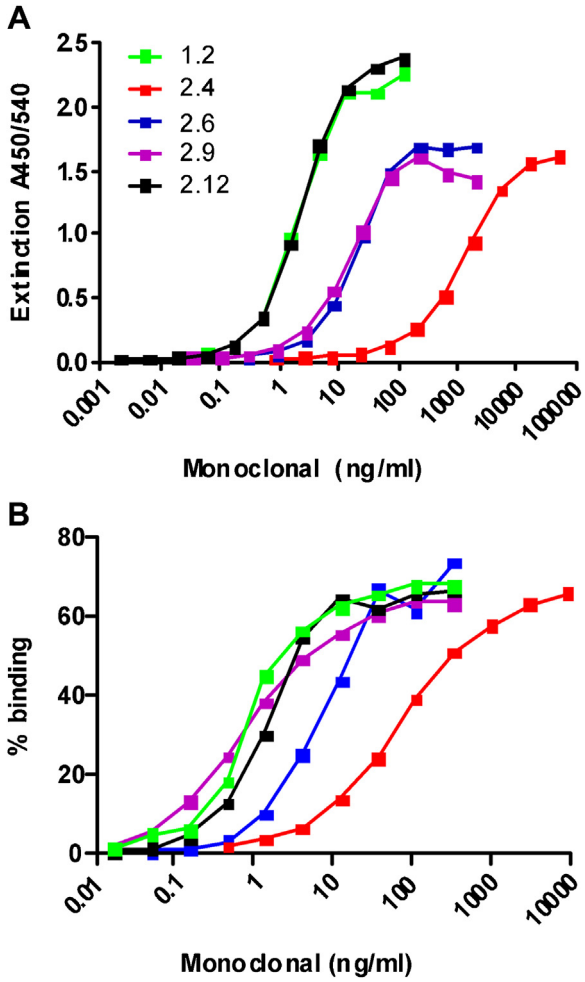

Fig. 1. The measurement of monoclonal anti-adalimumab antibodies in the bridging ELISA and ABT.
The measurement of five anti-adalimumab specific mAb's in the bridging ELISA (A) and the ABT (B) for the detection of ADA against adalimumab.

signal in the bridging ELISA is the result of both the quantity and the affinity of the antibody measured. On the other hand, the differences in EC50 values in the ABT did not significantly correlate with the affinity of the antibodies (Fig. 3B (spearman $r = 0.58$; $p = 0.06$)). Since different mAbs generated different signals for a given concentration, other factors may influence detection in the ABT. To investigate whether this could be a difference in preference to engage in a bivalent interaction, we repeated the ABT measurements using radiolabelled adalimumab Fab instead of F(ab') 2 for detection (Fig. 2C). A significant correlation between EC50 values in the Fab ABT and the affinity was found, demonstrating that affinity also affects the results of the ABT (Fig. 2C; spearman $r = 0.73$; $p < 0.05$). Comparing the fold increase in EC50 between the F(ab') 2 ABT and the Fab ABT shows that all antibodies can bind bivalently, but that there is a large variety in preference to undergo a bivalent interaction (median fold difference 6.23 (range 2.24–150.93)). Together this suggests that the output in the ABT is the result of a combination of quantity of the antibody, it's affinity and the preference of the antibody to engage in a bivalent interaction.

3.3. Non-parallel lines in the bridging ELISA and the ABT

Next, we wondered if the non-parallel lines in Fig. 1A and B are specific for measuring mAb's or whether this is also found in the measurement of patient sera or rabbit polyclonal anti-