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Drug interference in immunogenicity assays depends on valency

Theo Rispens[a,b,*], Margreet H. Hart[a,b], Pleuni Ooijevaar-de Heer[a,b], Astrid van Leeuwen[c], Anke Vennegoor[d], Joep Killestein[d], Gerrit-Jan Wolbink[a,b], Desiree van der Kleij[c]

[a] Sanquin Research, Amsterdam, The Netherlands
[b] Landsteiner Laboratory, Academic Medical Centre, University of Amsterdam, The Netherlands
[c] Sanquin Diagnostic Services, Amsterdam, The Netherlands
[d] Department of Neurology, VU University Medical Center, Amsterdam, The Netherlands

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ABSTRACT

Direct comparison of immunogenicity data is hampered by differential drug interference in different assay formats. In this paper we identify a drug-related factor that influences the extent of drug interference. We systematically investigated the influence of drug valency of different antibody-derived biologicals on the drug interference, using mono- and bivalent formats of adalimumab as a model system. Our results indicate that compared to regular bivalent antibodies, antibody-derived drugs that are monovalent result in less drug interference. Two real-life examples were examined: natalizumab, an IgG4 antibody that becomes effectively monovalent in vivo due to Fab arm exchange, and certolizumab pegol, a pegylated Fab fragment. For both drugs it was demonstrated that drug interference is less pronounced in an antigen-binding test compared to similar assays for other therapeutic antibodies. When comparing immunogenicity data obtained for different biologicals this phenomenon should be taken into account.

1. Introduction

Antibody responses to a biological can result in loss of efficacy [1–9]. Incidences of antibody formation to different biologicals differ greatly [10]. However, direct comparison of immunogenicity data is hampered by differential drug interference in different assay formats [11–13]. In this paper we show that even if the same assay format is used to assess immunogenicity, the results for different biologicals may not be directly comparable.

Drug interference occurs in situations where drug and anti-drug antibody are simultaneously present in a serum sample. Because many therapeutic antibodies are administered in high doses, it is possible that a patient does make antibodies, but in amounts not exceeding the amounts of drug. Anti drug antibodies (ADA) will then be present in complex with drug. Part of these complexes may spontaneously dissociate, allowing detection in an assay for measuring ADA. Alternatively, acid-dissociation protocols have been developed to allow a larger part of ADA to dissociate and be detected [14–21]. Because the detection of ADA depends to a large extent on the balance between bound and unbound antibody to drug, factors that influence association and dissociation between drug and ADA will directly influence the test results.

We recently conducted a study on antibody formation to natalizumab [22]. We found a substantially larger percentage of patients that make antibodies compared to a number of other studies [23,24]. Besides factors such as differences in patient population and sampling times, the use of different assays in these studies is likely to contribute to these observed differences. Because natalizumab is an IgG4 antibody that will exchange half-molecules with patient IgG4 [25,26] (Fig. 1B), we wondered if this would result in less drug interference in the assay format that we used, i.e., the antigen-binding test [27] (Fig. 1A).

In the antigen-binding test, IgG from a serum sample is captured by protein A Sepharose beads, followed by detection of specific antibodies using radiolabeled F(ab′)2 fragments of the drug. This assay format was found to be susceptible to drug interference when used to assess antibody formation to adalimumab, albeit less compared to a bridging ELISA (Fig. 2A) [13]. However, we also found that a variation to this assay that only measures IgG4 antibodies

Abbreviations: ADA, anti-drug antibody; ABT, antigen binding test; GSH, reduced glutathione.

* Corresponding author at: Department of Immunopathology, Sanquin Research, Plesmanlaan 125, 1066 CX Amsterdam, The Netherlands. Tel.: +31 20 5123171; fax: +31 20 512 3171.
E-mail address: t.rispens@sanquin.nl (T. Rispens).

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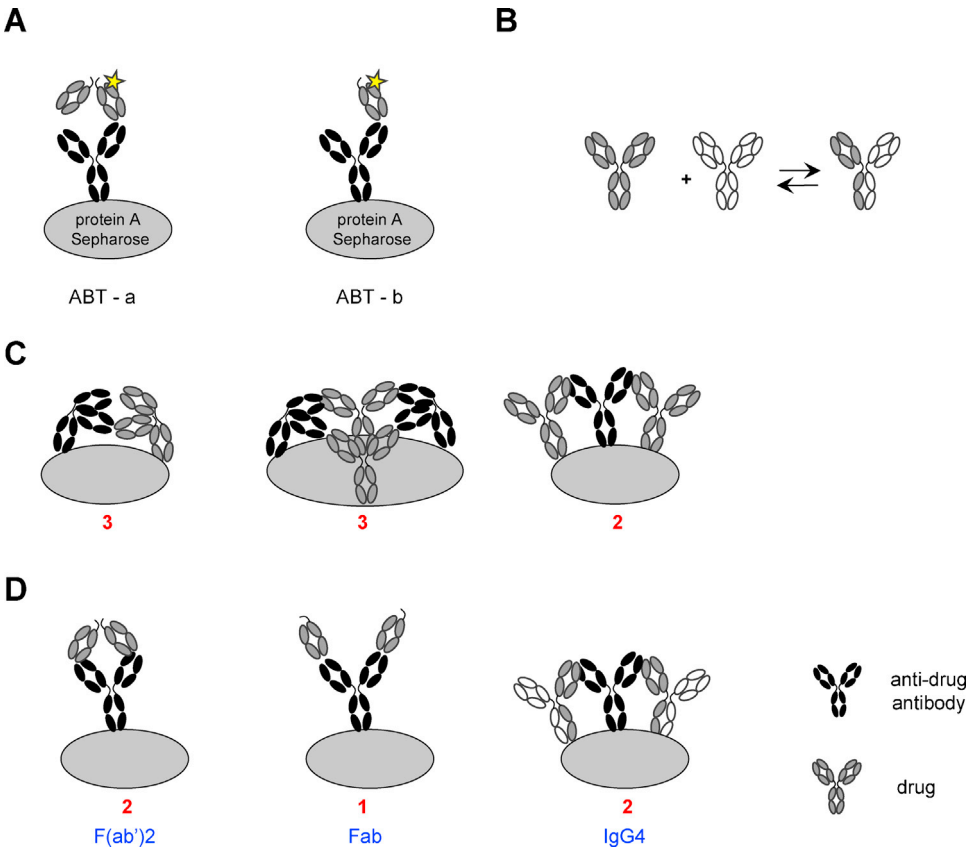

Fig. 1. Drug interference in antigen binding tests. (A) Antigen binding test (ABT-a) in which IgG from serum is captured with protein A Sepharose and specific anti-drug antibodies (black) detected using radiolabeled F(ab′)2 fragments of e.g. adalimumab (gray). ABT-b is a modification of this assay in which radiolabeled Fab fragments are used for detection. (B) Therapeutic IgG4 antibodies such as natalizumab will exchange half-molecules with serum IgG4 in vivo. (C) If drug and anti-drug-antibodies are present in serum, complexes containing both will be captured by protein A. In case the drug is an IgG1 antibody, such as adalimumab, it may be attached to the Sepharose by up to three sites: 2× via a Fab arm and 1× via its Fc tail. For each configuration, the number of sites of attachment is indicated in red. (D) F(ab′)2, Fab, and the IgG4 version of a therapeutic antibody (after exchange with other IgG4) can similarly attach by up to 2, 1, and 2 sites, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

to adalimumab was less susceptible for drug interference. As will be explained in Section 4, this difference in drug interference was found to depend on the total number of sites that is involved in the binding of the drug to the solid phase.

Therefore, in this study, we systematically investigated the influence of valency of different antibody-derived biologicals on drug interference. In particular, we compared F(ab′)2 and Fab fragments of adalimumab, as well as an IgG4 variant of adalimumab, to the intact IgG1 antibody. We demonstrate that in case the effective valency of a therapeutic antibody is changed from two to one, less drug interference is observed in both an antigen-binding test as well as a bridging ELISA. Two real-life examples were also examined. Natalizumab, an IgG4 antibody that is used to treat relapsing multiple sclerosis [28], becomes effectively monovalent in vivo due to Fab arm exchange [26]. Certolizumab pegol is a pegylated Fab fragment that binds TNF and is used to treat inflammatory diseases such as rheumatoid arthritis [29].

2. Materials and methods

2.1. Materials

Serum samples containing high titers of anti-adalimumab antibodies and no detectable adalimumab were selected from a prospective observational cohort of 300 patients as previously described by Bartelds et al. [6]. A serum sample containing anti-certolizumab antibodies was drawn under informed consent from a patient treated with certolizumab pegol. These studies were approved by the ethics committee of the BovenIJ Hospital, the Academic Medical Center/University of Amsterdam, Slotervaart Hospital and Reade, Amsterdam, The Netherlands. Serum samples from individuals with relapsing multiple sclerosis treated with natalizumab containing anti-natalizumab antibodies and no detectable natalizumab were drawn under informed consent after approval of the ethics committee of the VU medical center (Amsterdam). The samples were drawn just before the next infusion of natalizumab. Since the Fab arm exchange reaction is complete after several days [25,30] and natalizumab is infused every four weeks, this means that most natalizumab will be present as effectively monovalent natalizumab, typically ca. 98%.

Monoclonal therapeutic antibodies used in this study are natalizumab (Tysabri, Biogen Idec, Inc.), adalimumab (Humira, Abbott), and certolizumab pegol (Cimzia, UCB Pharma).

2.2. Production of adalimumab IgG4

An IgG4 version of adalimumab was prepared by cloning constructs coding for VH and VL of adalimumab [31] and the constant