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Available online September 30, 2016.
http://dx.doi.org/10.1016/j.jaci.2016.08.028

Neutralizing capacity of monoclonal and polyclonal anti-natalizumab antibodies: The immune response to antibody therapeutics preferentially targets the antigen-binding site

To the Editor:

Many therapeutic mAbs have been found to elicit an immune response to various degrees. Antidrug antibodies (ADAs) can diminish efficacy by neutralization or enhanced clearance of the therapeutics, and occasionally cause hypersensitivity reactions or other adverse events.[1] Therapeutic antibodies are classified as chimeric, humanized, or "fully human," in order of increasing homology of the variable domains with human germline sequences, and thereby their expected decrease in immunogenicity.

Recently, our group investigated the antibody repertoire in patients with an immune response against any of 4 different anti-TNF therapeutic antibodies.[2,3] For the human(ized) adalimumab, certolizumab, and golimumab, more than 94% were found to bind the TNF-binding site, demonstrating that these ADAs are essentially all neutralizing. Strikingly, the antibody response to the chimeric infliximab, of which the entire variable domains are of mouse origin, was also more than 90% directed to the TNF-binding site. These results prompted the question whether this restricted response is due to specific immunogenic properties of the anti–TNF-binding site, or that the antigen-binding site (or paratope) is an inherently immunodominant part of the antibody, irrespective of its target. If so, antidrug antibodies to any therapeutic antibody might be expected to be predominantly neutralizing.

To shed light on this question, we investigated the antibody response to natalizumab, a humanized antibody that differs in target (α4-integrin), subclass (IgG_4), and patient group (relapsing-remitting multiple sclerosis [RRMS]) from anti-TNF therapeutics. Natalizumab contains several nonhuman germline determinants outside of its paratope (Fig 1, *A*) and can thus theoretically elicit a broad nonneutralizing antibody response. In this study, we examined the neutralizing capacity of the anti-natalizumab response of patients with RRMS.

We therefore first developed human monoclonal anti-natalizumab antibodies. Variable domain sequences of in total 3 natalizumab-specific peripheral blood B cells were obtained from 2 patients (see Table E1 in this article's Online Repository at www.jacionline.org). These sequences were recombinantly expressed in HEK293F cells, resulting in 3 human monoclonal anti-natalizumab antibodies. Analysis of the clones using ImMunoGeneTics/V-QUEry and STandardization (IMGT/V-Quest)[5] revealed a distinct V(D)J gene usage for each clone. Using surface plasmon resonance, the affinity of anti-natalizumab 1.1 and 2.1 was found to be moderate, whereas anti-natalizumab 2.2 had a high affinity (1×10^{-11} mol/L) (see Fig E1 and Table E2 in this article's Online Repository at www.jacionline.org).

To investigate the natalizumab-binding site for each clone relative to the others, the antibodies were tested in a competitive ELISA. In this assay, the binding of a labeled clone was assessed in the presence of a competing nonlabeled clone (Fig 1, *B*). The results showed that the mAbs all competed with each other for binding to natalizumab (Fig 1, *C*), indicating that all 3 mAbs bind to a restricted site on natalizumab.

We further examined whether the mAbs could block the binding of natalizumab to its target α4-integrin. This was tested using fluorescently labeled natalizumab that binds α4-integrin expressed on Jurkat cells. Preincubation of natalizumab with any of the 3 mAbs abrogated the binding of natalizumab to α4-integrin (Fig 1, *D* and *E*). Similar results were found for the binding of exchanged (ie, monovalent; see "IgG_4 half-molecule exchange" section in this article's Online Repository at www.jacionline.org) natalizumab to α4-integrin (see Fig E2 in this article's Online Repository at www.jacionline.org). This indicates that all 3 monoclonal anti-natalizumab antibodies compete with α4-integrin for binding to natalizumab, and are thus all neutralizing.

Next, we investigated the neutralizing capacity of polyclonal anti-natalizumab antibodies from 15 ADA-positive patients. A competitive ELISA was set up in which α4-integrin blocks the paratope of natalizumab (Fig 2, *A*). For all patients, the binding of ADA to natalizumab was reduced with increasing concentrations of α4-integrin (Fig 2, *B*). With the highest amount of α4-integrin tested, the signal was reduced by at least 91% compared with the uninhibited signal (Fig 2, *C*). This indicates that for all patients tested, at least 91% of all ADAs to natalizumab are neutralizing.

We further analyzed which parts of natalizumab are targeted by the anti-natalizumab response using a similar competitive assay, but now using neutralizing, but less bulky, Fab fragments of monoclonal anti-natalizumab 2.2 to block the binding site of natalizumab (Fig 2, *D*). Again, the binding of patient ADAs to natalizumab could be reduced in a concentration-dependent manner, ultimately preventing at least 92% of ADAs from binding natalizumab using this single mAb (Fig 2, *E* and *F*). This implies that most ADAs from patients with RRMS bind determinants of natalizumab located in or in proximity to the paratope.

The results from this present study on the anti-natalizumab response, in combination with our previous findings on the neutralizing capacity of ADAs to anti-TNF therapeutics,[2,3] indicate that the immune response preferentially targets the paratope of at least several classes of therapeutic antibodies. The antigen specificity of the drug seems irrelevant for this response. In addition, the nonhuman germline determinants outside of the paratope found in natalizumab, as well as those in the chimeric infliximab (and even in the other humanized and fully human anti-TNFs), apparently are less immunogenic. This observation is also consistent with the lack of immune response toward mismatched allotypes of therapeutic antibodies.[6] The paratope of therapeutic antibodies, but perhaps of any antibody, might be intrinsically more immunogenic than other determinants outside of the paratope. It is however unknown whether the paratope displays specific structural features that facilitate its potential role as epitope.

For infliximab, attempts were made to more precisely locate immunogenic amino acid sequences using synthetic peptides. Homann et al[7] found reactivity of ADA-positive patients to 4 peptide sequences located in or in proximity to the complementarity-determining regions, although 2 of these peptides were also recognized by healthy controls.[7] In contrast, a study by Kosmač et al[8] showed no reactivity to synthetic peptides nor to denatured

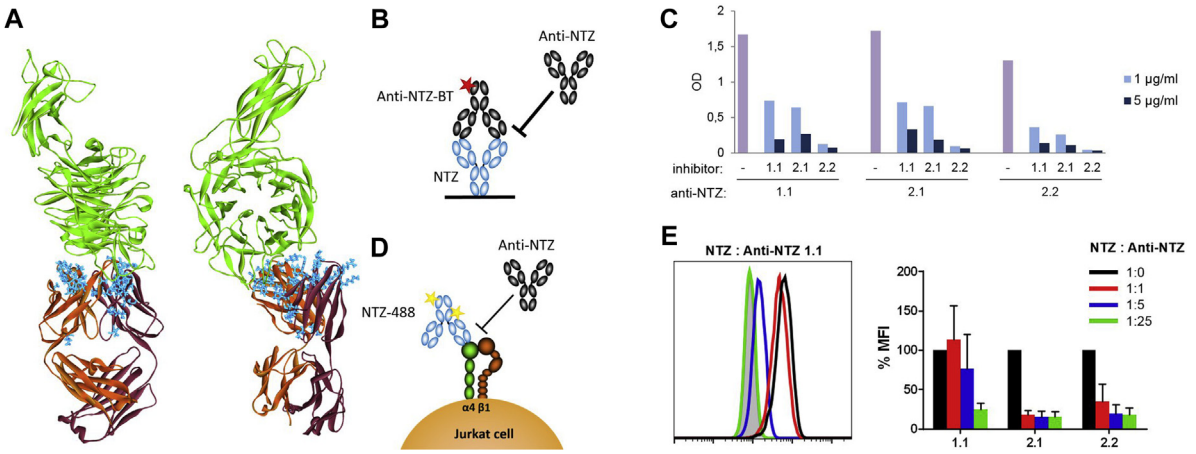

FIG 1. Monoclonal anti-natalizumab antibodies compete with each other and with α4-integrin for binding natalizumab. **A,** Crystal structure of natalizumab Fab *(orange* and *purple)* in complex with the α4 headpiece *(green)*. Blue residues differ from human germline. Adapted from Yu et al.[4] **B,** Schematic representation of the competitive ELISA. **C,** Monoclonal anti-natalizumab antibodies bind natalizumab and compete with each other. **D,** Fluorescently labeled natalizumab (NTZ-488) binds α4-integrin expressed on Jurkat cells; neutralizing antibodies inhibit this binding. **E,** *Left:* Representative histogram of the ratio-dependent reduction of natalizumab binding to α4-integrin induced by mAb 1.1. *Right:* Inhibition of natalizumab binding to cell surface α4-integrin by all 3 mAbs determined by FACS. *BT*, Biotinylated; *FACS*, fluorescence-activated cell sorting; *MFI*, mean fluorescence intensity; *NTZ*, natalizumab.

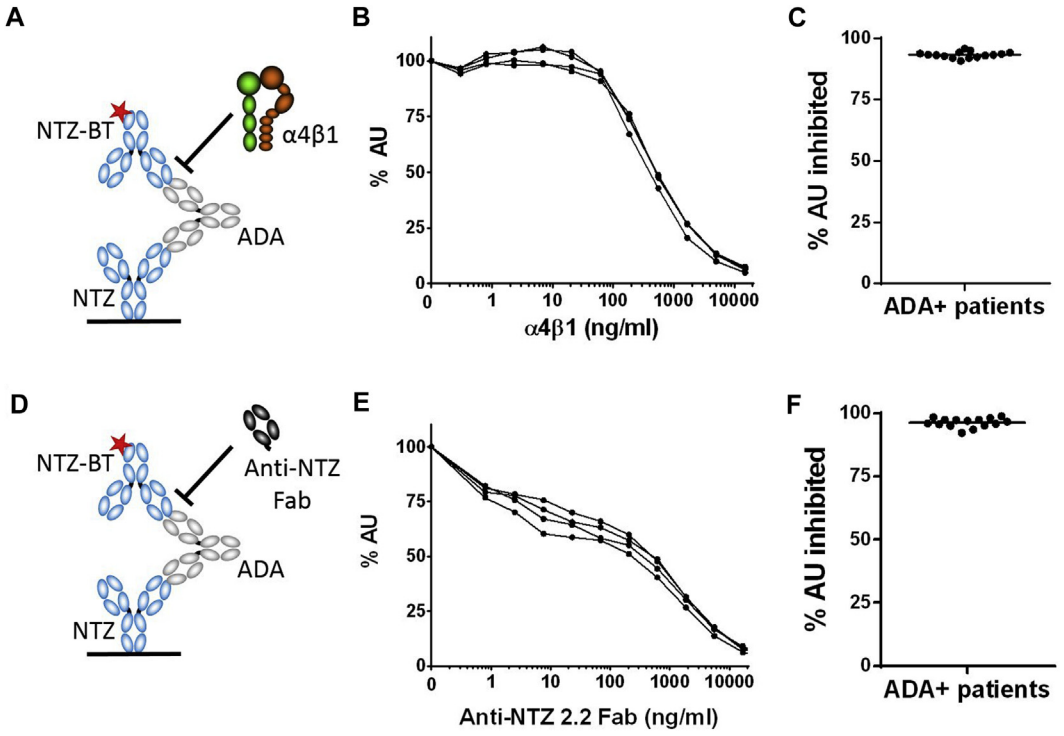

FIG 2. Most serum anti-natalizumab antibodies neutralize natalizumab. **A** and **D,** Schematic representation of the competitive ELISA. **B** and **E,** Increasing amounts of α4β1 (Fig 2, *B*) or anti-natalizumab 2.2 Fab (Fig 2, *E*) decrease ADA binding to natalizumab. Each *line* represents 1 patient. A selection of representative patients is shown. **C** and **F,** Percentage of AUs inhibited by 15 μg of α4β1 (Fig 2, *C*) or anti-natalizumab 2.2 Fab (Fig 2, *F*). Each *dot* represents a single patient. Median and range for inhibition with α4β1 93% (91% to 96%) and anti-natalizumab 2.2 Fab 97% (92% to 99%) (n = 15). *AU*, Arbitrary unit; *BT*, biotinylated; *NTZ*, natalizumab.

infliximab Fab, suggesting that anti-infliximab antibodies target conformational rather that linear epitopes. It therefore remains unclear which precise determinants on infliximab are targeted. For adalimumab, we demonstrated that anti-adalimumab antibodies bind to multiple overlapping, but distinct epitopes in the paratope.[3]

Human(ized) and chimeric therapeutic antibodies all have fully human constant domains. Although there is little evidence for