SUPPORTING INFORMATION

Given the therapeutic efficacy of murine mAb GNC92H2, the next challenge in transitioning a mAb candidate to advanced preclinical or clinical study was to reduce its immunogenicity to humans. Traditional methods have included humanization, antibody engineering via phage display technology, and the generation of human-mouse chimeras, in which the murine variable regions are joined to human constant regions; such techniques are either technically nontrivial, time-consuming, fail to evade the human anti-chimeric mAb response, and/or diminish the binding affinity/specificity of the resulting mAb. Herein, through a fortuitous collaboration with Dr. Chadwick King of Abgenix Biopharma, now Amgen Inc., XenoMouse® mice were immunized with our GNC hapten in order to obtain fully human anti-cocaine antibodies for our study on the immunopharmacotherapeutic reversal of cocaine overdose. To provide a brief background on Abgenix's XenoMouse® technology, the vaccination of XenoMouse® strains with human antigens results in a robust antigen-specific immune response in mice and the potential to elicit the entire range of human heavy chain, kappa light chain and lambda light chain antibodies as well as the different IgG classes: IgG1, IgG2, IgG4, based on the specific XenoMouse®.[1,2] Furthermore, the affinity values for mAbs from XenoMouse® animals are amongst the highest published values for human antibodies against human antigens, including those from other engineered mice[3] or those from combinatorial libraries[4].

Upon the generation of GNC-binding antibody repertoire using XenoMouse, KinExA (Kinetic Exclusion Assay) was employed to measure the binding constants of the superior anti-GNC mAb clones during the isolation and selection of mAb GNCgzk. The KinExA system (Sapidyne Instruments Inc.) measures the K_d, k_{on}, and k_{off} binding constants in the *solution phase* to characterize bimolecular binding events.[5] It thereby avoids the mass transport limitations and mobility effects inherent to traditional methods that measure binding events between a solution phase and a solid phase, and thus provides a *very* accurate K_d measurement.[6] In a standard set-up, the antigen and antibody are mixed in solution for a predetermined time period or until the formation of the antigen-antibody complex has reached

equilibrium. This solution is passed through resin that contains either immobilized antigen or a mAb capture reagent, and the KinExA flow spectrofluorimeter measures the level of free mAb using a fluorescently labeled polyclonal antibody against the mAb target (e.g., anti-human-IgG pAb for the detection of mAb GNCgzk). To estimate the K_d for a mAb, the amount of antigen that is allowed to equilibrate with a specific mAb concentration (i.e., k_{on}[mAb][antigen] = k_{off}[mAb-antigen complex]) is varied such that the fluorescent signal for free mAb ranges from 0 to 100% of the maximum signal. The initial concentrations of antigen and mAb and the % of free mAb may be used to calculate the K_d. Depending on the assay method, k_{on} may be obtained via plotting the free mAb as a function of time or of varying antigen concentrations. These values for the association rate constant, k_{on}, and the equilibrium dissociation constant, K_d, of the antibody-antigen complex may be carried over to the calculation of k_{off} ($k_{off} = k_{on} \times K_d$).

Other methods commonly used to kinetically and thermodynamically characterize antibody-antigen binding include equilibrium dialysis, competition ELISA, Biacore, FACs, and radioligand binding assays. Indeed, we have previously measured the cocaine binding affinity of mAb GNC92H2 using other established protocols, including: equilibrium dialysis (K_d = 200 nM)[7] and competition ELISA for cocaine ($K_{d,app}$ ~ 13 nM). Likewise, the affinity of other anti-cocaine mAbs for cocaine and its major metabolites (e.g., benzoylecognine and ecognine methyl ester, and its transesterfication product with ethanol: cocaethylene) has been determined through these alternative methods and reported in the literature (see Table 1). However, with respect to the determination of accurate quantitative K_d's, all such biophysical methods possess their own experimental weaknesses, for which we will provide a brief overview here.[6] Equilibrium dialysis offers a benefit similar to KinExA in that the antibody and antigen are permitted to interact freely in solution. Its major limitations, namely the solubility and size of the antigen (i.e., such that the antigen diffuses freely across the dialysis membrane), are not applicable to the assessment of cocaine-binding mAbs. Biacore, which is based on the automated determination of binding kinetics and thermodynamics via surface plasmon resonance, is both sensitive and capable of

handling a wider range of antigens (*vs.* equilibrium dialysis). Furthermore, the growth in its popularity over the last decades has promoted significant advances in its instrumentation, broadened its applicability to a variety of biomolecular interactions, and improved the data analysis methods. Also, the change in mass of the species coating the sensor chip is recorded in real time, which allows one to "observe" the association and dissociation binding kinetics on sensorgrams. Like competition ELISA, the immobilization of one species on a chip or ELISA plate may interfere with both the activity of the bound species (effective display of the key epitopes, binding activity of the antibody) and the interaction between binding partners.

The KinExA binding results are reported in the current study in order to remain consistent with our original use of the KinExA method for the selection of GNCgzk from among superior clones, for the evaluation of mAb GNCgzk-binding to numerous targets (e.g., cocaine and its major metabolites), and for its direct comparison to murine mAb GNC92H2. To summarize (see Table S1), the binding affinities of mAb GNCgzk and of mAbGNC92H2 for cocaine were calculated to be K_d = 0.18 nM and K_d = 2.0 nM, respectively.[8] GNCgzk and GNC92H2 displayed modest affinity for the psychoactive metabolite benzoylecognine.

Table S1. Anti-cocaine antibody affinity for cocaine and its major metabolites[a]

	Competitive Elisa[b]				**KinExA**[c]	
Antibody	Cocaine	Cocaethylene	Benzyl ecgonine	Ecgonine methyl ester	Cocaine	Benzyl ecgonine
GNCgzk	3.4	0.5	0.4×10^3	79×10^3	0.18	50
GNC92H2	13	5.0	8.0×10^3	50×10^3	2.0	1.4×10^3
	Double Ab. Precipitation Method[d]					
Human 2E2	4.4	3.4	4.3	5.2×10^3		
Murine 3P1A6	0.22	0.86	14	0.24×10^3		

a All data are in nM.

b Data represents $K_{d,app}$.

c KinExA experiments were performed by Dr. Chadwick King of Abgenix Biopharma, now Amgen Inc.

d See Paula et al., 2003[9] and 2004[10].

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