Isolation and Characterization of Human Monoclonal Antibodies to Digoxin

William J. Ball, Jr., Rama Kasturi, Purabi Dey, Michael Tabet, Susan O'Donnell, Debra Hudson and Dianne Fishwild

*J Immunol* 1999; 163:2291-2298; ;
http://www.jimmunol.org/content/163/4/2291

---

**References**  This article cites 42 articles, 10 of which you can access for free at: http://www.jimmunol.org/content/163/4/2291.full#ref-list-1

**Subscription**  Information about subscribing to *The Journal of Immunology* is online at: http://jimmunol.org/subscription

**Permissions**  Submit copyright permission requests at: http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**  Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/alerts
Isolation and Characterization of Human Monoclonal Antibodies to Digoxin

William J. Ball, Jr., Rama Kasturi, Purabi Dey, Michael Tabet, Susan O'Donnell, Debra Hudson, and Dianne Fishwild

Fab preparations of sheep polyclonal anti-digoxin Abs have proven useful for reversal of the toxic effects of digoxin overdoses in patients. Unfortunately, the use of foreign species proteins in humans is limited because of the potential for immunological responses that include hypersensitivity reactions and acute anaphylaxis. Immunization of recently developed transgenic mice, whose endogenous μ heavy and κ light chain Ig genes are inactivated and which carry human Ig gene segments, with a digoxin-protein conjugate has enabled us to generate and isolate eight hybridoma cell lines secreting human sequence anti-digoxin mAbs. Six of the mAbs have been partially characterized and shown to have high specificity and low nanomolar affinities for digoxin. In addition, detailed competition binding studies performed with three of these mAbs have shown them to have distinct differences in their digoxin binding, and that all three structural moieties of the drug, the primary digitoxose sugar, steroid, and five-member unsaturated lactone ring, contribute to Ab recognition. The Journal of Immunology, 1999, 163: 2291–2298.

Because the clinical usefulness of anti-digoxin polyclonal Fab treatment (11–13) and the feasibility of generating drug-specific mouse mAbs have been well established, this immunological treatment strategy is potentially applicable to additional drug overdose situations. Indeed, in addition to sheep polyclonal anti-digoxin Abs can quickly reverse digoxin cardiotoxicity by binding to free drug in plasma and effecting the redistribution of the drug from a patient’s tissues back to the vascular circulation (8, 9). Further, because Fabs are relatively rapidly excreted in urine, high affinity Fabs that retain bound drug can provide a route of drug elimination as well as a means of its neutralization (10).

Because both the clinical usefulness of anti-digoxin polyclonal Fab treatment (11–13) and the feasibility of generating drug-specific mouse mAbs have been well established, this immunological treatment strategy is potentially applicable to additional drug overdose situations. Indeed, in addition to sheep polyclonal anti-digoxin Fabs, goat Fabs directed against colchicine (14) have been used to treat life-threatening overdoses of this toxic alkaloid. Additionally, therapeutic mouse mAbs directed against the tricyclic anti-depressant, desipramine (15), and the abused psychosis-inducing drug, phencyclidine (16), are under development. Unfortunately, in general, sheep and goat polyclonal Fabs can present problems with respect to the ability to reproducibly generate high affinity Abs, which then must be purified from collections of animal sera. Further, the use of these foreign proteins in patients leads to an immunological response that can cause hypersensitivity reactions and acute anaphylaxis. Mouse mAbs, while providing a single well-characterized product, have very short half-lives in humans and are especially prone to generate anti-idiotype, activating Abs as well as anti-constant region responses. Therefore, drug-targeted immunological intervention has been restricted by the lack of reliable methods to produce drug-specific human Abs that would reduce the risk of sensitizing patients and allow for more than a one time only intervention during life-threatening crises. To address this general problem, in recent years, considerable efforts have been made to generate mouse-human chimeric, humanized, and primatized mAbs (17–20) that are more suitable for human use.

As an alternative and potentially more useful approach, recently we (21–26) and others (27–30) have adopted the strategy of humanizing the mouse humoral immune system. We have been able to develop several unique strains of genetically altered mice with inactivated endogenous μ heavy and κ light chain loci and inserted human heavy (constant regions, Cμ and Cy) and κ light chain transgenes (21–24). This allows the generation of mouse B cells that are capable of responding to immunization and undergo heavy chain class switching and somatic mutation to generate human IgG1 κ Abs. Our initial results indicate that these animals are capable of generating human Abs against a variety of human and nonhuman proteins and that standard hybridoma technology can be used to obtain human mAbs with affinities and quantities that compare favorably with those of murine mAbs (24–26).

We now report the first use of one of these transgenic mouse strains, HC2/KCo5 (24), to generate hybridoma-secreted human mAbs directed against the low m.w., nonpeptidic hapten, digoxin. In this paper we describe the partial characterization of six of the eight human anti-digoxin mAbs we have obtained to date. Three of these mAbs have been studied more thoroughly, and determination...
of the ability of eight digoxin-related cardioactive steroids and 10 steroid hormones to compete with digoxin for mAb binding has shown them to have a fine specificity of binding comparable to that of normal mouse mAbs. Further, radioligand binding studies have shown them to have low nanomolar binding affinities for digoxin that may prove sufficient for them to be clinically useful.

Materials and Methods

Transgenic mice

The mice [C57BL/6 × CBA/JF] used in this study have undergone four distinct genetic modifications, resulting in double-transgenic/double-deletion mice that have been described previously (25). The disruption of the endogenous mouse \( \mu \) heavy chain production (designated the \( \mu D \) strain) results from the insertion of a neomycin resistance gene into the \( \mu \) coding region (N. Lonberg, D. Fishwild, and L. D. Taylor, manuscript in preparation). The \( \kappa \) light chain disruption (\( \kappa C \) strain) results from recombinant deletion of \( \kappa C \) and \( \kappa R \) gene segments (24). The constructed human sequence heavy chain minigene, transgene, designated HC2, that rescues B cell development in the mutant background animals, includes four \( \kappa V \), 16 D, six \( \kappa J \) gene segments, and \( \kappa C \) and \( \kappa Y \). The \( \kappa C 05 \) light chain transgene contains the \( \kappa C 04 \) transgene of four \( \kappa V \), with all five \( \kappa J \) gene segments and \( \kappa C \) as well as a 450-kb yeast artificial chromosome (YAC) that contains most of the remaining distal portion of the human \( \kappa R \) region (25).

Synthesis of hapten-carrier conjugates

Digoxin was conjugated to BSA, chicken OVA, and keyhole limpet hemocyanin (KLH) using the method described by Butler and Chen (6). The approximate extent of digoxin covalent coupling per mole (or milligram) of protein was determined spectrophotometrically in 83% \( \text{H}_2\text{SO}_4 \), with the absorption of the hapten-protein conjugate at 388 and 465 nm compared with that of protein and digoxin (6). The extent of hapten coupling was 8 and 2 mol of digoxin/mol of BSA and OVA, respectively, and 0.1 mmol/mg KLH.

Immunizations and cell fusion

Fifteen transgenic mice were immunized initially with either 100 \( \mu \)g of a digoxin-KLH conjugate suspended in CFA via i.p. injection or 50 \( \mu \)g of digoxin-KLH suspended in TiterMax (TM) via s.c. injection. The mice receiving immunogen in CFA were then immunized i.p. twice with 20 \( \mu \)g of digoxin-KLH in IFA followed by weekly or biweekly injections of 20 \( \mu \)g of digoxin-ova in IFA. The mice receiving immunogen in TM were subsequently immunized s.c. approximately monthly with 50 \( \mu \)g of digoxin-ova in TM. All mice received a final s.c. boost of 20 \( \mu \)g of digoxin-ova in PBS 3 days before fusion. Splenic lymphocyte suspensions were fused to P3 × 63-Ag8.653 nonsecreting mouse myeloma cells (American Type Culture Collection, Manassas, VA; CRL 1480), and hybridomas were selected as previously described (25).

Hybridoma cell screening and cloning

An ELISA was used to screen for hybridoma-secreted human anti-digoxin Abs. Microtiter plate wells were coated overnight at 4°C with 100-\( \mu \)l aliquots of 10 \( \mu \)g/ml of digoxin-BSA in 10 mM PBS, pH 7.2. Thereafter, the ELISA was completed as previously described (24). The hybridomas from positive-testing plate wells were then subcloned by limiting dilution plating.

Purification of human mAbs

The stability of cloned hybridoma cell lines was established by several weeks of in vitro culturing and repeated testing of spent culture medium for the presence of secreted mAbs. Samples containing \( \sim 1 \times 10^6 \) cells in log phase growth were injected i.p. into pristane-treated SCID mice for log phase growth were injected i.p. into pristane-treated SCID mice for 3 days before fusion. Splenic lymphocyte suspensions were fused to P3 × 63-Ag8.653 nonsecreting mouse myeloma cells (American Type Culture Collection, Manassas, VA; CRL 1480), and hybridomas were selected as previously described (25).

ELISA analyses of human mAb avidities and binding specificities

Avidity determinations. The avidities of the six mAbs isolated for digoxin were determined using an ELISA in which the digoxin-BSA conjugate (5 \( \mu \)g/ml) was adsorbed to microtiter plates, which were then blocked using a 5% BSA/PBS solution followed by varying concentrations of culture medium or purified mAb. Then, biotinylated goat anti-human IgG Ab was added, followed by a streptavidin alkaline phosphatase conjugate and then substrate to detect bound mAb.

Competitive binding ELISAs. Determination of the relative binding specificities of the six mAbs, their binding to digoxin, additional additives, and a variety of steroids was accomplished through use of a competitive binding ELISA. Anti-human IgG Fc region-specific Ab was adsorbed to the microtiter plates, and then the human mAbs were captured to the plates. Subsequently, a fixed concentration of a digoxin-alkaline phosphatase conjugate was mixed with varying concentrations of digoxin, serving as the standard, or other competitors, and binding of the digoxin-AP conjugate was determined. Briefly, goat anti-human IgG Fc-specific IgG (5 \( \mu \)g/ml) was adsorbed onto polystyrene (Corning, Corning, NY) plates in 0.1 M \( \text{NaHCO}_3 \), pH 9.6, followed by a blocking step with 0.5% casein in 10 mM PBS buffer, pH 7.6, and 0.02% sodium azide and then the addition of 100 \( \mu \)l of mAb (2 \( \mu \)g/ml). The captured mAbs were incubated for 1 h at 37°C with 100 \( \mu \)l of a mixture of a 1:150 dilution of a digoxin-alkaline phosphatase conjugate (O.E.M. Concepts, Toms River, NJ) and varying competitor concentrations in 10 mM PBS, pH 7.2, and 0.5% casein. Following washing of the plate, 50 \( \mu \)l/well of the substrate solution (1 mg/ml p-nitrophenylphosphate, 50 mM \( \text{NaHCO}_3 \), pH 9.8, and 1 mM MgCl\(_2\)), was added to the plates and incubated at room temperature for 10 min. Ab-bound digoxin was quantitated colorimetrically (405 nm) after adding 50 \( \mu \)l/well of 0.1 N \( \text{NaOH} \). Data were analyzed using a nonlinear regression curve-fitting program Inplot (GraphPad, San Diego, CA).

Ligand binding assays

First, radioligand binding assays using varying concentrations of \( ^{[3]} \)Hdigoxin were performed with the three purified anti-digoxin mAbs to obtain the dissociation constants (\( K_d \)). Next, competition binding studies were performed to obtain the IC\(_{50}\) or inhibition constants of digoxin, digoxigenin, and progesterone relative to that of digoxin. These assays used a double antibody precipitation technique, previously described

\(^{[3]} \)Hligand-mAb complexes on glass-fiber filters. For \( K_d \) determinations, 0.06–0.3 \( \mu \)g/0.5 ml assay of purified mAb was incubated at room temperature for 1 h with varying concentrations of \( ^{[3]} \)Hdigoxin (sp. act.
FIGURE 1. Determination of digoxin-specific human Ab responses in transgenic mice. ○ and △ Human IgG anti-digoxin serum responses (dilutions, 1/1000) for mice 14739 and 14747, respectively, following immunization with digoxin-KLH conjugate. ○ and △ Human IgM responses.

= 15 Ci/mmol; DuPont-NEN, Boston, MA) in 0.5 ml of PBS, pH 7.4, containing 0.05% BSA. Then excess affinity-purified goat anti-human IgG Fc specific (ICN Biomedicals, Costa Mesa, CA) and rabbit anti-goat IgG Abs were added to each assay tube, and the binding reactions were allowed to go to completion (33). To determine nonspecific binding, a Na\(^+\),K\(^+\)-ATPase-directed mAb (M7-PB-E9) (34) was substituted for the anti-digoxin mAbs. The \(K_d\) values for digoxin were obtained by analyzing the data using PRIZM (GraphPad, San Diego, CA), a nonlinear regression curve-fitting program. The competition curves were obtained by performing binding studies with a fixed 60-nM concentration of \(^{[3}\text{H}]\text{digoxin}\) and varying concentrations of cold competitor. The \(IC_{50}\) values were obtained by fitting the radioligand binding data using Inplot (GraphPad). These \(IC_{50}\) values were converted to inhibitory dissociation constants (\(K_i\) values), using the Cheng and Prusoff (35) equation: 

\[
K_i = IC_{50}/(1 + [L]/K_d),\]

where [L] is the concentration of \(^{[3}\text{H}]\text{digoxin}\), and \(K_d\) is the dissociation constant for digoxin.

Results
Characterization of the immune responses in the transgenic mice
In this work, HC2/KCo5 mice were immunized with the digoxin-carrier conjugates as described in Materials and Methods. All 15 animals generated a hapten-directed response, with eight being designated high responders by having serum titer values of >1/1250 when tested for human IgG Ab binding to digoxin-BSA by ELISA.

As illustrative of the animals’ responses, serum samples from two mice that were considered good producers of anti-digoxin Abs were characterized with respect to the timing and level of their responses. Mouse IgG as well as the human Ab responses were expected due to trans-switching from the recombined human VDJ sequence to the murine endogenous \(\gamma\) heavy chain and because the endogenous \(\lambda\) locus was not disrupted (24). As shown in Fig. 1, both initial human IgM and IgG anti-digoxin responses occurred within the first week after immunization, with the IgG levels peaking at about 2 wk and severalfold higher than the IgM response. In addition, the mouse IgG anti-digoxin response was monitored and was found to be about one-third to one-half that of the human IgG response. Further, serum samples obtained from these two transgenic animals at the time of cell fusions as well as that for two normal CB6F1/J mice (one immunized with digoxin-KLH and one control) were analyzed and compared. These results indicated that the sera from the transgenic mice 14739 and 14747 contained about 190 and 42 \(\mu\)g/ml, respectively, of total mouse IgG with essentially no mouse IgM. In comparison, the two normal mice had mouse IgG levels of 7.2 and 2.3 \(\mu\)g/ml and IgM levels of 0.89 and 0.39 \(\mu\)g/ml. The serum levels of human IgG for both transgenic mice were ~11 \(\mu\)g/ml, while human IgM levels were 244 and 400 \(\mu\)g/ml, respectively. Thus, while the production of mouse IgG in the transgenic animals appeared to be reduced to only 2–3% that in normal mice, these levels still surpassed those of the human IgG. Endogenous IgM production was well below that of the human IgM. Interestingly, despite the fact that total endogenous mouse IgG levels surpassed those of the human IgG, the transgenic animals’ immunological response to immunization with the digoxin-carrier conjugate was predominately a human IgG response.

Determination of the species of origin and specificity of the hybridoma secreted human anti-digoxin mAbs
The spleens from six of the 15 immunized animals were used for cell fusions. Hybridomas from two of the six animals generated detectable human IgG k anti-digoxin Abs in the culture medium. Altogether, nine anti-digoxin Ab-producing hybridoma cell populations were detected in parental wells, while all eight of the hybridomas chosen for further growth and subcloning were successfully isolated. Six of these hybridoma cell lines were then conditioned to grow as T-flask cultures, and late growth phase samples of the culture medium were tested for human mAb binding to the digoxin-BSA, -KLH, and -OVA conjugates. The human mAbs secreted by these cell lines bound equally well to all three hapten-protein conjugates. Further all six anti-digoxin mAbs were fully human IgG k IgGs, with no detection of mouse or human mouse mixed chain Abs. In addition, no mAb binding was detected to bovine, chicken, or human serum albumin; KLH; chicken or turkey OVA; or casein. Ab binding dilution curves obtained using culture medium samples showed that the mAbs were secreted at concentrations that gave titer values, or half-maximal binding to carrier-linked digoxin at dilutions of ~1/200 to 1/400. Ab concentrations were determined by capturing the mAbs from solution with plate-adsorbed Ab and comparing these levels to those obtained using purified human IgG as a standard. Human mAb concentrations in the medium were ~5–25 \(\mu\)g/ml of human mAb. Dilution curves of mAb binding to digoxin-BSA showed all of them to have an estimated apparent binding affinity or avidity of about 0.5–1 \(nM\) (data not shown). These avidity values were in agreement with the values obtained for several high affinity mouse mAbs (obtained from Michael Margolies, Massachusetts General Hospital, Boston, MA) monitored using the same ELISA procedure (our unpublished observations).

Characterization of binding avidities and specificities of three purified human anti-digoxin mAbs
Three mAbs, designated SC2-4, 7F2-31, and 11E6-7 were obtained from mouse ascites samples and purified, and their binding avidities and specificities for digoxin were determined. As found previously for mAbs in cell culture supernatants, the ELISA procedure using digoxin-BSA as Ag gave essentially identical titer values of about 1 \(nM\) for all three mAbs (data not shown).

Next, a competitive ELISA protocol was used to determine the mAbs’ apparent affinities for digoxin. In this procedure, adsorbed anti-human IgG Fc region-specific Igs captured a fixed amount of mAb to the plates, and the \(IC_{50}\) values for digoxin were obtained by having digoxin compete in solution with digoxin-alkaline phosphatase for mAb binding. The \(IC_{50}\) values of mAbs SC2-4, 7F2-31, and 11E6-7 for digoxin were ~0.28, 0.15, and 0.15 \(\mu\)M, respectively (see representative Fig. 2A). These results suggested that mAbs 7F2-31 and 11E6-7 were similar to each other but distinct from SC2-4.
Once the mAbs’ affinity values for digoxin were obtained, their fine binding specificities for eight additional cardiotonic steroids (see Fig. 3) were investigated by having these compounds compete with the digoxin-alkaline phosphatase conjugate for binding to immobilized mAb. Table I (and representative data, Fig. 2, A and B) presents the compounds relative binding affinities. In brief, the three mAbs were similar in that each had about a 3-fold lower affinity for digitoxin (distinguished by the absence of the C12-OH moiety of digoxin) than digoxin. In addition, the digitoxose sugars clearly contributed to the binding of each mAb, since average 5- and 8-fold losses in the mAb binding affinities to the digoxin and digitoxin aglycones, respectively, were observed. The similarity between mAbs 7F2-31 and 11E6-7 was further extended in that they both were more sensitive to the reduction of the double bond in the lactone ring (dihydrodigitoxin) than 5C2-4, while 5C2-4 binding was more affected by acetylation of the aglycone, i.e., acetyl strophanthidin. Interestingly, all three mAbs showed a similar decreased affinity for the toad-derived bufalin with its six-hydroxyl groups and a single sugar, showed virtually no competition with digoxin.

In addition to determining the binding specificities of the mAbs to various cardiac glycosides we determined the extent to which these mAbs cross-react with steroid hormones (see Fig. 3). As shown in Table II (and representative data, Fig. 2, A and B), the mAbs showed little or no binding to either the steroid precursor cholesterol or most of the 10 steroid hormones tested. Progesterone and testosterone, however, were recognized by all three mAbs with about a 100-fold reduction in affinity relative to digoxin, while 5C2-4 was distinct from the other two mAbs, with a 2-fold higher affinity for corticosterone.

Although the data are not shown, the (nonpurified) mAbs from the three additional cloned hybridomas, designated 3E4, 10B1, and 5D8 were similarly tested for their binding avidities to the plate-adsorbed digoxin-BSA conjugate, and their binding specificities were determined using the competitive ELISA procedure. These Abs each gave similar, ~1 nM titer values for binding to the digoxin-BSA conjugate and IC 50 ELISA values of 0.17, 0.12, and 0.18 µM, respectively, for digoxin. In the competition ELISA they had similar digoxin relative IC 50 ratios for digitoxin and digoxigenin of ~3.3 and 1.7, respectively, with no binding to ouabain. For the steroids, determination of their relative IC 50 values showed testosterone to have an ~150-fold lower affinity than digoxin, with cortisol being weakly inhibitory. In addition, cholesterol, β-estradiol, and androsterone showed essentially no competition with digoxin. Thus, pending a more complete repetition of these ELISAs with purified Ab and determination of their K d values and amino acid sequences, these three mAbs, while not identical, were more similar to 7F2-31 and 11E6-7 than to 5C2-4.

**Discussion**

A sizable number of patients have been successfully rescued from digoxin overdoses by the infusion of one of two commercial preparations of polyclonal anti-digoxin Fabs (Digibind, Glaxo Wellcome, Research Triangle Park, NC; and Digidot, Boehringer Mannheim, Indianapolis, IN) (10, 11, 36, 37). In addition, because of the diversity of Abs present in these preparations they have also been used to treat poisoning by toad venoms (38), a Chinese herbal medicine/aphrodisiac, and the oleander plant (3). However, despite
FIGURE 3. Chemical structures.
their clinical successes these preparations appear underutilized, perhaps due to their cost and concerns about the use of foreign species Abs in patients (39). In addition, many mouse anti-digoxin mAbs have been generated and used for the quantitation of serum digoxin levels in patients. A few mouse mAbs such as 35-20 and 26-10 isolated by Margolies et al. (40, 41) have been also been shown to be effective for the reversal of digoxin intoxication in animal models (42, 43). However, no anti-digoxin mAb has been used in the clinic.

In this work we report the production of eight mouse hybridomas, which secrete human sequence anti-digoxin mAbs, and initial avidity and fine binding specificity determinations for six of these mAbs. ELISA competition binding studies using digoxin and related cardioactive steroids as well as steroid hormones demonstrated the importance of all three structural components of digoxin, the digitoxose sugars, steroid moiety, and lactone ring for mAb recognition. In addition, radioligand binding studies showed three of the mAbs, those designated 5C2-4, 7F2-31, and 11E6-7, to have affinities for digoxin from about 2–20 nM with relatively good recognition of digitoxigenin and digitoxin but greatly reduced affinities for progesterone, the steroid hormone that showed the highest degree of mAb binding.

Analysis of the ELISA competition binding data obtained for the three more fully characterized mAbs enables us to make some initial conclusions about their binding specificities. The results show that the sugar moiety (at least the primary sugar) contributes to binding of the mAbs, because the aglycones, digitoxigenin and digitoxigenin, had ~5- and 8-fold lower binding affinities than digoxin and digitoxin, respectively. As for the contributions of substituents on the steroid moiety of digoxin, the removal of the C-12 hydroxyl group (digitoxin) results in an ~3-fold reduction in the binding affinity for all three mAbs. In contrast, with acetylstrophanthinidin, the presence of an acetyl and hydroxyl group at the C-12 hydroxyl group (digitoxin) results in an ~3-fold reduction in the binding affinity for all three mAbs. In contrast, with acetylstrophanthinidin, the presence of an acetyl and hydroxyl group at the C-12 hydroxyl group (digitoxin) results in an ~3-fold reduction in the binding affinity for all three mAbs. In contrast, with acetylstrophanthinidin, the presence of an acetyl and hydroxyl group at the C-12 hydroxyl group (digitoxin) results in an ~3-fold reduction in the binding affinity for all three mAbs. In contrast, with acetylstrophanthinidin, the presence of an acetyl and hydroxyl group at the C-12 hydroxyl group (digitoxin) results in an ~3-fold reduction in the binding affinity for all three mAbs. In contrast, with acetylstrophanthinidin, the presence of an acetyl and hydroxyl group at the C-12 hydroxyl group (digitoxin) results in an ~3-fold reduction in the binding affinity for all three mAbs. In contrast, with acetylstrophanthinidin, the presence of an acetyl and hydroxyl group at the C-12 hydroxyl group (digitoxin) results in an ~3-fold reduction in the binding affinity for all three mAbs. In contrast, with acetylstrophanthinidin, the presence of an acetyl and hydroxyl group at the C-12 hydroxyl group (digitoxin) results in an ~3-fold reduction in the binding affinity for all three mAbs. In contrast, with acetylstrophanthinidin, the presence of an acetyl and hydroxyl group at the C-12 hydroxyl group (digitoxin) results in an ~3-fold reduction in the binding affinity for all three mAbs. In contrast, with acetylstrophanthinidin, the presence of an acetyl and hydroxyl group at the C-12 hydroxyl group (digitoxin) results in an ~3-fold reduction in the binding affinity for all three mAbs. In contrast, with acetylstrophanthinidin, the presence of an acetyl and hydroxyl group at the C-12 hydroxyl group (digitoxin) results in an ~3-fold reduction in the binding affinity for all three mAbs. In contrast, with acetylstrophanthinidin, the presence of an acetyl and hydroxyl group at the C-12 hydroxyl group (digitoxin) results in an ~3-fold reduction in the binding affinity for all three mAbs. In contrast, with acetylstrophanthinidin, the presence of an acetyl and hydroxyl group at the C-12 hydroxyl group (digitoxin) results in an ~3-fold reduction in the binding affinity for all three mAbs. In contrast, with acetylstrophanthinidin, the presence of an acetyl and hydroxyl group at the C-12 hydroxyl group (digitoxin) results in an ~3-fold reduction in the binding affinity for all three mAbs. In contrast, with acetylstrophanthinidin, the presence of an acetyl and hydroxyl group at the C-12 hydroxyl group (digitoxin) results in an ~3-fold reduction in the binding affinity for all three mAbs. In contrast, with acetylstrophanthinidin, the presence of an acetyl and hydroxyl group at the C-12 hydroxyl group (digitoxin) results in an ~3-fold reduction in the binding affinity for all three mAbs. In contrast, with acetylstrophanthinidin, the presence of an acetyl and hydroxyl group at the C-12 hydroxyl group (digitoxin) results in an ~3-fold reduction in the binding affinity for all three mAbs. In contrast, with acetylstrophanthinidin, the presence of an acetyl and hydroxyl group at the C-12 hydroxyl group (digitoxin) results in an ~3-fold reduction in the binding affinity for all three mAbs. In contrast, with acetylstrophanthinidin, the presence of an acetyl and hydroxyl group at the C-12 hydroxyl group (digitoxin) results in an ~3-fold reduction in the binding affinity for all three mAbs. In contrast, with acetylstrophanthinidin, the presence of an acetyl and hydroxyl group at the C-12 hydroxyl group (digitoxin) results in an ~3-fold reduction in the binding affinity for all three mAbs. In contrast, with acetylstrophanthinidin, the presence of an acetyl and hydroxyl group at the C-12 hydroxyl group (digitoxin) results in an ~3-fold reduction in the binding affinity for all three mAbs. In contrast, with acetylstrophanthinidin, the presence of an acetyl and hydroxyl group at the C-12 hydroxyl group (digitoxin) results in an ~3-fold reduction in the binding affinity for all three mAbs. In contrast, with acetylstrophanthinidin, the presence of an acetyl and hydroxyl group at the C-12 hydroxyl group (digitoxin) results in an ~3-fold reduction in the binding affinity for all three mAbs. In contrast, with acetylstrophanthinidin, the presence of an acetyl and hydroxyl group at the C-12 hydroxyl group (digitoxin) results in an ~3-fold reduction in the binding affinity for all three mAbs. In contrast, with acetylstrophanthinidin, the presence of an acetyl and hydroxyl group at the C-12 hydroxyl group (digitoxin) results in an ~3-fold reduction in the binding affinity for all three mAbs. In contrast, with acetylstrophanthinidin, the presence of an acetyl and hydroxyl group at the C-12 hydroxyl group (digitoxin) results in an ~3-fold reduction in the binding affinity for all three mAbs. In contrast, with acetylstrophanthinidin, the presence of an acetyl and hydroxyl group at the C-12 hydroxyl group (digitoxin) results in an ~3-fold reduction in the binding affinity for all three mAbs. In contrast, with acetylstrophanthinidin, the presence of an acetyl and hydroxyl group at the C-12 hydroxyl group (digitoxin) results in an ~3-fold reduction in the binding affinity for all three mAbs. In contrast, with acetylstrophanthinidin, the presence of an acetyl and hydroxyl group at the C-12 hydroxyl group (digitoxin) results in an ~3-fold reduction in the binding affinity for all three mAbs. In contrast, with acetylstrophanthinidin, the presence of an acetyl and hydroxyl group at the C-12 hydroxyl group (digitoxin) results in an ~3-fold reduction in the binding affinity for all three mAbs. In contrast, with acetylstrophanthinidin, the presence of an acetyl and hydroxyl group at the C-12 hydroxyl group (digitoxin) results in an ~3-fold reduction in the binding affinity for all three mAbs. In contrast, with acetylstrophanthinidin, the presence of an acetyl and hydroxyl group at the C-12 hydroxyl group (digitoxin) results in an ~3-fold reduction in the binding affinity for all three mAbs. In contrast, with acetylstrophanthinidin, the presence of an acetyl and hydroxyl group at the C-12 hydroxyl group (digitoxin) results in an ~3-fold reduction in the binding affinity for all three mAbs. In contrast, with acetylstrophanthinidin, the presence of an acetyl and hydroxyl group at the C-12 hydroxyl group (digitoxin) results in an ~3-fold reduction in the binding affinity for all three mAbs. In contrast, with acetyl...
with either a ketomethyl (progesterone) or a hydroxyl group (testosterone) enables the steroid moiety to inhibit digoxin binding, albeit with a 100-fold lower affinity than digoxin.

In the absence of confirmatory sequence information we do not know the extent to which the six mAbs reported here differ or are closely related, because they originate from one animal. However, 5C2-4 appears distinct from 7F2-31 and 11E6-7 as its IC50 and Kd values for digoxigenin, digitoxin, and progesterone appear to be unique with respect to its high affinity anti-digoxin responses. Further testing of all six mAbs will allow us to determine whether this mouse yielded only three or up to six distinct mAbs, a possible testament to the repertoire diversity of the transgenic animal.

We can, however, make some general comparisons between these mAbs and the mouse anti-digoxin mAbs characterized by others. First, the low nanomolar affinities of these human mAbs for digoxin compare very well with mAbs obtained from the BALB/c mouse strain (46, 47). These values are, however, at least 10-fold poorer than the majority of mAbs obtained by Margolies and colleagues (40, 41) from the A/J strain of mice, which they report to be unique with respect to its high affinity anti-digoxin responses. Next, we can compare the competition binding data for the human mAbs with the results obtained by Margolies et al. (40) with 14 mouse mAbs. First, with respect to the ability of the human mAbs to distinguish between digoxin and digitoxin we found that the 3-fold loss in the affinity of the human mAbs for digitoxin more than matches the average 1.4-fold difference in affinity observed for nine of the mouse mAbs. Then, with respect to the recognition of the sugar moiety by the human mAbs we found that the 5- and 8-fold reductions in their binding to the digoxin and digitoxin aglycones, respectively, are comparable with those of a group of six mouse mAbs, which averaged ~2.6- and 9-fold decreases in their affinities for the aglycones. Further, the decreased affinities of the three human mAbs for acetylstrophanthinid with essentially no binding to ouabain, oleandrin, and most steroids are also consistent with the range of specificities observed for the A/J mouse-derived Abs. Interestingly, a chimpanzee-derived anti-digoxin mAb (48) has also been characterized and found to have a 4-fold affinity difference between digoxin and digitoxin but no recognition of the digitoxose sugars.

Finally, we would like to consider the likelihood of mAb 7F2-31 having the potential to be clinically useful for digoxin detoxification. Although most animal detoxification studies have used mouse mAbs (42, 43) with about a 10-fold higher affinity than mAb 7F2-31, one study has directly compared the in vivo effects of three mouse mAbs with differing digoxin affinities. Cano et al. (46) tested the abilities of these mAbs to raise total plasma digoxin and reduce free digoxin levels by reversing the drug’s normal tissue distribution in rats. Interestingly, they report that at a stoichiometric mAb/drug ratio, mAb 6C9 with a digoxin affinity of 3.2 nM was able to reduce free plasma levels by 90% compared with the 99% achieved by mAb 1C10, which has an affinity of 0.17 nM. Furthermore, by raising the concentration of mAb 6C9 to a 5:1 ratio vs drug, it was found to be equally as effective as 1C10. In contrast, the third mAb, with a Kd value of 40 nM, was essentially ineffective.

Comparison of the affinity of mAb 7F2-31 with clinically used Fab preparations provides encouraging information. Sheep Fabs with affinities ranging from 1–0.01 nM (49, 50) have all been effective when administered at stoichiometric concentrations relative to patient’s plasma drug levels. In addition, Fab preparations that may have had Kd values above 1 nM have proven effective (37). Therefore, mAb 7F2-31 may prove adequate for initial treatment of the massive drug overdose levels of digoxin (or digitoxin), for which anti-digoxin Fab intervention is currently recommended (50). Clearly, both mAbs 7F2-31 and 11E6-7 have affinities that are at least 10- to 30-fold greater than the Kd values of the Na+ pump for digoxin under in vivo ligand conditions (51, 52), and they should compete effectively with the Na+ pump for digoxin.

Acknowledgements

We thank Mr. Lamar Gerber for his technical assistance and construction of Fig. 3.

References


