TOLERANCE TO RAT MONOCLONAL ANTIBODIES

Implications for Serotherapy

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There are now numerous reports (1–5) showing that mAbs can be effective as therapeutic agents in a variety of diseases. A major complication encountered in many of these studies has been the antiglobulin response that such xenogeneic Igs elicit (6–10). This acts both to curtail the prospect of long-term therapy and to risk potentially harmful hypersensitivity reactions. Two strategies have been suggested that might be used to mitigate such unwanted responses. The first involves induction of tolerance to the xenogeneic mAb before commencing therapy. The second requires the use of either human mAbs or chimeric mAbs constructed by combining human constant gene segments with mouse or rat variable gene segments (11–13). Natural tolerance to human determinants would be expected to reduce the immune response to such mAbs when used as therapeutic agents in clinical practice. Both strategies have a common problem. Simply put, it is whether tolerance can ever be attained to the unique variable region (i.e., idiotype) of a given therapeutic mAb, and if not, will tolerance to constant region epitopes be sufficient to prevent the response to the whole molecule?

The concept of tolerance reducing an immune response to Igs is supported by a vast literature involving the induction of tolerance to polyclonal heterologous Igs in mice (14), and there is some evidence that such tolerance may reduce the antiglobulin response to therapeutic polyclonal antilymphocyte globulins (ALG) (15, 16). Therapeutic mAbs differ from polyclonal Ig in two major features, which make it impossible to predict their behavior as tolerogens. First, they bind to specific antigens on cells or in solution. This would have a substantial influence on their uptake and presentation as immunogens. Second, they are monoclonal and therefore highly homogeneous. Each injection of mAb would contain a relatively high immunogenic dose of the idiotypic determinants. That such homogeneity can affect immunogenicity is well demonstrated by classical experiments (17–19) that have shown that relatively pure antiallotype, antiidiotype, or antisubclass immune responses can be elicited in laboratory animals, by immunization of one strain with Igs of restricted heterogeneity (i.e., myeloma proteins or mAbs) from another, or by first rendering an animal tolerant to one Ig type

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and then immunizing with another. Adjuvants have generally been used to elicit such responses. Two implications can be drawn from these experiments. First, tolerance to one part of an Ig may not always prevent an immune response to the rest of that Ig. Second, contrived circumstances of antigen presentation (e.g., adjuvant) may be necessary to elicit such responses. In contrast, recent studies (20) involving tolerance to hen egg lysozyme (HEL) have shown that certain epitopes on a molecule may evoke suppressor mechanisms that render the animal tolerant to the whole molecule. There is no *a priori* reason why such a tolerance mechanism should not operate with respect to mAbs to prevent the antiglobulin response.

In view of such conflicting data, it cannot be predicted whether tolerance, be it natural or acquired, could prevent antiglobulin responses to mAbs used in therapy. In this study, we have addressed a number of questions that we feel are central to resolving this uncertainty. (*a*) Is it possible to induce tolerance to mAbs? (*b*) If it is possible, will the antigen binding potential of an mAb influence its ability to tolerize? (*c*) Will tolerance to the constant region of an mAb influence whether or not a response can be generated to idiotypic determinants?

The results described here lead us to conclude that operational tolerance to xenogeneic mAbs can be achieved in mice by classical deaggregation methods, and also by the use of an anti-L3T4 mAb in a novel tolerance-inducing regimen. This is the case for mAbs that do not have specificity for cell surface antigens (non–cell-binding mAbs), however the situation for mAbs that bind to cell surface antigens (cell-binding mAbs) is different, as full tolerance is difficult to achieve. Indeed, even in the presence of tolerance to constant region determinants, cell-binding mAbs still elicit strong antiidiotypic responses. This suggests that the antiidiotypic response will remain a constraint on extended serotherapy directed towards cellular antigens, even with the use of human and chimeric mAbs. Further strategies must be developed to prevent this complication.

Materials and Methods

Mice. CBA/Ca mice were bred and maintained at the animal facility of the Department of Pathology, University of Cambridge. Male mice 6–8 wk old were used in groups of five in all experiments.

Monoclonal Antibodies. Rat mAbs were produced by a modified protocol of Galfre and Milstein (21) by fusion of the rat myeloma Y3.Ag 1.2.3 (22) and DA rat spleen cells. mAbs were purified from ascitic fluids produced in Pristane-(Aldrich T2 280-2; Gillingham, United Kingdom) primed (DA × LOU)F_1 rats. The Ig fraction was prepared by precipitation with 50% saturated ammonium sulfate, and then ion-exchange chromatography on a DEAE-cellulose column (Whatman DE-52; Maidstone, United Kingdom) in 0.01 M sodium phosphate buffer, pH 8.0. The mAbs were then dialyzed into PBS, the protein concentration was estimated by measuring the absorbance at 280 nm, and the mAbs were stored at −20°C until use. The binding activity of each purified mAb was checked by immunoperoxidase staining on mouse spleen sections. None of the anti–human mAbs crossreacted on mouse cells. The anti–mouse lymphocyte antibodies were previously described by Cobbold et al. (23). All the mAbs used were of the IgG2b subclass and are listed in Table I.

Induction of Classical Tolerance to YTH 3.2.6. Mice were tolerized by i.p. injection of 0.5 mg of deaggregated YTH 3.2.6 (Campath 2) (24) in 1 ml sterile PBS, according to the method of Dresser (25). Deaggregation was achieved by ultracentrifugation at 150,000 *g* for 150 min (L8-70M; Beckman Instruments, Palo Alto, CA) of 5 ml aliquots of mAb

TABLE I
mAbs Used

mAbs*	Specificity
YTS 154.7	Anti-Thy-1
YTS 169.4	Anti-Lyt-2
YTS 121.1	Anti-Lyt-1
YTS 191.1	Anti-L3T4
YBM 15.1.6	Anti–mouse neutrophil
YTH 3.2.6 (Campath 2)	Anti–human CD 7
YTH 12.5.14 (Campath 3)	Anti–human CD 3
YTH 24.5	Anti–human T200 antigen
YTH 65.3.33	Anti–human T200 antigen
YTH 53.1.4	Anti–human RBC

* All mAbs are of the rat IgG2bκ subclass except YTH 12.5.14, which is IgG2bλ. mAbs were previously described (23, 24, 27, 44), except YTH 53.1.4, which is our unpublished data.

at 10 mg/ml. After ultracentrifugation, the top one-third of each aliquot was carefully removed, the protein concentration was determined, and after appropriate dilution in sterile saline, the mAb was injected into mice. This procedure was kept as short as possible to avoid reaggregation of the mAb.

Induction of Tolerance to Rat IgG2b Determinants by Anti-L3T4 Therapy. As previously described (26), mice were rendered tolerant to rat IgG2b determinants by injections of 2 mg (~0.4 mg active mAb) YTS 191.1 (anti-L3T4 antigen) on day 0 (i.v.), and on days 1 and 2 (i.p.). This mAb was purified from ascites by ammonium sulfate precipitation as above, dialyzed against sterile saline, and then stored at −20°C until use. Controls received either an irrelevant IgG2b mAb (YTH 65.3.33 [27]) or saline by the same protocol. These mice were then maintained on antibiotics (oxytetracycline 50 mg/liter) (Terramycin; Pfizer, Sandwich, United Kingdom), and were free of any obvious disease.

Immunization. After classical tolerance induction to YTH 3.2.6, mice were immunized with 0.5 mg doses of the immunizing mAbs on day 9 (i.v.) and day 19 (i.p.), and bled on day 24. After tolerance induction by anti-L3T4 therapy, mice were immunized on days 42 (i.p.) and 52 (i.p.), and bled on day 58. The sera were separated and stored at −20°C until assay. The immunizing mAbs were used in their soluble form except where this gave inconsistent immunization in normal mice. For this reason, YBM 15.1.6, YTH 3.2.6, YTH 53.1.4, and YTH 12.5 were partially aggregated to increase their immunogenicity by heating the purified mAb at 10 mg/ml for 25 min at 63°C followed by placing on ice overnight, in a modification of the protocol of Weigle (14). This partially aggregated preparation was then stored at −20°C until use.

Measurement of Serum Antibodies to Rat IgG. Anti–rat IgG antibody titers were assessed by a solid-phase ELISA. Microtitration trays were coated with the immunizing mAb by incubation of 50 μl/well of a 20 μg/ml solution in PBS for 60 min, and were then washed three times in 0.05% (vol/vol) Tween 20 (Sigma Chemical Co., Poole, United Kingdom) in PBS. Trays were then blocked overnight with a 1% (wt/vol) solution of BSA (Sigma Chemical Co.) in PBS plus 0.02% (wt/vol) sodium azide. The trays were washed before use and between each step in the assay as above. Test serum samples were added to the trays at 50 μl/well, serially diluted in 0.1% (wt/vol) BSA/PBS, and were then incubated for 60 min at 20°C, followed sequentially by biotinylated species-specific sheep anti–mouse Ig (Amersham Corp., Amersham, United Kingdom) and strepavidin–horseradish peroxidase complex (Amersham Corp.) each diluted 1:1,000 and incubated for 35 min at room temperature. Bound enzyme was then detected by the addition of the substrate *o*-phenylenediamine, incubated at 100 μl/well for 5 min, and the reaction stopped by adding 3 M H_2SO_4 (30 μl/well). Absorbance was read at 490 nm, and values for each sample were plotted graphically against the dilution. The titer was then read relative to a standard positive control consisting of a mixture of 1:10 ascites of the following mouse anti–rat Ig

mAbs: NORIG 1.1.6, NORIG 7.16.2 (both anti-rat IgG2b) (28, 44) and MAR 18.5 (anti-rat κ light chain) (29). Normal mouse serum was used as a negative control and consistently gave titers of <1:5.

Detection of Serum Antiidiotype Antibodies. Antibodies to rat mAb idiotypes were detected indirectly by comparing the relative titers of a given anti-serum to either the immunizing mAb or two irrelevant rat IgG2b mAbs, as determined by the ELISA technique above (9, 10).

Measurement of Rat IgG2b in Mouse Serum. Circulating free rat IgG2b in mouse serum was detected by an ELISA. Microtitration trays were coated with NORIG 7.16.2 (mouse anti-rat IgG2b), blocked overnight, and serial dilutions of serum samples were applied and incubated as above. Bound IgG2b was then detected by biotinylated NORIG 1.1.6 (mouse anti-rat IgG2b) at 2 μg/ml. This was followed by strepavidin-horseradish peroxidase complex (Amersham Corp.) and the substrate *o*-phenylenediamine, as above. Positive controls were constructed by adding known amounts of YTH 3.2.6 to normal CBA/Ca mouse serum, and a standard curve was generated from which test concentrations were determined.

Preparation of H_2L_2 and H_2K_2 variants of YTS 169.4. The myeloma line Y3 Ag 1.2.3, used to produce all the above rat mAbs, secretes an Ig light chain of the LOU rat κ 1a allotype (22). After fusion with a DA rat spleen cell to produce a hybridoma cell, the Ig chains from both parental cells are codominantly expressed (30), and the individual chains can associate randomly to form a complex mixture of molecules. The association of the spleen cell-derived H chain with the spleen cell-derived L chain (κ 1b or λ) usually has antigen specificity, while its association with the myeloma-derived light chain (K)[1] does not (31). The hybridomas thus produce a mixture of bivalent nonspecific (H_2K_2), monovalent (H_2LK), and bivalent specific (H_2L_2) mAbs. The H_2K_2 and H_2L_2 forms of YTS 169.4 were purified from serum-free culture supernatants dialyzed into 20 mM Tris HCl (Sigma Chemical Co.), pH 7.5, and then subjected to HPLC (Ultra Pac column TSK DEAE 5PW; LKB-Produckter, Bromma, Sweden). The first three peaks eluted off this column by a 0–400 mmol NaCl gradient were shown to contain the H_2L_2, H_2LK, and H_2K_2 forms, respectively, by an inhibition of passive hemagglutination assay, which detected the relative amounts of the heavy chain isotype and light chain allotypes in each peak (32).

Results

Induction of Tolerance to Rat mAb by Classical Methods. Tolerance to polyclonal IgG can classically be induced in mice by i.p. injection of the IgG in the deaggregated form (25). Such tolerance has been shown (14) to be fully developed within 5 d of injection, to last up to 120 d and to be specific for the injected IgG. In our initial experiments, we showed that, in an analogous fashion, mice can be rendered tolerant to a rat IgG2b mAb. We found that mice pretreated with an i.p. injection of 0.5 mg of deaggregated YTH 3.2.6 (anti-human CD 7), and then immunized 9 d later with the immunogenic heat-aggregated form of the same mAb, produced 95–98% less antiglobulin response after 15 d than controls that had not been so pretreated. This result was highly reproducible, and is exemplified in a control group in Fig. 1 (immunizing mAb YTH 3.2.6). Later bleeds confirmed that there had been no delayed immune response. Unresponsiveness persisted for at least 31 d after injection of deaggregated material. It was not possible to induce tolerance by this method to a number of mAbs with binding specificity for mouse cell surface antigens (cell-binding mAb), as even after deaggregation, these elicited strong antiglobulin responses (data not shown).

One criticism of using serological assays such as the ELISA to measure

[1] *Abbreviations used in this paper:* HGG, human γ globulin; K, myeloma-derived light chain.

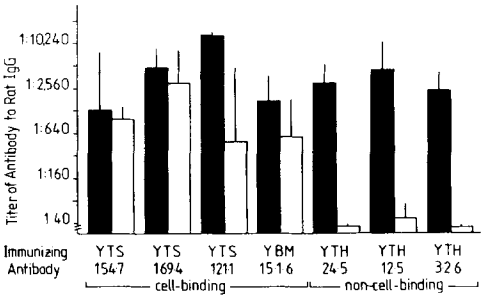

FIGURE 1. The antiglobulin response of mice tolerant to YTH 3.2.6 after immunization with various different IgG2b mAbs. Mice were rendered YTH 3.2.6 unresponsive (open bars) by i.p. injection of 0.5 mg deaggregated YTH 3.2.6 on day 0. Controls (filled bars) received saline. Randomly selected mice were then immunized on days 9 (i.v.) and 19 (i.p.) with 0.5 mg of one of the immunizing mAbs shown, then bled on day 24. Antiglobulin responses were measured by ELISA, and the geometric means and SDs of the antibody titers from five mice per group are shown.

tolerance has been the potential for artifactual inhibition of the titer by free serum tolerogen (14). We excluded this possibility by directly measuring tolerogen in serum with a sandwich ELISA (see Materials and Methods), which was sensitive enough to detect 250 ng/ml rat IgG2b mAb in mouse serum, and yet was unable to detect any YTH 3.2.6 in any of the sera at the time of assay. Furthermore, in an inhibition assay where immune sera were preincubated for 2 h after artificial addition of free YTH 3.2.6 mAb and the antiglobulin titer then assayed, it was found that ~50 μg/ml free mAb was needed to cause 50% inhibition of the measured titer (data not shown). From this we concluded that the titers as measured by the ELISA technique are a true reflection of tolerance.

Antiglobulin Response of Mice Tolerant to One IgG2b mAb on Challenge with Other IgG2b mAbs. Mice rendered tolerant to YTH 3.2.6 must necessarily be unresponsive to the rat IgG2b determinants on the constant region of this mAb. As shown in Fig. 1, we determined how this tolerance would affect the ability of the immune system to mount an immune response to various other IgG2b mAbs. Mice were rendered tolerant to YTH 3.2.6 by pretreatment with deaggregated mAb on day 0, and together with unpretreated controls, were immunized 9 d later with one of either four cell-binding mAbs or three non–cell-binding mAbs. Fig. 1 shows that control mice all made strong antiglobulin responses, confirming the immunogenicity of the IgG2b subclass in mice. Mice tolerant of YTH 3.2.6 responded in one of two ways. They failed to respond to any of the non–cell-binding mAbs but responded vigorously to the cell-binding ones. This result implies that tolerance to shared constant region determinants was sufficient to prevent the immune response to the whole molecule in the case of non–cell-binding mAbs, but was insufficient in the case of cell-binding mAbs.

Characterization of Immune Response to Cell-binding mAbs. The strong immune responses to cell-binding mAbs in tolerant mice in Fig. 1 raised the question of whether these mAbs actually overcame the unresponsiveness to constant region determinants, or whether the responses measured were restricted to the unique variable region (i.e., the idiotype) of the mAbs. To detect any antiidiotypic component, the sera assayed in Fig. 1 were further analyzed in an ELISA for

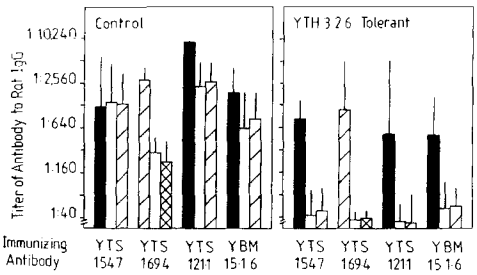

FIGURE 2. Characterization of the antiglobulin responses to cell-binding mAbs in control and YTH 3.2.6 tolerant mice. The antiglobulin responses to cell-binding mAbs in control and YTH 3.2.6 tolerant mice from Fig. 1 were further analyzed by assaying the reactivity of the sera to the immunizing mAb (filled bars) and also to two of the following different IgG2b mAbs: YTS 169.4 H_2L_2 (striped bars); YTH 3.2.6 (open bars); or YTS 169.4 H_2K_2 (cross-hatched bars). Figure shows the geometric means and SDs of the antiglobulin titers from five mice per group.

their reactivity to either the immunizing mAb or to irrelevant IgG2b mAbs. In this way the antiidiotypic component would manifest as a preferentially high titer to the immunizing mAb as compared to the irrelevant IgG2b mAbs. Fig. 2 shows that the response in normal mice to YTS 169.4 and YTS 121.1 both have a significant component directed against their idiotype, whereas this is not obvious with YTS 154.7 and YBM 15.1.6. Tolerant mice, in contrast, make a response to all four cell-binding mAbs that is predominantly antiidiotypic. A closer analysis of the response to YTS 169.4 showed it to be directed mainly to the H_2L_2 and not the H_2K_2 form of this mAb. All the mAbs used to immunize were mixtures of the bivalent specific (H_2L_2), monovalent specific (H_2LK), and bivalent nonspecific (H_2K_2) forms (see Materials and Methods). The antiidiotypic response could therefore apparently discern the idiotype actually involved in cell binding (i.e., the HL idiotype). These results imply that the ability of an mAb to bind to cells renders the idiotypic determinants associated with cell binding major antigenic stimuli, even in the presence of constant region tolerance.

Therapy with mAb YTS 191.1 Renders Mice Tolerant to Rat IgG2b Determinants. The rat mAbs YTS 191.1 (23) and GK 1.5 (33), directed against the L3T4 antigen found on T helper cells (34), are highly immunosuppressive when injected in vivo, and are unique as examples of IgG2b mAbs, as they do not elicit antiglobulin responses to themselves (1, 3, 23, 35, 36). We have recently shown (26) that mice treated with YTS 191.1 regain full immunocompetence within 42 d of therapy, but remain specifically tolerant to rat IgG2b determinants. This tolerance was demonstrated by the failure of treated mice to make an immune response to two non–cell-binding mAbs, at a time point when they were capable of a normal response both to heat-aggregated human γ globulin (HGG) and chicken γ globulin (CGG). Furthermore mice injected with aggregated HGG at the time of anti-L3T4 therapy not only failed to respond to this antigen, but were also found to be specifically unresponsive (i.e., tolerant) on later rechallenge with the same antigen. These findings raise a number of questions with respect to the present study. First, how would tolerance to IgG2b determinants induced by anti-L3T4 therapy affect the immunogenicity of cell-binding mAbs? Second, would anti-L3T4 therapy given concomitantly with cell-binding mAbs prevent

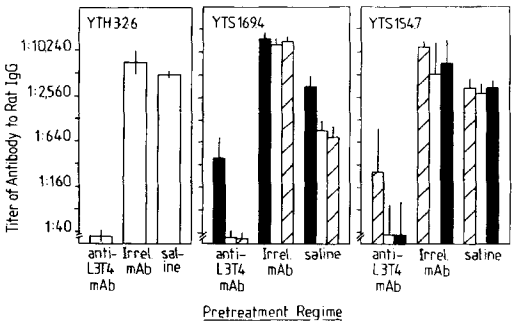

FIGURE 3. Antiglobulin responses in mice rendered rat IgG2b–tolerant by treatment with anti-L3T4 mAb. Mice were rendered IgG2b-tolerant by injections of 2 mg of YTS 191.1 (anti-L3T4 mAb) on days 1 (i.v.) or 2 and 3 (i.p.), or received an irrelevant IgG2b mAb (*Irrel. mAb*) (YTH 65.3.33) or saline by the same protocol. Randomly selected mice were then immunized i.p. on days 42 and 52 with either YTH 3.2.6 (non–cell-binding), YTS 154.7 (anti-Thy-1) or YTS 169.4 (anti-Lyt-2). Mice were bled on day 58, and the antiglobulin responses of each serum were determined on plates coated with either YTH 3.2.6 (open bars), YTS 154.7 (striped bars), or YTS 169.4 (filled bars) by ELISA. Figure shows the geometric means and SDs of the antiglobulin titers of five mice per group.

the antiglobulin response to these, and if so, would the mice be tolerant on later rechallenge with the same cell-binding mAbs?

Cell-binding IgG2b mAbs Elicit Antiidiotypic Responses in Mice Rendered IgG2b Tolerant by Anti-L3T4 Therapy. In Fig. 3, mice were rendered IgG2b tolerant, as previously described (26), by pretreatment with injections of 2 mg (~0.4 mg active mAb) of YTS 191.1 on day 0 (i.v.), and days 1 and 2 (i.p.). Controls received an irrelevant mAb or saline. They were then immunized on days 42 and 52 (i.p.) with either YTS 169.4 (anti-Lyt-2) or YTS 154.7 (anti-Thy-1), or as a control with YTH 3.2.6 (non–cell-binding), and bled on day 58. The figure shows that anti-L3T4–pretreated mice failed to mount an antiglobulin response to YTH 3.2.6, which confirms that this pretreatment renders mice IgG2b unresponsive. The two cell-binding mAbs, on the other hand, both elicited predominantly antiidiotypic responses in pretreated mice, confirming our findings above that cell binding renders the idiotype of an mAb a major antigenic determinant even in the presence of constant region tolerance.

Anti-L3T4 Therapy Does Not Induce Tolerance to Idiotype of Cell-binding mAbs. We next asked whether the same anti-L3T4 protocol that we have previously shown is able both to ablate the normal immune response and to render mice tolerant to heat-aggregated HGG would have the same effect on rat IgG2b mAbs. In Fig. 4, mice were pretreated with YTS 191.1, an irrelevant mAb, or saline on days 0, 1, and 2 as before, and were then injected (i.p.) on days 2 and 3 with 0.5 mg of either YTS 169.4 (anti-Lyt-2); YTS 154.7 (anti-Thy-1); YTH 3.2.6 or YTH 53.1.4 (both non–cell-binding). These mice were then bled on days 12 and 42, and their antiglobulin titers were measured by an ELISA. Fig. 4 shows that by day 12, anti-L3T4–treated mice failed to respond to any of the injected mAbs, while controls produced strong responses. This confirms a recent abstract report (37) that antiglobulin responses can be ablated by concommitant anti-L3T4 therapy. However, a late bleed on day 42 showed that, while the antiglobulin responses in control mice had begun to fall, mice

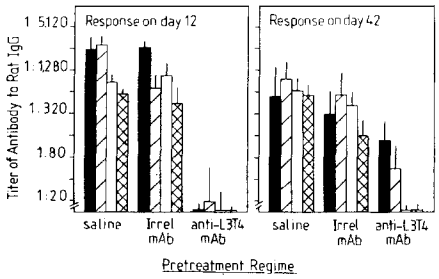

FIGURE 4. Anti-L3T4 mAb therapy abrogates the early antiglobulin response to other rat IgG2b mAbs. Mice were pretreated with YTS 191.1 (anti-L3T4 mAb), or an irrelevant mAb (*Irrel. mAb*), or saline on days 0, 1, and 2, as before, and were then randomly selected and injected i.p. on days 2 and 3 with 0.5 mg of either YTS 169.4 (filled bars), YTS 154.7 (striped bars), YTH 3.2.6 (open bars), or YTH 53.1.4 (crosshatched bars). All mice were bled on days 12 and 42 and their antiglobulin titers were measured by ELISA on plates coated with the respective immunizing Ig. Figure shows the geometric means and SDs of the titers from five mice per group.

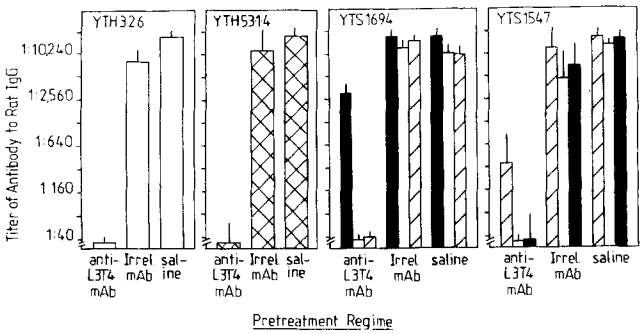

FIGURE 5. Antiglobulin responses on late rechallenge of mice pretreated with anti-L3T4 therapy plus IgG2b mAbs. Mice from the experiment shown in Fig. 4 were rechallenged on days 42 and 52 with i.p. injections of 0.5 mg of the same IgG2b mAbs as were injected on days 2 and 3, and were bled on day 58. Antiglobulin responses were assayed by an ELISA, and the sera reactivities were measured on plates coated with either YTH 3.2.6 (open bars), YTH 53.1.4 (crosshatched bars), YTS 169.4 (filled bars), or YTS 154.7 (striped bars). Figure shows the geometric means and SDs of the antiglobulin titers from five mice per group.

that had received anti-L3T4 pretreatment had made a late response to the two cell-binding mAbs, but not to the non–cell-binding ones. These mice were then rechallenged on days 42 and 52 wih the same mAbs as had been injected on days 2 and 3, and were again bled on day 58. Fig. 5 shows that the anti-L3T4–pretreated mice injected with the two non–cell-binding mAbs (YTH 3.2.6 and YTH 53.1.4) failed to respond to late rechallenge, while those injected with the cell-binding mAbs (YTS 169.4 or YTS 154.7) produced strong antiidiotype responses. We therefore conclude that with this anti-L3T4 protocol it is possible to induce tolerance to rat IgG2b constant region determinants, but not to the idiotypic determinants of cell-binding mAbs. We have recently been informed of the basically similar results of Gutstein, Wofsy, and Seaman (personal communication), who note that anti-L3T4–pretreated mice given a rat IgG2b mAb anti–mouse T200 also produce a predominantly antiidiotype response.

Discussion

In this study we have investigated whether tolerance may prevent the antiglobulin response to mAb when used as therapeutic agents. We report that, in mice, it is possible to induce tolerance to rat IgG2b mAbs both by means of classical deaggregation methods and by the newly described (26) method of anti-L3T4 therapy, but that the ability of an mAb to bind to a cell surface antigen limits induction by either method. Furthermore, mice that are tolerant to rat IgG2b constant region determinants fail to respond when challenged with a short immunizing course of other non–cell-binding mAbs of the same subclass, regardless of whether these mAbs are heat aggregated or not, but produce rapid, predominantly antiidiotypic responses on immunization with cell-binding ones.

Two other interesting observations arise from these results. First, the doses of cell-binding mAbs used to immunize mice were sufficient to cause >85% peripheral depletion of their target cell subsets (23), and yet none of the four mAbs tested were able to break completely the unresponsive state to constant region determinants, even though they elicited antiidiotypic responses. This may imply that none of Lyt-1$^+$, Lyt-2$^+$, Thy-1$^+$ T cells nor neutrophils are exclusively responsible for maintaining this unresponsiveness by some suppressor mechanism. Second, in the case of the antiidiotype response to YTS 169.4, the reactivity was to the HL (specific) not the HK (nonspecific) idiotype. As both the H_2L_2 and monovalent H_2LK forms would be expected to bind to cells and to cause depletion (38), one might have expected a response to both sets of idiotypic determinants. It is possible that the greater avidity of the H_2L_2 competed out the binding of the H_2LK form and so prevented it from becoming cell-bound and therefore immunogenic. It will be interesting to determine whether the purified monovalent (H_2LK) form elicits a response to both idiotypes in tolerant mice.

The strong antiidiotypic responses to cell-binding mAbs in tolerant mice imply that binding of a mAb to a cell surface antigen leads to effective uptake, processing, and presentation of its idiotypic determinants. Cell-binding could theoretically enhance this process in a number of ways, for example, it would cause aggregation of the mAb on the cell surface; it would increase its immunogenic half-life; it could, especially in the case of rat IgG2b mAbs, lead to rapid cellular destruction by either complement activation or antibody-dependent cellular cytotoxicity (23, 39), with subsequent uptake and processing of the mAb bound to cellular debris; and finally, binding of an mAb to a cell surface receptor may directly modulate cellular functions. All these factors may, in certain circumstances, serve to enhance immunogenicity. Whatever the exact mechanism, we believe that the antiidiotypic response to cell-bound antibodies may be of great significance physiologically. We have found that an injection of as little as 1 μg of YTS 169.4 is able to provoke an antiidiotype response in tolerant mice (data not shown). We propose that, at this concentration, the immune system, which is tolerant to constant region epitopes, would see a cell-bound mAb in the same way as it would see an autoantibody. The antiidiotypic response, which could be both humoral and/or cellular in nature, may then be a predictable reaction to any autoantibody directed to cell surfaces, in this way serving as a natural regulatory mechanism to limit autoimmunity.

Another problem raised is how T and B cells interact to give a relatively pure immune response to the idiotypic determinants of an mAb in the presence of constant region tolerance. If we were to consider idiotypes in this context to be analogous to haptens on a carrier molecule, then we would expect carrier tolerance to prevent a response to the hapten (40). However, idiotypes are composed of many different idiotopes duplicated on each Fab arm. Therefore we could imagine idiotope 1 (plus MHC) providing help for B cell response to idiotope 2. Alternatively, the classical dogma applicable to haptens may not strictly apply here, and like-like help cannot be excluded.

The findings of this study with regard to the immunogenic behavior of therapeutic mAbs in the presence of constant region tolerance are important, as they may predict the nature of the antiglobulin responses that human and chimeric mAbs might elicit in clinical practice. That tolerance to constant region determinants is able to prevent an antiglobulin response to a short immunizing course of non–cell-binding mAbs, either soluble or aggregated, would predict that human and chimeric mAbs to soluble antigens (e.g., to digoxin [41] or snake venom factors) would fail to elicit an antiglobulin response even though they would be functionally aggregated in vivo by forming immune complexes. From the point of view of clinical therapy, the antiidiotype response that cell-binding mAbs elicit in the presence of constant region tolerance is much more ominous, as such a response to human and chimeric mAbs would negate their proposed advantages. Our work therefore calls into doubt the suggestion that human or chimeric mAbs will be the panacea for all antiglobulin responses, and raises the question whether the antiidiotype response to cell-binding mAbs can be prevented. Cytotoxic drugs and steroids are the most readily available means of nonspecifically suppressing unwanted immune responses, and have been shown to be effective in limited clinical trials with therapeutic mAbs (42), but this form of therapy exposes the patient to the dangers of generalized immunosuppression and also mitigates the highly selective therapeutic potential of mAbs. Another strategy stems from the previous observation that anti–mouse globulin responses in clinical trials often have a significant antiidiotypic component (10). These authors suggest the sequential use of a number of mAbs against the same antigen but with different idiotypes, to avoid the complications of the antiglobulin response without preventing its occurrence. Our data support this suggestion, the only proviso being that the mAbs be of an isotype suitable to ensure therapeutic effectiveness in vivo (23, 39, 43, 44). Another possibility is the use of anti-L3T4 (CD 4) mAb therapy. In this paper we have shown that such therapy is able to induce tolerance to the constant region determinants on rat IgG2b mAbs and prevent the early antiidiotypic response to cell-binding mAbs. It may be that with more aggressive anti-L3T4 therapeutic protocols, the complete abolition of the antiidiotype response, or even tolerance, to cell-binding mAbs would be possible. We are presently investigating this possibility, mindful of the obvious disadvantage that such a protocol would expose the patient to the dangers of a potently immunosuppressive regimen.

An alternative way of looking at the antiidiotype problem is to determine why rat IgG2b mAbs, which are highly effective at depleting their target T cell subsets in vivo, do not also bind specifically to and deplete B cells that carry Ig surface

receptors directed against their idiotype (i.e., antiidiotypic B cells), and so by clonal deletion prevent the antiidiotype response. It may be that such B cells are in privileged sites within the immune system, and avoid destruction, or it may be that many are destroyed but a small number escape to give rise to the antiidiotypic response. It is a likely possibility that an mAb binding to antiidiotypic B cells would cause the Ig receptors of these cells to be rapidly modulated and internalized (46). This would protect the B cell from destruction and may even constitute a triggering signal. If this were the case, then it should be possible to find ways to clonally delete antiidiotypic B cells, say by preventing such modulation using monovalent mAbs (38), or by using toxin-linked mAbs (45) that could kill the antiidiotypic B cells after internalization.

In conclusion, the antiglobulin response is a major complication of mAb therapy that cannot as yet be predictably avoided in clinical practice by the use of human or chimeric mAbs.

Summary

The antiglobulin response is a major complication of mAb therapy. It has been suggested that, in clinical practice, this might be avoided by using human or chimeric mAbs, or by prior induction of tolerance to the therapeutic mAb. In this study, we show that it is possible to induce tolerance in mice to the constant regions of rat IgG2b mAbs by both classical deaggregation methods and by anti-L3T4 mAb therapy. Mice tolerant to IgG2b constant region determinants failed to make an antiglobulin response when immunized with a number of mAbs of the same isotype that had no binding specificity for mouse cells, but produced vigorous antiidiotypic responses to cell-binding mAbs. Binding of antibodies to hemopoietic cells rends their idiotypic determinants major immunogens even in the presence of tolerance to constant region epitopes. These findings suggest that the use of human or chimeric mAbs will not be sufficient to eliminate the antiglobulin response, and that additional methods need to be investigated.

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