Modulation by Adjuvants and Carriers of the Immunogenicity in Xenogeneic Hosts of Mouse Anti-idiotype Monoclonal Antibody MK2-23, an Internal Image of Human High Molecular Weight-Melanoma Associated Antigen

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ABSTRACT

The mouse anti-idiotype monoclonal antibody (mAb) MK2-23 recognizes an idiotope in the antigen-combining site of the immunizing anti-human high-molecular-weight melanoma-associated antigen (HMW-MAA) mAb 763.74. Administration with an adjuvant of mAb MK2-23 conjugated to a carrier has been shown to induce anti-HMW-MAA antibodies both in syngeneic hosts and in patients with malignant melanoma. Adjuvant and carrier are required for the induction of anti-HMW-MAA antibodies in BALB/c mice immunized with mAb MK2-23. Whether both adjuvant and carrier are required also in patients with malignant melanoma is not known and cannot be deduced from results obtained in a syngeneic animal model system. Therefore, the present study has evaluated for the first time the effect of a carrier and an adjuvant on the immunogenicity of mAb MK2-23 in a xenogeneic host. Rabbids were selected for this purpose, since they have a constitutive expression of HMW-MAA in their normal tissues with a distribution similar to that in humans. The combined use of an adjuvant and a carrier enhances the immunogenicity of mAb MK2-23 in rabbits markedly more than each of them individually. Specifically, all the rabbits immunized with mAb MK2-23 conjugated to keyhole limpet hemocyanin (KLH) and mixed with Freund's adjuvant (FA) produced antibodies which were shown with serological and immunochromatographic assays to be specific for HMW-MAA and to be both IgG and IgM. In contrast anti-HMW-MAA antibodies were detected in only one of the 3 rabbits immunized with mAb MK2-23 mixed with FA and were not detected in the rabbits immunized with mAb MK2-23 conjugated to KLH or with mAb MK2-23 without KLH and FA. These results indicate that active specific immunotherapeutic effects can be obtained in patients with malignant melanoma. Therefore, mAb MK2-23 conjugated to KLH induced anti-HMW-MAA antibodies in about 60% of the immunized patients. This immunity was associated with prolongation of patients' survival (20), suggesting that active specific immunotherapy with anti-id mAb represents a useful approach for the treatment of malignant melanoma. These findings have stimulated interest in investigations to optimize the immunogenicity of mAb MK2-23. The adjuvant and the carrier were used in patients with malignant melanoma, since a recent study (21) showed that the combined use of a carrier and an adjuvant markedly enhances the immunogenicity of mAb MK2-23 in BALB/c mice. Whether the adjuvant and the carrier are required for optimal immunogenicity of mAb MK2-23 in patients with malignant melanoma cannot be assumed on the basis of the results in BALB/c mice since the animal model system differs from the clinical situation in at least two aspects. First, mAb MK2-23 is syngeneic to BALB/c mice but xenogeneic to patients with malignant melanoma. Second, HMW-MAA is expressed both in normal tissues and in melanoma lesions in patients with malignant melanoma (for a review, see Ref. 22) but is not detectable in BALB/c mice.

To the best of our knowledge, in no antigenic system has the effect of a carrier and/or an adjuvant on the immunogenicity of an anti-id mAb in a xenogeneic host which expresses the corresponding antigen been investigated. Since this information is very useful to design the immunization protocol with mAb MK2-23 in patients with malignant melanoma, in the present study we have characterized the effect of conjugation to a carrier and administration with an adjuvant on the immunogenicity of mAb MK2-23 in rabbits. The latter were selected, since they are xenogeneic to mAb MK2-23 and express HMW-MAA in normal tissues with a distribution similar to that in humans.

INTRODUCTION

Jerne's prediction (1) that anti-id antibodies can induce immunity to nominal antigens has been proved in a number of antigenic systems (for a review, see Ref. 2), including several human tumor-associated antigen systems (3–16). Anti-tumor-associated antigen immunity elicited by anti-id antibodies has been shown to have a beneficial effect on the course of malignant diseases in animal model systems (17–19). Recently, it has been demonstrated that anti-id mAbs as immunogens to implement active specific immunotherapy with mAb MK2-23 in patients with malignant melanoma is likely to benefit from the use of a carrier and an adjuvant, providing additional evidence that mAb MK2-23 bears the internal image of HMW-MAA, and suggesting that the immune response elicited by mAb MK2-23 is T-cell dependent.

MATERIALS AND METHODS

Animals. Twelve-month-old male New Zealand White rabbits were purchased from Hazleton Research Products (Denver, PA).

Cell Lines. Cultured M14/13 human melanoma cells and their autologous L14 B-lymphoid cells were grown in RPMI 1640 supplemented with 10% serum plus (JRH Biosciences, Lenexa, KS) and 2 mM L-glutamine.

mAb and Conventional Antisera. mAbs 149.53, 225.28, KLH and TP61.5 to distinct and spatially distant determinants of HMW-MAA were developed and characterized as described elsewhere (24, 25). The anti-mAb MF9-10, MF9-36, MKI-94, MKI-104, and MKI-140 elicited with anti-HMW-MAA mAb 149.53; the anti-id mAbs MF11-30, MF11-97, TK1-92, TK1-93, and TK1-31 elicited with anti-HMW-MAA mAb 225.28; the anti-id mAbs MK2-23 and MK2-120 elicited with anti-HMW-MAA mAb 763.74; the anti-id mAbs MK1-94, MKI-104, and MKI-140 elicitcd with anti-HMW-MAA mAb 149.53; the anti-id mAbs MF11-30, MF11-97, TK1-92, TK1-93, and TK1-31 elicited with anti-HMW-MAA mAb 225.28; the anti-id mAbs MK2-23 and MK2-120 elicited with anti-HMW-MAA mAb 763.74; the anti-id mAbs TK6-18, TK6-74, TK6-123, and TK6-123 elicited with anti-HMW-MAA mAb TP61.5; and the anti-id mAbs MK2-23 and MK2-120 elicited with anti-HMW-MAA mAb 225.28 were developed and characterized as described elsewhere (9, 14).

mAbs were purified from ascitic fluid by sequential precipitation with caprylic acid and ammonium sulfate (26). The purity of mAb preparations was monitored by SDS-PAGE (27). mAbs were chemically cross-linked to KLH with glutaraldehyde (28).

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2 To whom requests for reprints should be addressed.

3 The abbreviations used are: anti-id, anti-idiotype; FA, Freund's adjuvant; HMW-MAA, high molecular weight-melanoma associated antigen; KLH, keyhole limpet hemocyanin; mAb, monoclonal antibody; PBS, phosphate-buffered saline; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

4 S. Ferrone, unpublished results.
Anti-idiotype sera were obtained from rabbits immunized with anti-id mAbs MF9-10, MF11-30, and TK7-371, using an immunization schedule similar to the one previously described (14). Affinity-purified goat antibodies to rabbit IgG, IgM, and Ig were purchased from Jackson Immunoresearch Laboratories (Avondale, PA) and Fisher Scientific (Pittsburgh, PA). Antibodies were radiolabeled with $^{125}$I using the lodogen method (29).

Preparation of Anti-anti-id Antisera. Four groups of rabbits (3/group) were immunized s.c. with mouse anti-id mAb (500 µg/injection) on days 0, 14, 28, and 42. In group I mAb MK2-23 was neither conjugated to KLH nor mixed with FA; in group II mAb MK2-23 coupled to KLH was used for all the injections; in group III mAb MK2-23 was mixed with CFA for priming on day 0, with IFP for boosting on day 14 and with PBS on days 28 and 42; in group IV mAb MK2-23 coupled to KLH was mixed with CFA for priming on day 0, with IFP for boosting on day 14 and with PBS for boosting on days 28 and 42. Sera were harvested weekly.

Serological Assays. The binding of rabbit anti-anti-id antisera to cells with $^{125}$I-labeled anti-rabbit IgG, IgM, or Ig xenon antibodies, the inhibition by anti-id mAbs of the binding of anti-anti-id antisera to target cells, and the inhibition by anti-anti-id antisera of the binding of $^{125}$I-labeled mAb to target cells were performed in 96-well round-bottomed flexible microtiter plates (Becton Dickinson Labware, Oxnard, CA) as described (9, 14). The binding of rabbit anti-anti-id antisera to anti-id mAb with $^{125}$I-labeled anti-rabbit IgG, IgM, or Ig xenon antibodies and the inhibition by anti-anti-id antisera of the binding of $^{125}$I-labeled anti-id mAbs to mAbs were performed in 96-well round-bottomed polystyrene microtiter plates (Dynatech Laboratories, Inc., Alexandria, VA) as described (9, 14). Absorption of rabbit antisera with cells was performed by incubating rabbit serum with cells (1 ml of serum/4 x $10^7$ cells) for 4 h at 4°C on a rotator. Serum was harvested by centrifugation.

Immunological Methods. Mouse IgG mAbs were conjugated to Affigel 10 (Bio-Rad Laboratories, Richmond, CA) at the concentration of 15 or 20 mg/ml gel following the manufacturer’s instructions. Absorption of anti-mouse IgG or anti-anti-id antibodies from rabbit sera was performed by passing the sera through mouse IgG mAb or mouse anti-id mAb column. Absorption of anti-mouse IgG or anti-anti-id antibodies from rabbit serum was monitored by testing its reactivity with mouse IgG mAb or anti-id mAb in a binding assay with $^{125}$I-anti-rabbit Ig xenon antibodies.

Labeling of cells with $^{125}$I, solubilization with Nonidet P40, indirect immunoprecipitation, SDS-PAGE, and autoradiography were performed as described (9).

RESULTS

To investigate the effect of conjugation to a carrier and/or of administration with an adjuvant on the immunogenicity of mAb MK2-23 in a xenogeneic host, the humoral immune response of the 3 rabbits immunized with mAb MK2-23 conjugated to KLH and administered with FA (group IV) was compared to that of 3 rabbits immunized with mAb MK2-23 without carrier and adjuvant (group I), of 3 rabbits immunized with mAb MK2-23 conjugated to KLH (group II), and of 3 rabbits immunized with mAb MK2-23 mixed with FA (group III).

Antibodies reacting with melanoma cells were detected in sera harvested 1 week following the second booster from one rabbit of group III and from the 3 rabbits of group IV. The titer of antibodies was increased in the sera obtained 2 weeks after the second booster and reached the peak in the sera obtained 2 weeks after the third booster from the 3 rabbits of group IV. The titer of antibodies reacting with melanoma cells was increased in the sera obtained 2 weeks after the third booster from one rabbit of group III and reached the maximal value of 1:400. The mean maximal titer of antibodies was 1:460, with a range of 1:200 and 1:800 in the sera from the 3 rabbits of group IV. Antibodies reacting with melanoma cells were not detected in the sera from the 3 rabbits of group I and from the 3 rabbits of group II. The kinetics of the development of antibodies in the 4 groups of rabbits is shown in Fig. 1.

Anti-anti-id antibodies, i.e., antibodies that inhibit the binding of $^{125}$I-mAb MK2-23 to mAb 763.74 by at least 90%, became detectable in the sera harvested 1 week after the first booster from the 3 rabbits of group III and from the 3 rabbits of group IV. The titer of anti-anti-id antibodies gradually increased in both groups of rabbits in the sera obtained during the following weeks and reached the peak in the sera obtained 2 weeks following the third booster in both groups of rabbits. They were 1:850, with a range of 1:640 to 1:1280 and 1:1060 with a range of 1:640 to 1:1280 in the rabbits of group III and in those of group IV, respectively. The titer of anti-anti-id antibodies was markedly reduced in the sera obtained 1 week later from all the rabbits of groups III and IV. Anti-anti-id antibodies became detectable...
in the sera harvested 2 weeks after the third booster from the 3 rabbits of group I and from 2 of the 3 rabbits of group II. The level of anti-anti-id antibodies did not markedly change through the ninth week of observation in these two groups of rabbits. The maximal mean titer of anti-anti-id antibodies was 1:120 with a range of 1:40 and 1:160 and 1:120 with a range of 1:80 and 1:160 in the sera harvested 3 weeks after the third booster from the 3 rabbits of group I and 2 weeks after the third booster from the 2 rabbits of group II. The kinetics of the development of anti-anti-id antibodies in the four groups of rabbits is shown in Fig. 2.

The rabbits in all 4 groups developed anti-mouse IgG antibodies after the first injection. The level of anti-mouse IgG antibodies was increased in sera obtained 2 weeks after the first immunization from rabbits of group III and from those of group IV, respectively. No marked change was observed in the sera obtained in the following weeks in spite of two additional boosters. The maximal mean titer was 1:40,960 and 1:51,200 in the 3 rabbits of group III and in the 3 of group IV, respectively. The level of anti-mouse IgG antibodies was decreased in the sera obtained 3 weeks after the third booster from the rabbits of both groups. The level of anti-mouse Ig antibodies was only slightly increased in sera obtained through the ninth week of study from rabbits of group I and of group II. The maximal mean titer was 1:1960 and 1:2560 in the 3 rabbits of group I and in the 3 of group II, respectively. The kinetics of the development of anti-mouse Ig antibodies in the 4 groups of rabbits is shown in Fig. 3.

A number of experiments analyzed the characteristics of the antibodies elicited in rabbits by immunization with mAb MK2-23 conjugated to KLH and mixed with FA. Sera from the 3 immunized rabbits displayed a higher reactivity with mAb MK2-23 than with the unrelated isotype-matched anti-id mAb MF9-10 and with cultured M14/13 human melanoma cells than with autologous L14 B-lymphoid cells (Figs. 4 and 5). The reactivity of immune sera with unrelated mouse anti-id mAb MF9-10 and with L14 cells was completely removed following absorption with mouse IgG and with L14 cells, respectively. Neither absorption affected the reactivity of the immune sera with anti-id mAb MK2-23 and with M14/13 melanoma cells. The reactivity pattern is specific, since serum from a rabbit immunized with anti-id mAb MF9-10 maintained its reactivity with the immunizing anti-id mAb but lost that with anti-id mAb MK2-23 following absorption with mouse IgG (Fig. 4). Furthermore, this immune serum lost its reactivity with M14/13 melanoma cells following absorption with cultured L14 B-lymphoid cells (Fig. 5). Last, preimmune sera from the 3 immunized rabbits displayed a low and similar reactivity in binding assays with the mouse anti-id mAb MK2-23 and the unrelated isotype-matched anti-id mAb MF9-10 and with HMW-MAA-bearing M14/13 melanoma cells and the autologous L14 B-lymphoid cells. The reactivity of preimmune sera with mAb MK2-23 and MF9-10 was completely removed by absorption with mouse IgG and that with M14/13 and L14 cells by absorption with L14 cells.

To determine the Ig class of the antibodies elicited by mAb MK2-23 in rabbits, the binding assays with anti-id mAb MK2-23 and with M14/13 melanoma cells were performed with 125I-labeled xenoantibodies specific for rabbit IgG and IgM. Both Ig classes were found in the antibody populations reacting with anti-id mAb MK2-23 and with M14/13 melanoma cells (Fig. 6).

The presence of anti-HMW-MAA antibodies in sera from rabbits immunized with anti-id mAb MK2-23 was conclusively proved by SDS-PAGE analysis. Rabbit immune sera immunoprecipitated from radiolabeled M14/13 melanoma cells two components with the same electrophoretic mobility as the two immunoprecipitated by mouse anti-HMW-MAA mAb 763.74 (Fig. 7). Furthermore, in sequential immunoprecipitation experiments rabbit immune sera and mAb 763.74 removed the molecules immunoprecipitated by each of them from a melanoma cell extract. The immunoprecipitation is specific, since immune sera lost their ability to immunoprecipitate HMW-MAA from radiolabeled melanoma cells following absorption with anti-id mAb MK2-23 but maintained it following absorption with mouse IgG. Furthermore, preimmune sera did not immunoprecipitate any component from radiolabeled melanoma cells.

![Fig. 2. Effect of conjugation to a carrier and/or administration with an adjuvant on the kinetics of the development of anti-anti-id antibodies in rabbits immunized with mouse anti-id mAb MK2-23.](image-url)
Cross-inhibition experiments compared the fine specificity of antibodies elicited by mAb MK2-23 with that of mAb 763.74. Rabbit immune sera inhibited the binding of 125I-mAb 763.74 to M14/13 melanoma cells in a dose-dependent fashion (Fig. 8). The maximal inhibition obtained with the lowest dilution of rabbit immune sera tested was about 60%. The inhibition is specific, because incubation of M14/13 melanoma cells with rabbit immune sera did not affect their ability to bind 125I-anti-HMW-MAA mAb TP61.5. The latter mAb defines a determinant distinct and spatially distant from that defined by mAb 763.74. Furthermore, the reactivity of 125I-mAb 763.74 with M14/13 melanoma cells was not affected by preincubation with sera from rabbits immunized with the anti-id mAb MF9-10 (data not shown).

Inhibition experiments determined whether HMW-MAA binding anti-anti-id antibodies express in their antigen-combining sites the idiotope defined by the immunizing mAb MK2-23. As shown in Fig. 9, rabbit anti-anti-id antiserum displayed decreased reactivity with M14/13 melanoma cells after incubation with mAb MK2-23. The inhibition is dose dependent. Furthermore, the inhibition is specific, since incubation with the unrelated anti-id mAb TK7-371 did not
Fig. 5. Effect of absorption with cultured L14 human B-lymphoid cells on the reactivity with autologous cultured human M14/13 melanoma cells and L14 B-lymphoid cells of sera from rabbits immunized with mouse anti-id mAb MK2-23. Sera were harvested on day 42 from rabbits immunized with mAb MK2-23 coupled to KLH and mixed with CFA on day 0, with IFA on day 14 and with PBS on day 28. Immune sera were absorbed with cultured L14 B-lymphoid cells (4 × 10^6 cells/ml serum). Unabsorbed (A) and absorbed (B) sera (Δ) (100 μl) were incubated with melanoma cells M14/13(-) (2 × 10^5 cells/well) for 2 h at 4°C. Plates were then washed 3 times. Following the addition of 125I-anti-rabbit IgG xenoantibodies (1 × 10^5 cpm/well) and an additional 2-h incubation at 4°C, plates were washed 5 times. Bound radioactivity was measured in a gamma counter. Results are expressed as the mean ± SD of bound cpm/well obtained with sera from three rabbits. Cultured L14 B-lymphoid cells ( ) which are autologous to melanoma cells M14/13, preimmune serum (O), and serum from a rabbit immunized with the unrelated isotype-matched anti-id mAb MF9-10 (□) were used as specificity controls.

Rabbit immune sera contain antibodies to idiotopes within the antigen-combining site of mAb MK2-23, since they inhibited its binding to anti-HMW-MAA mAb 763.74. Rabbit immune sera inhibited also the binding of unrelated 125I-anti-id mAb TK7-371 to its immunizing anti-HMW-MAA mAb TP61.5, although to a lower extent (Fig. 10). Following the absorption of anti-mouse IgG antibodies rabbit immune sera lost their ability to inhibit the binding of 125I-anti-id mAb TK7-371 to mAb TP61.5 but maintained the ability to inhibit the binding of 125I-anti-id mAb MK2-23 to mAb 763.74. An additional proof of the specificity of the inhibition is provided by the lack of inhibition of the binding of 125I-anti-id mAb MK2-23 to mAb 763.74 by sera from rabbits immunized with the unrelated anti-id mAb TK7-371 after the absorption of anti-mouse IgG antibodies.

To analyze the idiotypic profile of rabbit anti-anti-id antibodies elicited by mAb MK2-23, following the removal of anti-mouse IgG antibodies sera from rabbits immunized with mAb MK2-23 were tested with a panel of mouse anti-id mAb in a binding assay with 125I-labeled anti-rabbit IgG + M xenoantibodies. The anti-anti-id antibodies reacted with the immunizing mAb MK2-23 but did not react

Fig. 6. IgM and IgG components in antibody populations reacting with mouse anti-id mAb MK2-23 and with cultured M14/13 human melanoma cells in sera from rabbits immunized with mouse anti-id mAb MK2-23. Sera were harvested on day 42 from rabbits immunized with anti-id mAb MK2-23 coupled to KLH and mixed with CFA on day 0, with IFA on day 14, and with PBS on day 28. Immune sera were absorbed with insolubilized mouse IgG. Absorbed serum (100 μl/well) was added to anti-id mAb MK2-23-coated plates (1 μg/well) (A) and to M14/13 melanoma cells (B) (2 × 10^5/well). At the end of a 4-h incubation at room temperature (A) or 2 h at 4°C (B), plates were washed 3 times. Following the addition of 125I-anti-rabbit IgG (O) or IgM (Δ) xenoantibodies (1 × 10^5 cpm/well) incubation was continued for an additional 2 h at room temperature (A) or at 4°C (B). Plates were then washed 5 times, and bound radioactivity was measured in a gamma counter. Results are expressed as bound cpm/well. Preimmune serum ( --- - - ) was used as a control.
DISCUSSION

The present study has shown for the first time that the combined use of an adjuvant and a carrier markedly enhances the ability of a mouse anti-idiotype mAb to induce humoral immunity to a self antigen in xenogeneic hosts. Anti-HMW-MAA antibodies were developed by all the rabbits immunized with mAb MK2-23 conjugated to KLH and mixed with FA. On the other hand they were developed by only one of the rabbits immunized with mAb MK2-23 conjugated to KLH and of those immunized with mAb MK2-23 without KLH and FA. These findings parallel results obtained by analyzing the effect of KLH and FA on the immunogenicity of mAb MK2-23 in syngeneic hosts (21). This is a surprising conclusion, since the Fc portion of mouse mAb MK2-23 was expected to function in xenogeneic combinations as a carrier for the region of mAb MK2-23, which mimics the mAb 763.74-defined determinant of HMW-MAA. The latter possibility appears to occur for idiotope(s) of mAb MK2-23 which do(es) not mimic HMW-MAA, since FA markedly enhances its (their) immunogenicity, while KLH has no effect.

Recently Chattopadhyay et al. (15) described the development of anti-HMW-MAA antibodies in rabbits immunized with the anti-id mAb IM06 and IM32 mixed with the muramyl dipeptide-derived adjuvant. Both anti-id mAb recognize idiotopes in the antigen-combining site of the immunizing anti-HMW-MAA mAb MEM136, which defines a distinct and spatially distant determinant from that reacting with mAb 763.74 (30). The percentage of immunized rabbits which developed anti-HMW-MAA antibodies was not indicated. Therefore the immunogenicity of the anti-id mAb IM06 and IM32
higher titer than that of the mouse and human counterparts (20, 21). On the other hand, the HMW-MAA-binding rabbit anti-anti-id antibodies have a higher affinity than that of mAb MK2-23 and/or their reactivity with determinants of HMW-MAA. The latter phenomenon may reflect a lower-affinity constant of the anti-anti-id antibodies to melanoma cells, although incompletely (20, 21). The latter difference reflects the suboptimal characteristic of the immunization schedule in patients with malignant melanoma and/or abnormalities of their immune system because of the disease and/or previous chemotherapy and radiotherapy remains to be determined. Finally, HMW-MAA-binding rabbit anti-anti-id antibodies do not express the idiotope defined by anti-id mAb MK2-120. The latter recognizes an idiotope in the antigen-combining site of the immunizing anti-HMW-MAA mAb 763.74 (9) and of mouse HMW-MAA-binding anti-anti-id mAb elicited with mAb MK2-23.6

The anti-anti-id response elicited by the mAb MK2-23 is higher than that directed against constant regions of mouse IgG, since the rabbit immune sera display a higher reactivity with the immunizing anti-id mAb than with an unrelated isotype-matched anti-id mAb. Furthermore, absorption with mouse IgG does not affect the reactivity of rabbit immune sera with the immunizing anti-id mAb MK2-23 but removes that with an isotype-matched control. This pattern of immune response is not restricted to rabbits, since it has been found also in patients with malignant melanoma immunized with mAb MK2-23 conjugated to KLH and administered with Bacillus Calmette-Guérin (20). However, the level of anti-anti-id antibodies and of antibodies to constant portions of mouse IgG is markedly lower in patients with malignant melanoma than in rabbits.

Characterization of the immunogenicity of anti-id mAb MK2-23 in xenogeneic hosts with a constitutive expression of HMW-MAA strongly suggests that active specific immunotherapy with mAb MK2-23 in patients with malignant melanoma will benefit from the use of a carrier and an adjuvant. The validity of this possibility is being tested in a randomized clinical trial which is in progress.

Fig. 10. Specificity of the reactivity with idiotopes within the antigen-combining site of anti-id mAb MK2-23 of sera from rabbits immunized with mouse anti-id mAb MK2-23. Sera were harvested on day 42 from rabbits immunized with anti-id mAb MK2-23 coupled to KLH and mixed with CFA on day 0, with IFA on day 14, and with PBS on day 28. Immune sera were absorbed with insolubilized mouse IgG. Unabsorbed (A) and absorbed (B) immune sera (50 μl/well) were mixed with 125I-mAb MK2-23 (2 × 10^6 cpm/50 μl/well). At the end of a 4-h incubation at 4°C, the mixture was added to anti-HMW-MAA mAb 763.74-coupled plates, and incubation was prolonged for an additional 2 h at room temperature. Plates were then washed 5 times, and bound radioactivity was measured in a gamma counter. Results are expressed as percentage inhibition of the binding of 125I-anti-id mAb to the corresponding anti-HMW-MAA mAb as compared to the binding performed in the presence of preimmune serum. Serum from a rabbit immunized with anti-id mAb TK7-371 (Δ) and inhibition of the binding of 125I-anti-id mAb TK7-371 to its corresponding anti-HMW-MAA mAb TP61.5 (---) were used as specificity controls.


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