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Human high molecular weight melanoma-associated antigen (HMW-MAA) mimicry by mouse anti-idiotypic monoclonal antibody MK2-23: Induction of humoral anti-HMW-MAA immunity and prolongation of survival in patients with stage IV melanoma

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ABSTRACT **Twenty-five patients with stage IV melanoma were immunized with the mouse anti-idiotypic monoclonal antibody (mAb) MK2-23 (2 mg per injection), which bears the internal image of the determinant defined by anti-HMW-MAA mAb 763.74. Two patients were inevaluable, since they did not complete 4 weeks of therapy. Only 14 patients developed antibodies that were shown by serological and immunochemical assays to recognize the same or spatially close determinant as the anti-HMW-MAA mAb 763.74 and to express the idiotope defined by mAb MK2-23 in their antigen-combining sites. Side effects that are likely to be caused by bacillus Calmette–Guérin present in the immunogen consisted of erythema, induration, and ulceration at the sites of the injections. Occasionally, patients complained of flu-like symptoms, arthralgias, and myalgias. Three of the patients who developed anti-HMW-MAA antibodies achieved a partial response. It consisted of a decrease in the size of metastatic lesions and lasted 52 weeks in 1 patient and 93 weeks in the other 2 patients. Survival of the 14 patients who developed anti-HMW-MAA antibodies was significantly ($P = 0.0003$) longer than that of the 9 patients without detectable humoral anti-HMW-MAA immunity development. In the multivariate analysis, such an association between development of anti-HMW-MAA antibodies and survival prolongation was still significant ($P = 0.001$) after adjustment for difference in performance status, the only confounding factor found to be significantly related to survival. Lastly, a significant ($P = 0.03$ by likelihood ratio test) interaction between anti-HMW-MAA antibodies and patients' performance status was found, since the prolongation of survival associated with anti-HMW-MAA antibodies was more marked in patients with a performance status of ≤70% than in those with a higher one. These results suggest that anti-idiotypic mAb MK2-23 may represent a useful immunogen to implement active specific immunotherapy in patients with melanoma.**

The identification with monoclonal antibodies (mAbs) of human MAA that meet most, if not all, the criteria to be used as targets for immunotherapy (1–3) has stimulated interest in the development and application of immunotherapeutic approaches to melanoma. In the area of active specific immunotherapy, one immunogen is represented by anti-idiotypic mAb (i.e., antibodies to determinants on the variable region of anti-MAA antibodies, which bear the internal image of MAA). This approach is very attractive for several reasons. (*i*) The hybridoma methodology has facilitated the development of large amounts of anti-idiotypic mAb, thus providing immunogens with well-defined characteristics for clinical trials. (*ii*) Results in animal model systems (for review, see refs. 4 and 5) strongly suggest that the immunity to tumor-associated antigens elicited by anti-idiotypic mAb may have a beneficial effect on the disease. (*iii*) In a recent phase I clinical trial, repeated administrations to patients with melanoma of the mouse anti-idiotypic mAb MF11-30, which bears the internal image of the determinant defined by anti-HMW-MAA mAb 225.28 (6), have caused no side effects (7), thus facilitating the recruitment of patients to clinical trials.

In recent studies (8, 9), we have analyzed a large panel of anti-idiotypic mAbs elicited with syngeneic mAb recognizing distinct determinants of HMW-MAA. The latter has been selected as a target for immunotherapy because of its expression in a high percentage of melanoma lesions and its restricted distribution in normal tissues (for review, see ref. 10). The anti-idiotypic mAb MK2-23 elicited with the anti-HMW-MAA mAb 763.74 has been found to be more effective than mAb MF11-30 in eliciting anti-HMW-MAA immunity in animal model systems (ref. 8 and unpublished results). Therefore, in the present clinical trial we have characterized the humoral immune response induced by mAb MK2-23 in patients with melanoma. mAb MK2-23 conjugated to keyhole limpet hemocyanin (KLH) and mixed with bacillus Calmette–Guérin (BCG) has been used as an immunogen, since conjugation to a carrier and administration with an adjuvant have been shown to greatly enhance the immunogenicity of mAb MK2-23 in animal model systems (11).

PATIENTS, MATERIALS, AND METHODS

Patients. Patients entered into this study were required to have biopsy-proven malignant melanoma. They were required to have a Karnofsky performance status of at least 60%, normal liver function, serum creatinine of ≤2 mg/dl, a leukocyte count of ≥3000 cells per mm^3, and a platelet count of ≥100,000 cells per mm^3. All patients were required to have measurable disease by physical examination, x-ray, and/or computerized axial tomography scan and a life expectancy of at least 3 months. Tumor burden was measured by assigning a score of 1 to lesions in skin and soft tissues; 2 to lesions in lymph nodes; 3 to lesions in bone, lung, spleen, and urethra; and 4 to lesions in brain, gastrointestinal tract, and liver. Informed consent was obtained from each participating patient. Sentinel lesions were measured monthly. Complete blood count was obtained weekly and liver function tests were performed monthly. Serum was obtained weekly for analysis and stored at −20°C. Response was defined according to guidelines of the National Cancer Institute, as defined (7).

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Abbreviations: BCG, bacillus Calmette–Guérin; HMW-MAA, high molecular weight melanoma-associated antigen; KLH, keyhole limpet hemocyanin; mAb, monoclonal antibody; NP-40, Nonidet P-40.

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Cell Lines. Cultured human melanoma cells (Colo 38, EH-M, and M14/13) and B-lymphoid cells (L14 and LG-2) were grown in RPMI 1640 medium supplemented with 10% (vol/vol) fetal calf serum and 2 mM L-glutamine.

mAb and Conventional Antisera. Mouse mAbs 225.28 (an IgG2a), 149.53 (an IgG1), and 763.74 (an IgG1) to distinct and spatially distant determinants of HMW-MAA; the corresponding anti-idiotypic mAbs MF11-30, MF9-10, and MK2-23 (all IgG1); and the anti-intercellular adhesion molecule 1 mAb CL207.14 (an IgG1) were developed as described (8, 12–14). mAb VF19-LL71, elicited with interferon-γ-treated cultured human ovarian carcinoma cells SK-OV-3, immunoprecipitates 55-kDa and 44-kDa glycoproteins from cultured human melanoma Colo 38 cells.

mAbs were purified from ascites fluid by sequential precipitation with caprylic acid and ammonium sulfate (15). $F(ab')_2$ fragments were prepared as described (8). The purity of mAb and $F(ab')_2$ fragment preparations was monitored by SDS/PAGE (16). mAb MK2-23 was crosslinked to KLH (Sigma) with glutaraldehyde (17).

Affinity-purified goat anti-human IgG plus IgM antibodies, affinity-purified goat anti-human IgG antibodies, affinity-purified goat anti-human IgM antibodies, and affinity-purified goat anti-mouse IgG Fc antibodies were purchased from Jackson ImmunoResearch. Antibodies were labeled with ^{125}I utilizing the Chloramine-T (18) or the Iodogen (19) methods.

Serological Assays. The indirect binding assay with whole immunoglobulin- and $F(ab')_2$ fragments of mAb-coated microtiter plates (1 μg per well), the inhibition assay to measure human anti-anti-idiotypic antibodies, the indirect binding assay to measure antibodies reacting with cells, the cross-blocking assay to map determinants recognized by different antibodies, and the inhibition of anti-idiotypic mAb of the binding of anti-anti-idiotypic antibodies to target cells were performed as described elsewhere (7, 20). Absorption was performed by incubating patient's serum with cells (1 ml of serum per 4×10^7 cells) for 4 hr at 4°C on a rotator. Serum was then harvested by centrifugation.

Immunochemical Methods. Antibodies were conjugated to Affi-Gel 10 (Bio-Rad) at the concentration of 2 or 20 mg/ml of gel by following the manufacturer's instructions. Affinity chromatography to purify anti-anti-idiotypic antibodies from patients' sera was performed as follows. Sera were diluted with an equal volume of phosphate-buffered saline (PBS) and filtered through a 0.22-μm (pore size) filter (Costar). Then, sera were passed over a mouse IgG1 column to remove human anti-mouse IgG antibodies. Absorption of human anti-mouse IgG antibodies from serum was monitored by testing its reactivity with mouse IgG1 mAb in a binding assay with ^{125}I-labeled anti-human IgG plus IgM xenoantibodies. After removal of anti-mouse IgG antibodies, serum was passed over the immunizing anti-idiotypic mAb MK2-23 column. Bound antibodies were eluted with 0.1 M glycine hydrochloride (pH 2.9), neutralized with 1 M Tris (pH 10.0), and dialyzed against PBS. Reactivity and specificity of purified human anti-mouse IgG antibodies and anti-anti-idiotypic antibodies were monitored by testing with immunizing anti-idiotypic mAb MK2-23 and isotype-matched unrelated mouse mAb in a binding assay with ^{125}I-labeled anti-human IgG plus IgM xenoantibodies.

Labeling of cells with ^{125}I, solubilization with Nonidet P-40 (NP-40), indirect immunoprecipitation, SDS/PAGE, and autoradiography were performed as described (7, 8).

Statistical Analysis. The Kaplan–Meier product limit method (21) and the log-rank test (22) were used in the univariate analysis to assess the ability of individual prognostic variables to predict survival. The Cox proportional hazards regression model (23) was used in the multivariate survival analysis to identify a subset of variables, including any interactions between variables, that jointly predict survival. Likelihood ratio tests were used to assess the prognostic significance of the multivariate factors. Diagnostic hazards plots were performed whenever feasible to ascertain the appropriateness of the proportional hazards assumption.

RESULTS

The 25 patients with stage IV melanoma entered in this study included 17 males and 8 females with a median age of 53 years (range 31 to 78 years). The mean performance status was 70% with a range of 60–100%. Metastatic sites included skin, lymph nodes, bones, brain, lung, liver, gastrointestinal system, and genitourinary system. Four patients had received no prior therapy; the remaining had been treated with chemotherapy, immunotherapy, radiotherapy, and/or surgery.

Patients were immunized on days 0, 7, and 28 with subcutaneous injections of anti-idiotypic mAb MK2-23 (2 mg per injection) conjugated to KLH and mixed with 0.1 ml (1×10^7 organisms) of Tice BCG (Organon). Additional injections were given if the titer of anti-anti-idiotypic antibodies that inhibit the binding of ^{125}I-labeled mAb MK2-23 to anti-HMW-MAA mAb 763.74 at 1:8 dilution was lower than 90%.

Two patients were inevaluable since they had rapid disease progression and did not complete 4 weeks of therapy. In the remaining 23 patients, the number of immunizations per patient ranged from 3 to 12 with an average of 5.7. The average duration of treatment per patient was 38.7 weeks with a range of 9–93 weeks.

Preimmune sera from the 23 patients displayed reactivity with the whole IgG of mAb MK2-23 in a binding assay with a titer up to 1:1280. The reactivity of the preimmune sera with mAb MK2-23 was markedly lower with $F(ab')_2$ fragments than with the whole IgG. The titer of anti-mouse IgG antibodies did not show any detectable change throughout the duration of the treatment in 2 patients and increased in 1 patient after one immunization, in 15 after two immunizations, in 4 after three immunizations, and in 1 after six immunizations. The maximal titer of anti-mouse IgG antibodies was 1:240 in 1 patient, 1:320 in 3, 1:480 in 1, 1:640 in 5, 1:960 in 3, 1:1280 in 4, 1:1920 in 1, 1:2560 in 4, 1:5120 in 1, and 1:10,240 in 2. The kinetics of the development of anti-mouse IgG antibodies in 1 patient is presented in Fig. 1. It is noteworthy that the titer of sera is higher with the whole IgG of the immunizing mAb MK2-23 than with that of the isotype-matched mouse anti-idiotypic mAb MF11-30 (Fig. 1). Similar results were obtained by testing sera with $F(ab')_2$ fragments of mAb MK2-23 and of mAb MF11-30.

Anti-anti-idiotypic antibodies that inhibited the binding of ^{125}I-labeled anti-idiotypic mAb MK2-23 to the anti-HMW-MAA mAb 763.74 were not detected throughout the duration of the treatment in 5 patients. Anti-anti-idiotypic antibodies became detectable in 2 patients after two immunizations, in 8 after three, in 3 after four, in 4 after five, and in 1 after six. The kinetics of the development of anti-anti-idiotypic antibodies in 1 patient is shown in Fig. 1. The maximal titer of anti-anti-idiotypic antibodies (i.e., highest dilution of serum that inhibited the binding of ^{125}I-labeled mAb MK2-23 to mAb 763.74 by at least 90%) was 1:4 in 5 patients, 1:8 in 4, 1:16 in 5, 1:32 in 1, and 1:64 in 3. Sera from 18 patients also inhibited the binding of the unrelated mouse anti-idiotypic mAb MF11-30 and mAb KO3-34 to the immunizing anti-HMW-MAA mAb 225.28 and anti-HLA-DQw3 mAb KS13, respectively. The titer of the inhibition was lower than that found by inhibiting the binding of mAb MK2-23 to mAb 763.74 (Fig. 1). Furthermore, sera lost the ability to inhibit the binding of mAb MF11-30 to mAb 225.28 and of mAb KO3-34 to mAb KS13 after absorption of anti-mouse IgG antibodies with mouse IgG (data not shown).

Preimmune sera from all the patients displayed a low reactivity with HMW-MAA-bearing cultured melanoma cells

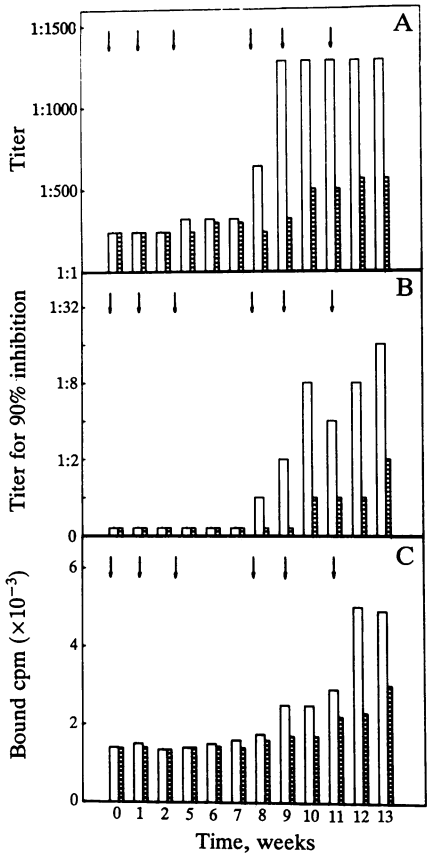

FIG. 1. Kinetics of the development of human anti-mouse IgG antibodies, anti-anti-idiotypic antibodies, and antibodies reacting with autologous melanoma cells in patient EH immunized with mouse anti-idiotypic mAb MK2-23. Patient EH was immunized in the weeks indicated by the arrows with subcutaneous injections of mAb MK2-23 (2 mg per injection) conjugated to KLH and mixed with BCG. Sera were drawn in the weeks indicated. (*A*) Human anti-mouse IgG antibodies were measured by testing sera with the immunizing mouse anti-idiotypic mAb MK2-23 (open bars) in a binding assay with ^{125}I-labeled anti-human IgG plus IgM xenoantibodies. Results are expressed as dilution of serum giving 50% of the maximal binding to mAb MK2-23. The specificity of the reaction was assessed by testing sera with the unrelated isotype-matched mouse anti-idiotypic mAb MF11-30 (hatched bars). The binding of mAbs MK2-23 and MF11-30 to microtiter plates was monitored by testing with ^{125}I-labeled anti-mouse IgG Fc xenoantibodies. (*B*) Anti-anti-idiotypic antibodies were measured by testing sera for the ability to inhibit the binding of ^{125}I-labeled mAb MK2-23 to anti-HMW-MAA mAb 763.74 (open bars) in an inhibition assay. Results are expressed as dilution of serum giving 90% inhibition of the binding of ^{125}I-labeled mAb MK2-23 to mAb 763.74 as compared to the binding in the presence of preimmune serum. The specificity of the inhibition was assessed by testing the sera for the ability to inhibit the binding of ^{125}I-labeled unrelated isotype-matched mouse anti-idiotypic mAb MF11-30 to anti-HMW-MAA mAb 225.28 (hatched bars). (*C*) Antibodies reacting with cultured autologous melanoma cells were detected by testing sera with cultured autologous melanoma cells EH-M (open bars) in a binding assay with ^{125}I-labeled anti-human IgG plus IgM xenoantibodies. Results are expressed as bound cpm per 2×10^5 cells. The specificity of the reaction was assessed by testing sera with cultured human B-lymphoid cells L14 (hatched bars). The latter do not express HMW-MAA.

Colo 38, EH-M, and/or M14/13 in a binding assay. The extent of reactivity was similar to that with cultured B-lymphoid cells LG-2 and L14, which do not express HMW-MAA. The reactivity of the preimmune sera with melanoma cells was lost after absorption with B-lymphoid cells (Fig. 2). The reactivity of the sera with cultured melanoma cells Colo 38, EH-M, and/or M14/13 did not change throughout the duration of immunization in nine patients, but increased in three patients after two immunizations, in five after three, in four after five, in one after six, and in one after nine. The maximal titer was 1:32 in six patients, 1:64 in four patients, 1:128 in three patients, and 1:256 in one patient. The kinetics of the development of antibodies reacting with cultured melanoma cells in one patient is shown in Fig. 1. The antibodies reacting with melanoma cells are both IgM and IgG (data not shown). The average dose of mAb MK2-23 required to increase the level of antibodies reacting with cultured melanoma cells was 8 mg with a range of 4–18 mg. The average time required to increase the level of antibodies reacting with cultured melanoma cells was 8.2 weeks with a range of 4–25 weeks.

The sera from the 14 patients with increased reactivity with cultured melanoma cells after immunization with mAb MK2-23 contained anti-anti-idiotypic antibodies with an average titer of 1:22 and a range of 1:4 to 1:64. Anti-anti-idiotypic antibodies were also found in sera from 4 patients without detectable changes in the reactivity with cultured melanoma cells throughout the immunization course; their average titer was 1:11 with a range of 1:4 to 1:32.

Sera with increased reactivity with cultured melanoma cells after immunization with mAb MK2-23 also displayed an increased reactivity with cultured B-lymphoid cells (Fig. 1). However, the reactivity of the sera with cultured B-lymphoid cells was markedly lower than that with cultured melanoma cells. Furthermore, absorption of sera with cultured B-lymphoid cells completely removed the reactivity with the absorbing cells but did not markedly affect that with cultured melanoma cells (Fig. 2).

Sera from the immunized patients did not immunoprecipitate any component from ^{125}I-labeled cultured melanoma cells probably because of the low affinity and/or titer of antibodies reacting with melanoma cells. To prove that the sera from the immunized patients contain anti-HMW-MAA antibodies, anti-anti-idiotypic antibodies were purified from sera by affinity chromatography on mAb MK2-23 after absorption of anti-mouse IgG antibodies. SDS/PAGE analysis of the components immunoprecipitated by human anti-anti-idiotypic antibodies from a NP-40 extract of radiolabeled Colo 38 melanoma cells detected two components with the

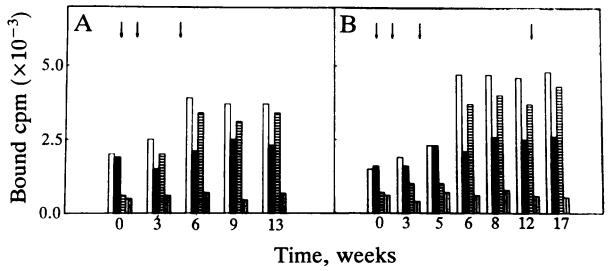

FIG. 2. Effect of absorption with cultured human B-lymphoid cells on the differential reactivity with cultured human melanoma cells M14/13 and autologous cultured human B-lymphoid cells L14 of sera from patients AK and AM immunized with mouse anti-idiotypic mAb MK2-23. Patients AK (*A*) and AM (*B*) were immunized in the weeks indicated by the arrows with subcutaneous injections of mAb MK2-23 (2 mg per injection) conjugated to KLH and mixed with BCG. Blood was drawn in the indicated weeks. Sera (100 μl) were absorbed with 4×10^6 cultured B-lymphoid cells by a 4-hr incubation on a rotator at 4°C. Antibodies reacting with cultured human melanoma cells were detected by testing sera with cultured human melanoma cells M14/13 (horizontal hatched bars) and their autologous cultured B-lymphoid cells L14 (vertical hatched bars) in a binding assay with ^{125}I-labeled anti-human IgG plus IgM xenoantibodies. Results are expressed as bound cpm per well. Unabsorbed sera tested with cultured human melanoma cells M14/13 (open bars) and with their autologous cultured B-lymphoid cells L14 (solid bars) were used as controls.

same electrophoretic mobility as the two immunoprecipitated by mouse anti-HMW-MAA mAb 763.74 (Fig. 3). That the two components immunoprecipitated by the purified anti-anti-idiotypic antibodies represent the two subunits of HMW-MAA was conclusively proven by immunodepletion experiments: the anti-anti-idiotypic antibodies did not immunoprecipitate any component from a NP-40 extract of radiolabeled cultured Colo 38 melanoma cells that had been immunodepleted with mAb 763.74. The immunodepletion is specific, since the anti-anti-idiotypic antibodies immunoprecipitated the two subunits of HMW-MAA from the melanoma cell extract that had been immunodepleted with the unrelated mouse mAb VF19-LL71.

Furthermore, after absorption of anti-mouse IgG antibodies, sera reacted with HMW-MAA purified from a Colo 38 melanoma cell extract by binding to anti-HMW-MAA mAb 149.53-coated plates. mAb 149.53 recognizes a distinct and spatially distant determinant from that recognized by mAb 763.74 (13). The reactivity is specific, since sera did not bind to intercellular adhesion molecule 1 purified from a Colo 38 melanoma cell extract by binding to anti-intercellular adhesion molecule 1 mAb CL207.14-coated plates. It is noteworthy that patients' sera did not react with HMW-MAA bound to mAb 763.74. The latter results suggest that patients' sera and mAb 763.74 recognize the same (or a spatially close) determinant. This interpretation is consistent with the specific inhibition by patients' sera of the binding of ^{125}I-labeled mAb 763.74 to melanoma cells. The anti-HMW-MAA antibodies induced by mAb MK2-23 express the corresponding idiotope in their antigen-combining sites, since mAb MK2-23 inhibited the binding to melanoma cells of patients' sera in a dose-dependent fashion.

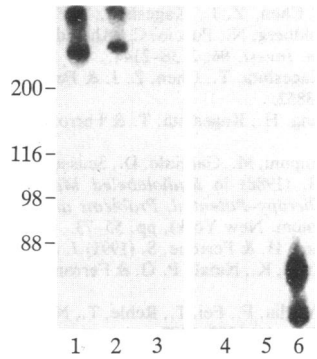

FIG. 3. Structural relationship of antigens immunoprecipitated from cultured human melanoma cells by anti-HMW-MAA mAb 763.74 and by sera from patient AM immunized with mouse anti-idiotypic mAb MK2-23. Sera were obtained on days 126, 133, 140, 147, 154, 161, 168, 175, 182, and 190 from patient AM immunized on days 0, 7, 28, and 112 with subcutaneous injections of mAb MK2-23 (2 mg per injection), conjugated to KLH, and mixed with BCG. Sera were pooled and utilized to purify anti-anti-idiotypic antibodies by affinity chromatography on mAb MK2-23 after removal of anti-mouse IgG antibodies. A NP-40 extract of ^{125}I-labeled cultured human melanoma Colo 38 cells was immunodepleted with mAb VF19-LL71 (lanes 1–3) or with anti-HMW-MAA mAb 763.74 (lanes 4–6). Each extract was then immunoprecipitated with insolubilized mAb 763.74 (lanes 1 and 4), human anti-anti-idiotypic antibodies (lanes 2 and 5), and anti-55-kDa and -44-kDa antigen mAb VF19-LL71 (lanes 3 and 6). Antigens were then eluted from the immunoadsorbents and analyzed by SDS/PAGE in a 5% slab gel in the presence of 2% (vol/vol) 2-mercaptoethanol. Gels were processed for autoradiography using Kodak XAR-5 film (Eastman Kodak). Positions of molecular mass standards are indicated on the left in kDa. mAb VF19-LL71 was used as a specificity control. No component was immunoprecipitated from the melanoma cell extract by antibodies purified from the immune serum by affinity chromatography on mouse IgG.

Repeated administrations of mAb MK2-23 conjugated to KLH and mixed with BCG were associated with local toxicity. Such toxicity was probably caused by BCG and consisted of erythema, induration, and occasional ulceration at the sites of immunization. Occasionally, patients developed flu-like symptoms and rarely complained of mild arthralgias and myalgias. Patients, however, experienced neither allergic nor anaphylactic reactions in spite of the development of anti-mouse IgG antibodies. Among the patients who developed anti-HMW-MAA antibodies, three patients achieved a partial remission, which consisted of a decrease in the size of paraortic lymph nodes in patient LM, a decrease in the size of multiple skin satellite metastases and a paraspinal mass in patient AM, and a decrease in the size of inguinal lymph nodes and disappearance of all subcutaneous nodules at the primary site in patient AK. These responses lasted 52 weeks in patient LM and 93 weeks in patients AM and AK.

The median survival of the 14 patients who developed anti-HMW-MAA antibodies was 52 weeks (range 19 to 93 weeks). However, the median survival of the remaining 9 patients without detectable anti-HMW-MAA antibodies in their sera was 19 weeks (range 9–45 weeks). The difference in survival between these two groups was statistically significant (P = 0.0003 utilizing the log-rank test) (Fig. 4). Univariate survival analysis utilizing the log-rank test was also performed on several other variables, including age, sex, prior therapy, tumor burden, and performance status categorized as >70% and ≤70%. Only performance status was significantly related to survival (P = 0.008 by log-rank test). Median survival was not reached in the 8 patients with performance status >70%; however, median survival was only 30 weeks for the 15 patients with performance status ≤70%. Multivariate analysis by the Cox regression showed that performance status was the only variable that significantly added to the prediction of survival by anti-HMW-MAA antibody development. Specifically, the multivariate analysis showed that the survival of patients who developed anti-HMW-MAA antibodies was significantly (P = 0.001 by likelihood ratio test) longer than that of those without detectable anti-HMW-MAA antibodies, even after adjusting for differences in performance status. Furthermore, the multivariate analysis revealed a significant interaction between anti-HMW-MAA antibodies and performance status. Specifically, the prolongation of survival associated with anti-HMW-MAA antibodies was significantly (P = 0.03 by likelihood ratio test) more marked in patients with a performance status ≤70% than in those with a higher performance status.

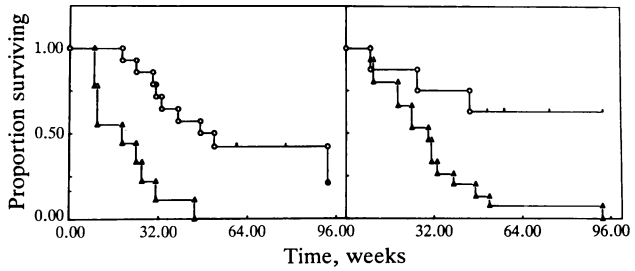

FIG. 4. Association between survival and development of humoral anti-HMW-MAA immunity or performance status in patients immunized with mouse anti-idiotypic mAb MK2-23. (*Left*) Survival of the 14 patients who developed anti-HMW-MAA antibodies (○) after immunization with mouse anti-idiotypic mAb MK2-23 was significantly (P = 0.0003) longer than that of the 9 patients who did not develop anti-HMW-MAA antibodies (△). (*Right*) Survival of the 8 patients with performance status higher than 70% (○) was significantly (P = 0.008) longer than that of the 15 patients with a performance status ≤70% (△).

DISCUSSION

Serological and immunochemical studies have shown that ≈61% of patients with melanoma immunized with the mouse anti-idiotypic mAb MK2-23, which bears the internal image of the determinant defined by the anti-HMW-MAA mAb 763.74, developed anti-HMW-MAA antibodies. Their level and affinity were low since immunoprecipitation of HMW-MAA from melanoma cells required purification of anti-anti-idiotypic antibodies by affinity chromatography on the immunizing mAb MK2-23. Furthermore, coating of melanoma cells with immunized patients' sera only partially inhibited the binding of ^{125}I-labeled mAb 763.74. The anti-HMW-MAA antibodies induced in patients by mAb MK2-23 recognize the same (or spatially close) determinant(s) as that recognized by mAb 763.74. Furthermore, like mAb 763.74, they express the idiotope recognized by mAb MK2-23 in their antigen-combining sites. Lastly, anti-anti-idiotypic antibodies that react with melanoma cells were detected in a lower percentage of immunized patients and at a later stage in the course of the immune response to mAb MK2-23 than anti-anti-idiotypic antibodies that do not bind to melanoma cells. All these findings parallel the results we have obtained in BALB/c mice immunized with mAb MK2-23 (11), except for the fact that sera from all the immunized BALB/c mice contained anti-HMW-MAA antibodies and immunoprecipitated HMW-MAA from radiolabeled melanoma cells. These differences may reflect abnormalities of the immune system in patients with melanoma because of the disease and/or therapy. Alternative, but not exclusive, explanations may be patients' reduced responsiveness because of tolerance to self HMW-MAA or optimization of the immunization schedule in mice, but not in patients.

The present study has shown that the survival of patients who have developed anti-HMW-MAA antibodies after immunization with anti-idiotypic mAb MK2-23 is significantly longer than that of those who have not. Although one cannot formally exclude that this association is casual, the more marked effect of anti-HMW-MAA antibodies on survival in patients with a performance status of ≤70% argues against development of humoral anti-HMW-MAA immunity as a marker of patients' better general health conditions. If the association between humoral anti-HMW-MAA immunity and prolonged survival reflects a cause–effect relationship, the mechanism underlying this association may be represented by the inhibition of the role that HMW-MAA has been suggested to play in the metastatic process of melanoma cells (24–27). Immune lysis of melanoma cells mediated by anti-HMW-MAA antibodies is not likely to play a significant role because of their low *in vitro* cytolytic activity (28). The latter phenomenon may also account for the lack of side effect, which one would expect to be associated with the lysis of normal cells recognized by anti-HMW-MAA antibodies. Whatever is the mechanism, association between induction of humoral immunity and favorable clinical course of melanoma is not unique to HMW-MAA. In a previous study (29), induction of high-titer anti-GM2 ganglioside antibodies by immunization with vaccines containing purified GM2 ganglioside was found to be associated with prolongation of a disease-free interval.

The present study confirms the results of a previous trial with the mouse anti-idiotypic mAb MF11-30 (7) in terms of characteristics of anti-anti-idiotypic antibodies and lack of side effects caused by anti-mouse IgG antibodies. In addition, the present investigation has provided the following information. (*i*) The anti-idiotypic mAb MK2-23 is significantly more effective than mAb MF11-30 in eliciting humoral anti-HMW-MAA immunity. (*ii*) Antibodies elicited by anti-idiotypic mAb can immunoprecipitate HMW-MAA from radiolabeled melanoma cells. (*iii*) Induction of anti-HMW-MAA immunity by mouse anti-idiotypic mAb MK2-23 is associated with prolongation of survival in patients with melanoma. (*iv*) About 40% of the patients immunized with mAb MK2-23 do not respond with a detectable anti-HMW-MAA immunity.

Besides measuring the cellular anti-HMW-MAA immunity induced by anti-idiotypic mAb MK2-23 and defining its effect on the course of the disease, future studies should determine the mechanism(s) underlying the inability of a subset of patients to develop humoral anti-HMW-MAA immunity after immunization with anti-idiotypic mAb MK2-23. The resulting information may suggest approaches to overcome the unresponsiveness and/or criteria to select patients who are likely to benefit from immunization with anti-idiotypic mAb MK2-23.

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