Human High Molecular Weight-Melanoma Associated Antigen Mimicry by an Anti-Idiotypic Antibody: Characterization of the Immunogenicity and the Immune Response to the Mouse Monoclonal Antibody IMel-1

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ABSTRACT

The mouse anti-idiotype (anti-id) monoclonal antibody (mAb) IMel-1 recognizes an idiotope in the antigen combining site of the immunizing anti-human high molecular weight melanoma-associated antigen (HMW-MAA) mAb 225.28. The mAb IMel-1 is able to induce an immune response against self cross-reacting HMW-MAA in rabbits that express HMW-MAA in normal tissues. Most of the rabbit anti-anti-id antibodies recognize a spatially distant determinant(s) from that defined by anti-HMW-MAA mAb 225.28. The immunogenicity of mAb IMel-1 is enhanced by its administration with the muramyl dipeptide-derived adjuvant. Anti-HMW-MAA antibodies were not detected in sera from rabbits immunized with HMW-MAA bearing cultured human melanoma cells. The differential immunogenicity of mAb IMel-1 and cell membrane bound HMW-MAA may account for the ability of anti-id mAb to induce anti-HMW-MAA immunity in patients who have not mounted such a response to HMW-MAA present in their lesions. Rabbit anti-HMW-MAA antibodies induced by anti-id mAb IMel-1 inhibited interactions of melanoma cells with elements of extracellular matrix. This may represent an additional mechanism by which anti-HMW-MAA immunity may affect the biology of melanoma cells in patients with melanoma immunized with anti-id mAb IMel-1.

INTRODUCTION

The use of mouse anti-id mAb as immunogens to implement active specific immunotherapy in patients with solid tumors is receiving increasing attention, since in animal model systems a beneficial effect on the course of the disease has been observed after induction of immunity to a relevant TAA by immunization with anti-id antibodies that bear the corresponding internal image (for review, see Refs. 1–3). Furthermore, hybridoma methodology has been successfully applied to develop anti-id mAb in various TAA systems (4–10) thus overcoming the practical difficulties to produce large amounts of anti-id antibodies with well defined and standardized characteristics that can be utilized as immunogens in clinical trials. Finally, the results of recent clinical trials, although preliminary in nature, corroborate the validity of investigations to evaluate active specific immunotherapy with anti-id mAb for several reasons. (a) Mouse anti-id mAb are able to induce immune responses to the corresponding TAA in patients (11, 12). (b) Clinical responses have been observed in some of the patients immunized with the relevant anti-id mAb (12). (c) Injections of mouse anti-id mAb have been found to cause no side effects: in particular, neither allergic nor anaphylactic reactions, despite the induction of high level anti-mouse immunoglobulin antibodies (11, 12), have been observed.

We are interested in exploring the potential of anti-id mAb bearing the internal image of the human HMW-MAA in active specific immunotherapy in patients with melanoma. The HMW-MAA has been selected among the many antigens identified with mouse mAb on melanoma cells (for review, see Refs. 13–15), since it meets most, if not all, of the criteria to be a target for immunotherapy (16). Specifically, the HMW-MAA is expressed in a large percentage of melanoma lesions with limited heterogeneity, has a high density on melanoma cells, and has a restricted distribution in normal tissues. Finally, the potential role of HMW-MAA in melanoma cell growth (17) and attachment to other cells and to cell substrates in tissues (18) suggests that anti-HMW-MAA immunity may affect the biology and metastatic properties of melanoma cells if it does not mediate their immune lysis.

Recently, one of us generated a large number of mouse anti-id mAb against the syngeneic anti-HMW-MAA mAb 225.28 (7). Among them, mAb MF11–30 recognizes an idiotope within the paratope of mAb 225.28, since it inhibits the binding of 125I-labeled mAb 225.28 to Colo38 melanoma cells (7). Anti-id mAb MF11–30 has been shown to induce or enhance humoral anti-HMW-MAA immunity in a low percentage of patients with melanoma when injected without adjuvants (11, 12). To determine whether the poor anti-HMW-MAA immune response in patients reflects the low immunogenicity of anti-id mAb MF11–30 or the inappropriate immunization schedule, in the present study we have investigated the effect of administration with the adjuvant MDP-A on the ability of anti-id mAb IMel-1, a subclone derived from MF11–30, to induce anti-HMW-MAA antibodies in rabbits. The latter express HMW-MAA in normal tissues (19). Therefore, the immune response of rabbits is likely to be predictive of the ability of the immunization with mAb IMel-1 to induce anti-HMW-MAA antibodies in patients with melanoma. Furthermore, we have compared the immunogenicity of mAb IMel-1 with that of HMW-MAA bearing human melanoma cells in rabbits since the resulting information may contribute to our understanding of the reasons why immunization with anti-id mAb MF11–30 induces anti-HMW-MAA immunity in patients with melanoma who have not mounted a detectable immune response to HMW-MAA expressed in their malignant lesions (11, 12). Finally, we have investigated the effect of anti-HMW-MAA antisera induced by mAb IMel-1 on the adhesion of melanoma cells to elements of the extracellular matrix. This information contributes to our understanding of the mechanisms by which humoral anti-HMW-MAA immunity may influence the biology of melanoma.

Received 4/11/91; accepted 9/3/91.

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1 This work was supported by Small Business Innovation Research Grant CA 44246–02; by USPHS Grant CA 37959 awarded by the National Cancer Institute, Department of Health and Human Services; and by Grant IM500 awarded by the American Cancer Society.

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3 The abbreviations used are: anti-id, anti-idiotype; TAA, tumor associated antigen; HMW-MAA, high molecular weight-melanoma associated antigen; mAb, monoclonal antibody; MDP-A, muramyl dipeptide derived adjuvant; PBS, phosphate buffered saline; BSA, bovine serum albumin; Ab3, anti-anti-idiotype antibody.
cells and the clinical course of the disease in patients with melanoma.

MATERIALS AND METHODS

Cells. The human melanoma cell lines Colo38, 397, M21, Meljur, and HSR* MeWo (A16), the human neuroblastoma cell line NMB7, and the human mammary carcinoma cell line MCF-7 were grown in RPMI 1640 supplemented with 10% calf serum, 1% glutamine, and 10 \( \mu \)g/ml gentamicin sulfate. The melanoma cell lines, except MeWo, express HMW-MAA, and NMB7 and MCF-7 cells did not express HMW-MAA and were used as controls. The MeWo cell line was generously provided by Dr. J. Roder of Toronto University.

mAb and Conventional Antisera. The anti-HMW-MAA mAb 225.28 (\( \gamma_2a,k \)) was prepared as described (20). The myeloma proteins, MOPC21 (\( \gamma_1k \)) and UPCI0 (\( \gamma_2a,k \)) (Bionetics, Charleston, SC), were used as isotype-matched controls. The anti-id mAb MF11-30 (\( \gamma_1k \)) to an idiotope within the antigen combining site of the immunizing mAb 225.28 was developed as described (7). The mAb IMel-l (\( \gamma_1k \)) is secreted by a subclone isolated from the hybridoma MF11-30 by limiting dilution at 1 cell per 10 well plates. IgG1 mAb were purified by chromatography on protein A-Sepharose and QAE-Sepharose columns. IgG2a mAb were purified by chromatography on a protein A-Sepharose 4B column. F(\( ab' \))2 fragments were prepared from IgG mAb by pepsin digestion as described (21). The purity of mAb and F(\( ab' \))2 fragments preparations was tested by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (22). mAb were labeled with \( ^{125}I \) utilizing the chloramine T method (23). Fluorescein isothiocyanate conjugated goat anti-rabbit IgG antibodies were purchased from Becton-Dickinson, Mountain View, CA.

Glycosaminoglycans. Chondroitin sulfate (mixed isomers) and bovine kidney heparan sulfate were purchased from Sigma Chemical Co., St. Louis, MO. Purity of matrix components was tested by cellulose acetate electrophoresis or sodium dodecyl sulfate-polyacrylamide gel electrophoresis as appropriate.

Immunization of Rabbits. Adult New Zealand White rabbits (3 to 5 in each group) were immunized with 4 injections at multiple s.c. sites at 2-week intervals of 500 \( \mu \)g of mAb IMel-1 with or without MDP-A (24). Control rabbits were immunized with 500 \( \mu \)g of MOPC21 with or without MDP-A, utilizing the same immunization schedule. Additional groups of rabbits were immunized with 4 i.p. injections of 1 x 106 of Meljur or NMB7 cells at 2-week intervals. Rabbits were bled 7 and 14 days after each immunization.

Seralological Assays. Indirect immunofluorescence was performed by incubating target cells (5 x 106 in 100 \( \mu \)l of PBS supplemented with 0.2% BSA and 0.02% NaN3 (PBS-BSA-NaNa) with dilutions of antisera or with different amounts of purified antibodies for 1 h at 4°C. Then cells were washed 6 times and incubated with 100 \( \mu \)l of a 1:200 dilution of fluorescein isothiocyanate-labeled anti-IgG xenogenic antibodies. Thereafter, cells were washed and analyzed on a FACScan (Becton Dickinson). The inhibition assay to map determinants recognized by different antibodies was performed by adding \( ^{125}I \)-labeled mAb 225.28 mixed with 25 \( \mu \)l of different dilutions of cold antibodies to Colo38 melanoma cells fixed on microtiter plates. At the end of a 16-h incubation at 4°C, plates were washed 10 times with PBS, pH 7.4, and bound radioactivity was quantitated in a \( ^{125}I \)-counter.

Analysis of the Anti-anti-id Antibody Response in Rabbits

RESULTS

Analysis of the Anti-anti-id Antibody Response in Rabbits

Immunized with Anti-id mAb IMel-1. Rabbits, which express HMW-MAA in normal tissues (19), were immunized to determine whether anti-id mAb IMel-1 can induce anti-HMW-MAA antibodies and whether an adjuvant was required for the induction of such an immune response. Immunization with the isotype-matched antibody, MOPC21, with and without MDP-A served as a control. An additional group was immunized with cultured human Meljur melanoma cells. Due to the lack of purified HMW-MAA, we used whole cells to immunize rabbits for the induction of anti-HMW-MAA responses. For control study, rabbits were also immunized with cultured NMB7 neuroblastoma cells. Sera obtained from all the immunized rabbits one week after the fourth immunization were tested for total anti-anti-id response by measuring their ability to inhibit the
binding of $^{125}$I-labeled mAb 225.28 to mAb IMel-1. Furthermore, sera from rabbits immunized with anti-id mAb IMel-1 were tested for reactivity with melanoma cells in indirect immunofluorescence. To avoid the interference of antibodies to unrelated structures with the detection of anti-HMW-MAA antibodies in sera from rabbits immunized with melanoma cells, immune sera were tested for their ability to inhibit the binding of $^{125}$I-labeled mAb 225.28 to Colo38 melanoma cells. Sera from rabbits immunized with mAb IMel-1 with and without the adjuvant MDP-A inhibited the binding of $^{125}$I-labeled mAb 225.28 to mAb IMel-1 (Fig. 1A). Sera from rabbits immunized with mAb IMel-1 in MDP-A, however, had higher anti-anti-id antibody titers than sera from rabbits immunized with mAb IMel-1 alone. A 1:650 dilution of sera from rabbits immunized with mAb IMel-1 in MDP-A inhibited by 50% the binding of mAb 225.28 to mAb IMel-1, whereas at the same dilution sera from rabbits immunized with mAb IMel-1 alone inhibited the reaction by only 10–12%. By comparing with a standard curve (Fig. 1B) of unlabeled affinity purified Ab3, the mean serum concentration of anti-anti-id antibodies was found to be 227.5 and 87.5 μg/ml in sera obtained after 4 immunizations from rabbits immunized with mAb IMel-1 with or without MDP-A, respectively. Antibodies reacting with melanoma cells in sera from rabbits immunized with mAb IMel-1 were measured by staining of HMW-MAA bearing Colo38 cells incubated with various dilutions of rabbit sera. Representative staining patterns of Colo38 cells incubated with 1:500 dilution of sera from rabbits immunized with mAb IMel-1 with or without MDP-A are shown in Fig. 2. The titer of sera from rabbits immunized with mAb IMel-1 with MDP-A is markedly higher than that of sera from rabbits immunized with mAb IMel-1 without adjuvant. A titer is defined as the final dilution of sera that gave a minimum of a 2-fold higher binding with Colo38 cells than with NMB7 cells. Rabbits immunized with mAb IMel-1 without MDP-A had a titer of 1:50 (data not shown), whereas sera from rabbits immunized with mAb IMel-1 in MDP-A had a titer of 1:1000 (data not shown). Furthermore, the intensity of staining of Colo38 cells incubated with a 1:50 dilution of sera obtained from rabbits immunized with mAb IMel-1 without MDP-A was lower than that of cells incubated with a 1:50 dilution of sera from rabbits immunized with mAb IMel-1 with MDP-A (data not shown). At a 1:50 dilution of sera from rabbits immunized with mAb IMel-1 without MDP-A, the percentages of Colo38 cells stained were 77, 22, and 35% using sera from 3 immunized rabbits. At the same dilution, the same sera stained 31, 21, and 1% of NMB7 cells. The percentage of cells

Fig. 1. Effect of adjuvant MDP-A on the induction of anti-anti-id antibodies in rabbits by anti-id mAb IMel-1. Sera from rabbits immunized with anti-id IMel-1 with (•) and without (O) MDP-A (A) and anti-anti-id antibodies (Δ) purified from sera from rabbits immunized with anti-id mAb IMel-1 in MDP-A (B) were tested for inhibition of the binding of $^{125}$I-labeled mAb 225.28 to F(ab')2 fragments of mAb IMel-1. Results are expressed as a percentage of inhibition as compared with assays performed in the presence of preimmune sera. Sera from rabbits immunized with myeloma protein MOPC21 (D) in MDP-A (A) and antibodies purified from sera from rabbits immunized with myeloma protein MOPC21 in MDP-A (Δ) (B) were used as specificity controls.

Fig. 2. IIF staining of Colo38 melanoma cells and NMB7 neuroblastoma cells with sera from rabbits immunized with anti-id mAb IMel-1 in MDP-A, IMel-1 without MDP-A, and MOPC21 in MDP-A. Colo38 (A, B, and C) and NMB7 (D, E, and F) cells were incubated for 1 h at 4°C with 100 μl of a 1:500 dilution of serum from a rabbit immunized with anti-id mAb IMel-1 in MDP-A (A and D), IMel-1 without MDP-A (B and E), and MOPC21 in MDP-A (C and F). Cells were then washed 6 times with 200 μl of PBS-BSA-NaNO3 buffer and incubated for 30 min at 4°C with 100 μl of a 1:200 dilution of fluorescein isothiocyanate (FITC)-goat anti-rabbit IgG antibodies. Cells were then washed 5 times with 200 μl of buffer and analyzed by a cytofluorograph.
cells, and MCF-7 mammary carcinoma cells (Fig. 3). (c) Absorption of purified anti-anti-id antibodies with NMB7 neuroblastoma cells, whereas absorption with Colo38 melanoma cells did not affect the binding of $^{125}$I-labeled mAb 225.28. These results suggest that anti-anti-id antibodies reacting with the determinant defined by mAb 225.28 are present in sera from rabbits immunized with mAb IMel-1, although in a low concentration. To corroborate this possibility, the amount of anti-anti-id recognizing the determinant defined by mAb 225.28 was quantitated; by comparing with an inhibition curve (Fig. 5B) obtained with known concentrations of mAb 225.28, it was estimated to be 0.6 $\mu$g/ml: approximately 0.26% of the anti-anti-id antibody population. On the other hand, the percentage of antibody molecules reacting with Colo38 cells was approximately 4% of the total anti-anti-id population (Table 1). The latter value was determined by measuring the percentage of $^{125}$I-labeled anti-anti-id antibodies bound by an excess of Colo38 melanoma cells after subtraction of background binding determined with $^{125}$I-labeled antibodies purified from sera from rabbits immunized with myeloma protein MOPC21.

Sera from rabbits immunized with cultured Meljur melanoma cells did not inhibit the binding of $^{125}$I-labeled mAb 225.28 to mAb IMel-1 and to Colo38 melanoma cells (data not shown). Unless the lack of inhibition reflects a markedly lower affinity of rabbit antibodies than of mAb 225.28, these results indicate that immunization of rabbits with cultured Meljur cells did not induce antibodies that mimic mAb 225.28 in terms of expression of the idiotope recognized by mAb IMel-1 and of specificity for HMW-MAA.

Effect of mAb 225.28 and Anti-anti-id Antibodies on the Interaction of Colo38 and NMB7 Cells with Complex Collagen I Gels. The ability of Colo38 and NMB7 cells to invade collagen I gels was enhanced by the addition of heparin sulfate (Table 2). Addition to the gel of chondroitin sulfate inhibited invasion by Colo38 cells, but had no effect on invasion by NMB7 cells (Table 2).

Therefore, a complex gel composed of collagen I and 10% chondroitin sulfate was selected as the most appropriate test matrix to assay the inhibitory effects of mAb 225.28 and of anti-anti-id antibodies. Cross-inhibition experiments were then performed to analyze the spatial relationship between determinants recognized by anti-anti-id antibodies and mAb 225.28. Coating of Colo38 melanoma cells with sera from rabbits immunized with mAb IMel-1 did not affect the binding of $^{125}$I-labeled mAb 225.28, whereas coating with a high concentration of purified anti-anti-id antibodies inhibited it (Fig. 5). The inhibition is specific, since coating of melanoma cells with antibodies purified from sera from rabbits immunized with myeloma protein MOPC21

![Graph](https://example.com/graph.png)

**Fig. 3.** Indirect immunofluorescence staining of different cells with sera from rabbits immunized with mAb IMel-1 in MDP-A. Different HMW-MAA expressing cells, Colo38 (○), M21 (□), Meljur (△), and nonexpressing cells, NMB7 (■), MCF-7 (▲), and MeWo (●) were incubated for 1 h at 4°C with 1:500 dilution of serum from a rabbit immunized with anti-mAb IMel-1 in MDP-A. Cells were then washed 6 times with 200 $\mu$L of buffer and incubated for 30 min at 4°C with 100 $\mu$L (1:200 dilution) of fluorescein isothiocyanate-goat anti-rabbit IgG antibodies. Cells were then washed 5 times with 200 $\mu$L of buffer and analyzed with a cytofluorograph.

**Fig. 4.** Indirect immunofluorescence staining of Colo38 melanoma cells with anti-anti-id antibodies purified from sera from rabbits immunized with anti-id mAb IMel-1 in MDP-A. Colo38 cells were incubated for 1 h at 4°C with anti-id antibodies purified from serum from a rabbit immunized with anti-id mAb IMel-1 in MDP-A. Cells were then washed 6 times with 200 $\mu$L of buffer and incubated for 30 min at 4°C with 100 $\mu$L (1:200 dilution) of fluorescein isothiocyanate-goat anti-rabbit IgG antibodies. Cells were then washed 5 times with 200 $\mu$L of buffer and analyzed with a cytofluorograph. Anti-anti-id antibodies absorbed with Colo38 melanoma cells or with NMB7 neuroblastoma cells and FITC-goat anti-rabbit IgG antibodies were used as specificity controls.
Fig. 5. Inhibition of the binding to Colo38 melanoma cells of 125I-labeled anti-HMW-MAA mAb 225.28 by anti-anti-id antibodies purified from serum from rabbits immunized with anti-id mAb IMel-1 in MDP-A. 125I-labeled mAb 225.28 (20,000 cpm) was mixed with different amounts of purified anti-anti-id antibodies purified from serum from rabbits immunized with anti-id mAb IMel-1 in MDP-A (△) or of unlabeled mAb 225.28 (○). The mixture was incubated for 16 h at 4°C with Colo38 cells fixed to microtiter plates. Then plates were washed 10 times with PBS. Bound radioactivity was counted in a γ-counter. Anti-MOPC21 antibody (△) obtained from rabbits immunized with anti-id mAb IMel-1 in MDP-A and isotype-matched control UPC10 (●) were used as specificity controls.

**Table 1** Binding to melanoma cells of anti-anti-id antibodies induced with anti-id mAb IMel-1

<table>
<thead>
<tr>
<th>125I-labeled antibodies</th>
<th>Specific activity (cpm/μg)</th>
<th>Added cpm (ng of antibody)</th>
<th>Bound cpm (ng of antibody)</th>
<th>Specifically bound cpm&lt;sup&gt;a&lt;/sup&gt; (% of bound antibody)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-anti-id antibodies&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13 × 10&lt;sup&gt;6&lt;/sup&gt;</td>
<td>10,748 (0.807)</td>
<td>1,021 (0.076)</td>
<td>451 (4.1)</td>
</tr>
<tr>
<td>Anti MOPC21 antibodies&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15 × 10&lt;sup&gt;6&lt;/sup&gt;</td>
<td>19,064 (1.43)</td>
<td>1,688 (0.126)</td>
<td>858 (4.2)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Various amounts of 125I-labeled anti-anti-id antibodies were incubated with 1% paraformaldehyde fixed Colo38 cells (2 × 10<sup>5</sup>) for 1 h at 4°C. Then cells were washed 6 times with PBS BSA-Na<sub>3</sub> and bound radioactivity was measured in a γ-counter.

**Table 2** Effect of matrix heparan sulfate and chondroitin sulfate on invasion of collagen I gels by Colo38 melanoma cells and by NMB7 neuroblastoma cells

<table>
<thead>
<tr>
<th>Secondary extracellular matrix gel component</th>
<th>Cell line</th>
<th>Invasion index&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>Colo38</td>
<td>1.00</td>
</tr>
<tr>
<td>Chondroitin sulfate (mixed isomers)</td>
<td>Colo38</td>
<td>0.50 (P = 0.012)</td>
</tr>
<tr>
<td>Heparan sulfate (bovine kidney)</td>
<td>Colo38</td>
<td>2.08 (P = 0.013)</td>
</tr>
<tr>
<td>None</td>
<td>NMB7</td>
<td>1.00</td>
</tr>
<tr>
<td>Chondroitin sulfate (mixed isomers)</td>
<td>NMB7</td>
<td>1.00</td>
</tr>
<tr>
<td>Heparan sulfate</td>
<td>NMB7</td>
<td>2.08 (P = 0.026)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Statistical analysis was carried out using Student’s t test applied to the log<sub>10</sub> data values; pooled variance of the data shown (log<sub>10</sub> scale) = 0.0132.

**Table 3** Effect of anti-HMW-MAA mAb 225.28 and of anti-anti-id antibodies induced by mAb IMel-1 on invasion of collagen I gels by Colo38 human melanoma cells

<table>
<thead>
<tr>
<th>Secondary extracellular matrix gel component</th>
<th>Test component in medium&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Invasion index&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chondroitin sulfate&lt;sup&gt;a&lt;/sup&gt;</td>
<td>MOPC21</td>
<td>1.0</td>
</tr>
<tr>
<td>Chondroitin sulfate&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Anti-MOPC21 antibodies</td>
<td>0.9</td>
</tr>
<tr>
<td>Chondroitin sulfate&lt;sup&gt;a&lt;/sup&gt;</td>
<td>mAb 225.28</td>
<td>0.72 (P = 0.32)&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Chondroitin sulfate&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Anti-anti-id antibodies</td>
<td>0.44 (P = 0.016)</td>
</tr>
</tbody>
</table>

<sup>a</sup> All test components were added at a final concentration of 30 μg/ml.

DISCUSSION

In the present study, serological evidence indicates that the mouse anti-id mAb IMel-1 developed against the syngeneic anti-HMW-MAA mAb 225.28 induces anti-HMW-MAA antibodies in rabbits. Approximately 4% of the anti-anti-id antibodies induced by mAb IMel-1 is directed against HMW-MAA on the melanoma cells. This result is in agreement with the notion that only a small fraction of the anti-anti-id induced by an anti-id is directed against the nominal antigen (28). However, only a small proportion of the anti-anti-id mimics the specificity of mAb 225.28. Therefore, anti-anti-id induced by mAb IMel-1 reacts with HMW-MAA, but appears to recognize determinant(s) that are spatially distant from that defined by mAb 225.28, provided that their lack of ability to inhibit the binding of mAb 225.28 does not reflect a markedly lower affinity constant. However, the latter possibility is unlikely inasmuch as the binding of 125I-labeled anti-anti-id to Colo38 cells could not be inhibited by cold mAb 225.28.

The mechanism(s) underlying the diversity in the fine specificity of anti-HMW-MAA antibodies induced by mAb IMel-1 is not known. The postulated role of the regulatory id network in the immune response suggests that mAb IMel-1 may induce an anti-HMW-MAA response by perturbing the idotype network (29–32). An alternative, although not mutually exclusive, possibility is that mAb IMel-1 activates primarily clones with a specificity different from that of mAb 225.28, as was found...
in other systems (33, 34). Whatever the mechanism(s), the present study is not the first example of differences in the fine specificity of antigen binding anti-idiotype antibodies and antibodies used to elicit anti-id antibodies. Viale et al. (4) reported that an anti-anti-id mAb recognizes a glycoprotein determinant on a tumor associated antigen of the human mammary carcinoma cell line MCF-7, whereas the original mAb recognizes a glycolipid determinant. Furthermore, 2 anti-anti-id mAb generated in the Schistosoma mansoni system (35) recognize determinant(s) distinct from that defined by the original mAb1. Finally, Shearer et al. (36) reported that anti-id mAb generated against a mAb specific for SV40T-Ag induced antibodies that recognize a determinant(s) of the viral encoded TAA distinct from that defined by the Ab1 used for the production of anti-id mAb.

An interesting result of our studies that is relevant to the rationale underly the use of anti-id antibodies as immunogens for active specific immunotherapy in cancer patients is represented by the differential immunogenicity of mAb IMel-1 and HMW-MAA bearing melanoma cells in rabbits. Since rabbits express HMW-MAA in normal tissues (19), our results indicate that mAb IMel-1 can induce immunity to self HMW-MAA. On the other hand, the results of the present investigation and of a previous report (37) indicate that cell membrane bound HMW-MAA is poorly immunogenic in rabbits.

The differential immunogenicity of cell membrane bound HMW-MAA and of anti-id mAb in rabbits may reflect the ability of anti-id antibodies to stimulate clones that are unresponsive to the nominal antigen, a phenomenon already described in other antigenic systems (34). It can be postulated that anti-id mAb mimic the HMW-MAA in an imperfect way and, therefore, may activate B-cell clones that have not been deleted during the establishment of self tolerance since they react with HMW-MAA with an affinity below the threshold required for deletion. Alternatively, it may be argued that anti-id mAb may induce T-cell help specific for id or Fc moiety of the anti-id molecule, which provide help to HMW-MAA specific B cell clones to induce anti-TAA antibody production. However, cell bound HMW-MAA cannot induce such T-cell help and therefore is not able to help anti-HMW-MAA clones to produce antibodies. Whatever the mechanisms, our results can account for the induction of anti-HMW-MAA immunity by anti-id mAb in patients without a detectable immune response to HMW-MAA expressed in their lesions (11). Furthermore, the present results argue in favor of the use of anti-id mAb as immunogens to elicit anti-TAA immunity in patients with solid tumors, especially since the convenient and easy way to prepare large amounts of well standardized anti-id mAb utilizing the hybridoma methodology contrasts with the practical difficulties to purify large amounts of immunologically functional TAA utilizing conventional biochemical and/or immunochemical techniques at least until the corresponding genes are cloned.

Earlier studies indicated that proteoglycans to which HMW-MAA belongs (38) are involved in a variety of cellular events related to biology of tumor cell growth, e.g., proliferation, migration, and adhesion (17, 18, 39–41). It has been reported that proteoglycans may influence tumor growth by acting as receptors (or ligand) for components of extracellular matrix including fibronectin (42–44), laminin (45, 46), collagen (47, 48), and other proteoglycans (49). The role of HMW-MAA in melanoma cell invasion is suggested by the inhibition of Colo38 cell invasion by antibodies elicited with mAb IMel-1. This effect
Human High Molecular Weight-Melanoma Associated Antigen Mimicry by an Anti-Idiotype Antibody: Characterization of the Immunogenicity and the Immune Response to the Mouse Monoclonal Antibody IMel-1


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