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Properties of Mouse Melanoma Antigen and Its Secretion Mechanism from the Cell Surface

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We analyzed the biochemical properties and biological significance of the melanoma antigen secreted in the culture supernatants of B16 melanoma cells. The 80 kilodalton (kd) molecule bearing the epitopes of mouse melanoma antigen was found to associate noncovalently with an 18 kd moiety in the culture supernatants as well as on the cell surface. Tunicamycin treatment of B16 cells did not affect the expression of the 69 kd nonglycosylated form of the 80 kd molecule but did abolish the association between the two molecules on the cell surface. We could not detect this antigen as a soluble form when the N-linked glycosylation was inhibited. Therefore, the glycosylation of the 80 kd molecule is essential for the formation of the 80 kd/18 kd complex and also for the secretion. Moreover, the affinity-purified melanoma antigen from the supernatants could induce anti-melanoma suppressor cells which block the generation of cytotoxic T lymphocytes against melanoma cells. Thus, the 80 kd glycoprotein as a soluble melanoma antigen performed a pivotal function in the escape mechanisms of melanoma cells from the host immune surveillance system.

Key words: Soluble tumor antigen — B16 melanoma — Secretion of melanoma antigen — Suppressor cell

The immune network acts as a host defense system against malignant cells, generating cytotoxic T lymphocytes (CTL[2]).[1, 2] This immune mechanism is initiated to execute its role by the recognition of a certain cell surface molecule as a tumor antigen. The tumor cells also shed or secrete soluble tumor antigens whose biological functions have not yet been clarified.[3] In addition, tumor cells actively suppress immune responses, resulting in the escape of tumor cells from the immune system.[4]

We have been studying B16 mouse melanoma cells as a model system in order to analyze these mechanisms. The establishment of syngeneic monoclonal anti-melanoma antibodies (M2590, M622 and M562) enabled us to characterize tumor antigens in melanoma cells. These antibodies are found to inhibit syngeneic CTL against B16 melanoma cells in the effector phase,[5] suggesting that the antigens recognized by these antibodies might constitute the target elements of CTL. Subsequent studies have revealed that the M2590 antibody recognizes a conformational change or a certain density of GM3 ganglioside as cross-species melanoma epitopes,[6, 7] and both the M622 and M562 antibodies specifically react to an 80 kd glycoprotein bearing mouse melanoma specific epitopes on the cell surface.[8] Recently we have identified anti-melanoma suppressor T cells induced by the spent culture medium of B16 cells which inhibit the generation of anti-melanoma CTL.[9]

We describe here the biochemical properties of the soluble form of the 80 kd molecule and its biological significance for inducing anti-melanoma suppressor cells for syngeneic CTL. We also identified the 18 kd molecule, which plays a key role in the secretion process of the melanoma antigen.

MATERIALS AND METHODS

Cells and antibodies Mouse melanoma cells (B16: C57BL/6 origin) were maintained in RPMI 1640 medium (GIBCO Laboratories, Grand Island, NY) containing 3% fetal calf serum (FCS)(GIBCO Laboratories) or in FCS-free RPMI 1640 supplemented with 10^{-8} *M* hydrocortisone (Sigma Chemical Co., St. Louis, MO), 10 μg/ml insulin (Sigma Chemical Co.) and 10 μg/ml transferrin (Sigma Chemical Co.). Mouse T lymphoma cells (EL-4: C57BL/6 origin) were maintained in RPMI 1640 supplemented with 3% FCS. The monoclonal anti-melanoma antibodies (M562 and M622, IgM, κ) were established by cell fusion between P3U1 BALB/c myeloma cells and C57BL/6 spleen cells primed with syngeneic B16 melanoma cells as previously described.[10] These antibodies specifically react to B16 mouse melanoma cells but not to other tumors. They do not show any

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[2] Abbreviations: CTL, cytotoxic T lymphocytes; kd, kilodaltons; PBS, phosphate-buffered saline; FCS, fetal calf serum; DSP, dithiobis(succinimidyl propionate); DTSSP, 3,3′-dithiobis-(sulfosuccinimidyl propionate); 2-DE, two-dimensional electrophoresis.

reactivities to normal tissues or melanoma cells of other species.[10] Monoclonal anti-Thy-1 antibody (CMS-1) (IgM, κ) raised in our laboratory was used as a control antibody. Polyclonal rabbit anti-mouse immunoglobulin was also prepared in our laboratory.

Metabolic labeling Subconfluent B16 melanoma cells in tissue culture-grade dishes (Falcon 3003, Becton Dickinson, Mountain View, CA) were labeled with 50 μCi/ml of [^{35}S]cysteine (Amersham International plc, Buckinghamshire, UK) in cysteine-free RPMI 1640 medium (GIBCO Laboratories) supplemented with 10% FCS at 37°C for 16 h. In some experiments they were labeled with 8 μCi/ml of [^{14}C]amino acid mixture (Amersham International plc) in Hanks' balanced salt solution enriched with 10% RPMI 1640 and 10% FCS for 16 h at 37°C. For experiments concerning the inhibition of glycosylation, cells were incubated with 2 μg/ml tunicamycin (Sigma Chemical Co.) for 4 h at 37°C, and then labeled with [^{14}C]amino acid mixture for a further 16 h in the presence of 2 μg/ml tunicamycin. Labeled cells were washed with phosphate-buffered saline (PBS), pH 7.2, and harvested for solubilization.

Cross-linking experiments with monoclonal antibodies Radiolabeled cells were washed with PBS and incubated with 100 μg/ml of antibody (M562, M622 or control anti-Thy-1 antibody) at 4°C for 2 h. They were then washed three times with PBS and resuspended in 1 m*M* $MgCl_2$/0.02% sodium azide/PBS, pH 8.3, at a cell density of 5×10^6/ml. The cross-linking reagent, dithiobis-(succinimidyl propionate) (DSP) (Pierce Chemical, Rockford, IL),[11] dissolved to 50 m*M* in dimethyl sulfoxide, was added at a final concentration of 0.5 m*M* to the cells that had been reacted with antibodies. They were then incubated at 23°C for 1 h with occasional mixing. After incubation, they were washed once with 100 m*M* Tris-HCl, pH 8.0/140 m*M* NaCl and solubilized as described below. Alternatively, radiolabeled cells were immediately subjected to cross-linking by 3,3′-dithiobis-(sulfosuccinimidyl propionate) (DTSSP) (Pierce Chemical)[12] under the above conditions, and then reacted with antibodies.

The culture supernatants were also used for the cross-linking experiments. The [^{35}S]cysteine-labeled conditioned medium was centrifuged at 100,000*g* for 2 h and the supernatant was incubated with antibody-coupled Sepharose 4B (Pharmacia Fine Chemicals, Uppsala, Sweden) at 4°C for 2 h. After incubation the beads were washed once with PBS, then immediately cross-linked by DSP under the conditions described above. The antibody-coupled Sepharose beads were prepared by coupling the antibody to cyanogen bromide-activated Sepharose 4B as described elsewhere.[13]

Solubilization procedures Labeled cells and cross-linked materials were lysed in 20 m*M* Tris-HCl, pH 8.0/140 m*M* NaCl/1 m*M* phenylmethylsulfonyl fluoride/1% (w/v) Triton X-100, and incubated at 4°C for 30 min. The cell lysates were centrifuged at 10,000*g* for 15 min and the supernatants were used for immunoprecipitation.

Immunoprecipitation Cell lysates or culture supernatants were reacted with protein A-Sepharose (Pharmacia Fine Chemicals) at 10% suspension, followed by incubation with rabbit anti-mouse immunoglobulin-bound protein A-Sepharose to block nonspecific binding (preabsorption procedure). At each preabsorption step they were incubated at 4°C for 2 h with constant mixing. The lysates or culture supernatants were then reacted with 100 μg/ml antibodies at 4°C for 2 h, followed by rabbit anti-mouse immunoglobulin-bound protein A-Sepharose at 4°C for 1 h. The beads were then washed extensively with 20 m*M* Tris-HCl, pH 8.0/140 m*M* NaCl/0.2% Triton X-100. Immunoprecipitated materials were analyzed by 5% to 15% gradient SDS-polyacrylamide gel electrophoresis under reducing conditions. The gels were processed for fluorography with Amplify (Amersham International plc). In the case of materials cross-linked with antibodies, they were directly subjected to immunoprecipitation procedures without preabsorption.

Two-dimensional electrophoresis (2-DE) 2-DE gel analysis was performed according to O'Farrell's procedure.[14, 15] [^{35}S]Cysteine-labeled B16 cell lysates were applied to a wheat-grem agglutinin agarose column (Seikagaku Kogyo Co., Tokyo), extensively washed with 20 m*M* Tris-HCl, pH 8.0/140 m*M* NaCl/1 m*M* phenylmethylsulfonyl fluoride/0.5% Triton X-100, and eluted with 0.2 *M* *N*-acetyl-D-glucosamine (Sigma Chemical Co.). The eluates were subsequently applied to the M562 or M622 antibody-coupled Sepharose and eluted with 3 *M* NaSCN/10 m*M* sodium phosphate buffer, pH 7.4. The materials were lyophilized after extensive dialysis against distilled water. They were then treated with 0.5 unit/ml crystallized neuraminidase[16] (from *Arthrobacter ureafaciens*, Nacalai Tesque, Inc., Kyoto) in 50 m*M* sodium acetate buffer, pH 5.2, at 37°C for 12 h and subjected to nonequilibrium pH gradient electrophoresis. The gels were then subjected to SDS-polyacrylamide gel electrophoresis as described above and processed for autoradiography.

Preparation of soluble melanoma antigen with M622 antibody The FCS-free culture supernatants of B16 melanoma cells were concentrated by saturated ammonium sulfate precipitation and centrifuged at 100,000*g* for 2 h followed by dialysis against PBS, pH 7.2. The supernatants were applied to an M622 or a control antibody-coupled Sepharose column, and the column was extensively washed with PBS, pH 7.2. The elution was carried out with 3 *M* NaSCN/10 m*M* sodium phosphate buffer, pH 7.2, and the eluates were dialyzed against PBS,

pH 7.2. The final volume of this preparation was 1/100 of the original culture supernatant.

Induction of suppressor cells and their assay system The methods for the induction of anti-melanoma suppressor cells and for their assay were previously described.[5] In brief, naive C57BL/6 spleen cells (1×10^7/ml) were cultured in 10% FCS-RPMI 1640 containing 5×10^{-5} *M* 2-mercaptoethanol/20 m*M* HEPES/2 m*M* glutamine for 24 h at 37°C in 5% CO_2 in air with purified soluble melanoma antigen. As a control, anti-Thy-1 antibody-bound materials were used. After incubation they were harvested, washed extensively, and used as suppressor cells. For the assay of suppressor activity, they were added to the *in vitro* primary CTL induction system from the beginning of the culture at a suppressor/responder ratio of 1/8. For the anti-melanoma CTL induction, 2×10^6 naive C57BL/6 spleen cells were cultured alone (as a background) or with either mitomycin C-treated B16 melanoma cells (2×10^4) or EL-4 lymphoma (4×10^4) as stimulators in RPMI 1640 with 10% FCS in a 96-well flat-bottomed microplate (Costar 3599, Costar Cooperation, Cambridge, MA) at 37°C in 5% CO_2 in air. After 4 days, ^{51}Cr-labeled target cells (B16 or EL-4) were incubated with the CTL population at a target/responder ratio of 1/40 at 37°C for 12 h, and the radioactivity from each well was counted. The specific percent lysis was expressed as the percentage of specific ^{51}Cr release calculated by the following formula: % specific ^{51}Cr release = cpm (experimental release − background release)/cpm (maximum release − background release) × 100. Background release was determined by incubating ^{51}Cr-labeled target cells with naive spleen cells of C57BL/6 mouse cultured without stimulators. Maximum lysis was obtained by disrupting the target cells with saponin.[17]

RESULTS

Immunoprecipitation from the cell surface We demonstrated in the immunoprecipitation experiments that both M562 and M622 antibodies specifically reacted to the 80 kd molecule in the [^{35}S]cysteine-labeled B16 cell lysates (Fig. 1). The 80 kd molecule migrated as a broad band, suggesting that it is heavily glycosylated. It was also shown that the M622 antibody could more effectively precipitate the 80 kd molecule than the M562 antibody did, probably due to the affinity difference between them. The precise analysis by 2-DE gel confirmed that the epitopes recognized by the M562 and M622 antibodies were present on the same molecule that migrated to a position corresponding to 72 kd after the treatment of affinity-purified 80 kd molecules with neuraminidase (Fig. 2). As shown in Fig. 1, we also detected two additional molecules, specifically reacting with the M622 antibody, which migrated at around 40 kd and 18 kd.

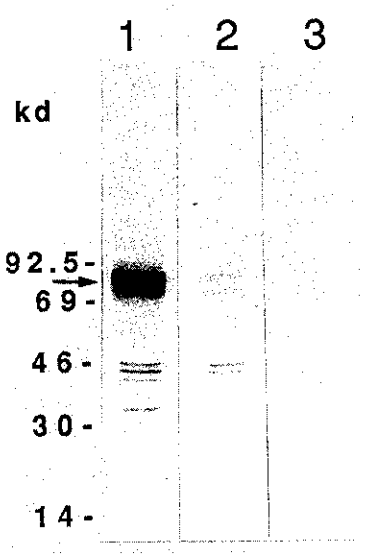

Fig. 1. Gel analysis of melanoma antigen from cell lysates. [^{35}S]Cysteine-labeled B16 cells were immunoprecipitated with M622 (lane 1), M562 (lane 2) and a control antibody (lane 3). They were subjected to 5% to 15% gradient SDS-polyacrylamide gel electrophoresis under reducing conditions. The sizes of molecular weights were determined by the use of standard size markers. The arrow indicates the 80 kd molecule.

In order to investigate further the relationship between these molecules, we used cross-linking reagents, DSP and DTSSP, for immunoprecipitation. B16 melanoma cells were reacted with anti-melanoma antibody and cross-linked by DSP to fix the interaction among nearby molecules. The lysates by Triton X-100 were then immunoprecipitated with a second antibody, rabbit anti-mouse immunoglobulin-bound protein A-Sepharose. As shown in Fig. 3A, the 80 kd and 18 kd molecules were clearly precipitated with both M562 and M622 antibodies, but the control antibody, anti-Thy-1 antibody, precipitated neither of the molecules.

The results of the experiment using another cross-linker, DTSSP, which can not permeate through the cell membrane in contrast to DSP,[18] are shown in Fig. 3B (in this case the cross-linking was performed on the cell surface before cell lysis and immunoprecipitation). The results we obtained were essentially the same as those in the experiment performed with DSP: both the 80 kd and 18 kd molecules were immunoprecipitated with both the M622 and M562 antibodies. These findings clearly show that the 18 kd molecule is physically but not covalently associated with the 80 kd molecule on the B16 melanoma cell surface. The DSP-treated immunoprecipitation experiment with [^{3}H]-glucosamine-labeled cell lysates showed that only the 80 kd molecule but not the 18 kd

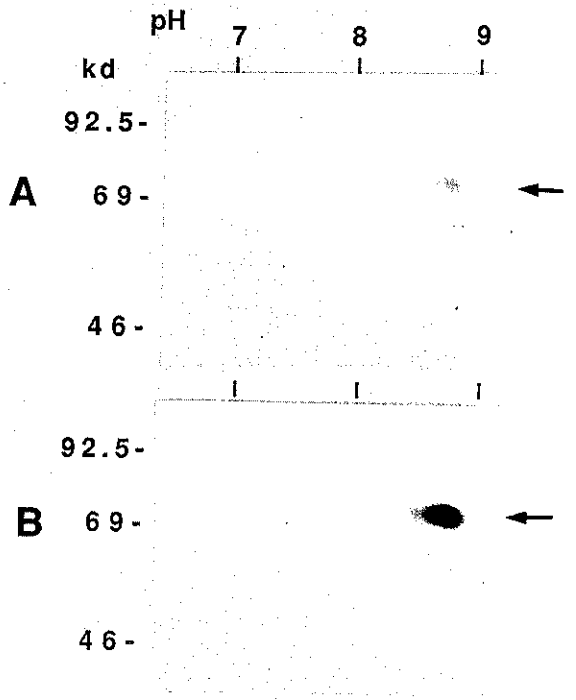

Fig. 2. 2-DE gel analysis of melanoma antigen purified with the M562 and M622 antibodies. [^{35}S]Cysteine-labeled materials purified with M562 (A) and M622 (B) were analyzed on 2-DE gel after treatment with neuraminidase. Note that a single spot (arrow) migrated to the same position (72 kd, pH 8.8) in both (A) and (B).

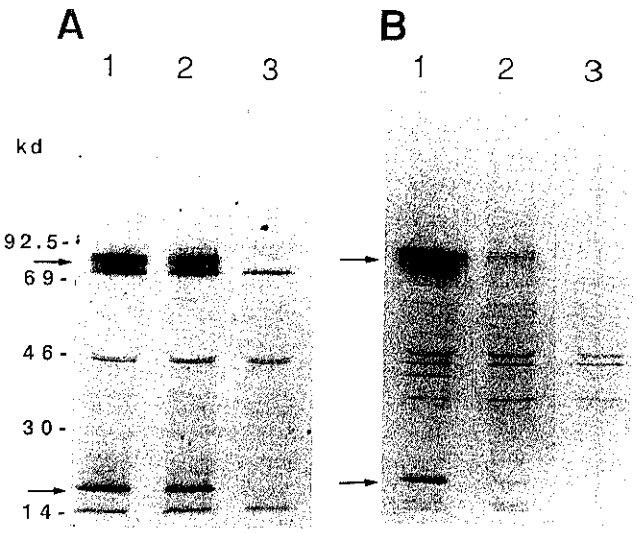

Fig. 3. Gel analysis of melanoma antigen from cell lysates with cross-linking reagent. [^{35}S]Cysteine-labeled B16 cells were immunoprecipitated with M622 (lane 1), M562 (lane 2) and a control antibody (lane 3) through cross-linking by DSP (A) or DTSSP (B) as described in "Materials and Methods." See the legend to Fig. 1. The arrows indicate the 80 kd and 18 kd molecules.

molecule was detected, suggesting that the 18 kd molecule is unglycosylated (data not shown).

Concerning the amount of 80 kd protein precipitated, however, the M622 antibody worked better than M562 in the DTSSP-treated case. In the DSP-treated case, very similar amounts of 80 kd were precipitated by both antibodies. This would be due to the difference of cross-linking procedures: cells were reacted to the antibody before the DSP treatment, but the cross-linking by DTSSP was done prior to the antibody reaction. Therefore, the fixation of the interaction between the antibody and the melanoma antigen can not be expected with the DTSSP treatment.

The M622 antibody also detected the 40 kd molecule together with the 80 kd/18 kd complex. However, the 40 kd molecule was not precipitated by the M562 antibody even after the treatment with cross-linkers. A film exposed for a long time did not show any 40 kd band even though the 80 kd band precipitated with M562 became of equal intensity to the 80 kd band reacted with M622 (Fig. 1). Therefore, it is possible that the 40 kd molecule possesses a cross reactive epitope with the M622 but not the M562 antibody.

Immunoprecipitation from culture supernatants The results in Fig. 4A show that the 80 kd molecule was precipitated from B16 culture supernatants as well as cell lysates, demonstrating that this molecule is present in a soluble form. In the soluble form the 80 kd molecule also seemed to be noncovalently associated with the 18 kd molecule, because the latter was apparently detectable when it was cross-linked (Fig. 4B). The cross-linking process under the experimental conditions used resulted in relatively large amounts of nonspecific binding to the antibody-Sepharose complex (Fig. 4B).

Effect of tunicamycin on the expression of the 80 kd molecule When the N-linked glycosylation was blocked by tunicamycin, the 80 kd molecule migrated at 69 kd and appeared as a distinct band (Fig. 5). The 18 kd protein was not precipitated with the M622 antibody even though cross-linking was performed by DSP. The immunoprecipitation on culture supernatants from tunicamycin-treated B16 cells detected neither the 80 kd nor the 18 kd molecule (Fig. 4A). These results indicate that the tunicamycin treatment interferes with the association between the 18 kd and 80 kd molecules on the cell surface and inhibits the secretion of melanoma antigen from the cell surface.

Suppressor cells induced by affinity-purified soluble melanoma antigen Our previous studies have demonstrated

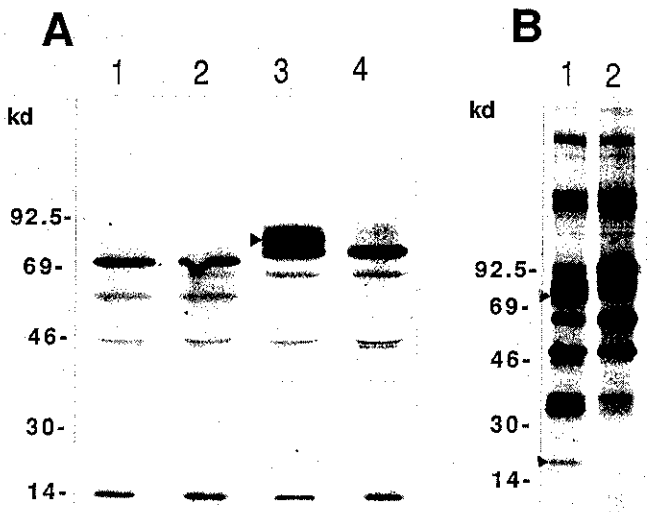

Fig. 4. Gel analysis of melanoma antigen from B16 culture supernatants. (A) The culture supernatants from [^{35}S]cysteine-labeled B16 cells with (lanes 1 and 2) or without (lanes 3 and 4) tunicamycin treatment were immunoprecipitated with M622 (lanes 1 and 3) or a control antibody (lanes 2 and 4). (B) [^{35}S]Cysteine-labeled B16 culture supernatants were reacted with M622 (lane 1) or a control antibody (lane 2) and then cross-linked by DSP. See the legend to Fig. 1. The triangles indicate the 80 kd and 18 kd molecules.

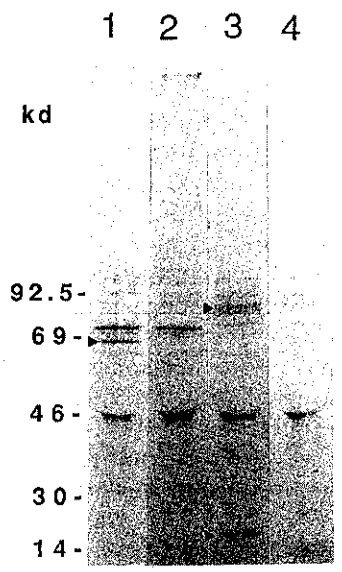

Fig. 5. Gel analysis of melanoma antigen from cells treated with tunicamycin. ^{14}C-Labeled B16 cells treated with (lanes 1 and 2) or without (lanes 3 and 4) tunicamycin. The labeled materials were reacted with M622 (lanes 1 and 3) or a control antibody (lanes 2 and 4) and then cross-linked by DSP. See the legend to Fig. 1. The triangles indicate the 80 kd, 69 kd and 18 kd molecules.

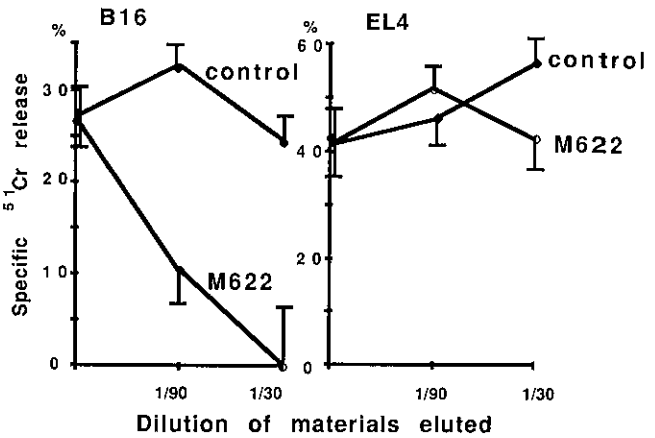

Fig. 6. Induction of anti-melanoma suppressor cells by affinity-purified soluble antigen. Various amounts of M622 and control antibody-purified materials were examined for the ability to induce suppressor cells. The suppressor activity was assayed in terms of specific ^{51}Cr release in anti-B16 and anti-EL-4 CTL induction systems. The data and bars indicate arithmetic means of the percent specific lysis for three cultures ± standard deviation.

that a concentrated B16 spent culture medium induces suppressor T cells which inhibit the generation of anti-melanoma cytotoxic T lymphocytes in the *in vitro* primary culture system.[9] Since the 80 kd molecule is secreted from B16 cells as a soluble form of melanoma antigen, we examined the possibility that this molecule could induce suppressor cells for CTL against melanoma cells. The 80 kd glycoproteins were affinity-purified by using the M622 antibody from culture supernatants of B16 cells, which had been maintained in FCS-free medium to avoid serum contamination. The results in Fig. 6 demonstrated that the M622-purified 80 kd molecule clearly induced anti-melanoma suppressor cells, inhibiting the generation of syngeneic anti-melanoma CTL in a dose-dependent manner. This suppression was specific as to the following points: 1) the materials bound to control antibody (anti-Thy-1 antibody)-coupled Sepharose could not induce suppressor cells, 2) suppressor cells induced by the 80 kd glycoprotein purified by the M622 antibody could not inhibit the generation of syngeneic CTL against EL-4. Thus, the 80 kd glycoprotein secreted from B16 melanoma is very influential in the generation of syngeneic anti-melanoma suppressor cells for syngeneic anti-melanoma CTL.

DISCUSSION

We have described here the biochemical properties of melanoma antigen, the 80 kd molecule, present on the

cell surface and in culture supernatants. The molecule bearing the mouse melanoma-specific epitopes was no longer detected in culture supernatants when B16 cells were treated with tunicamycin, a potent inhibitor of N-linked glycosylation (Fig. 4A), whereas the 69 kd molecule (the 80 kd antigen lacking N-linked glycosylation) was still expressed on the cell surface and retained the epitopes (Fig. 5). This finding indicates that the sugar moiety is the essential element for causing the malanoma antigen to be secreted in a soluble form. It is not clear, however, if the melanoma antigenic epitope is situated in the protein portion or O-linked sugar moiety.

We also showed that the 18 kd molecule was noncovalently associated with the 80 kd antigen. This 18 kd molecule was slightly precipitated from B16 cell lysates (Fig. 1), but was detected clearly when the cross-linkers were used to fix the physical linkage between the two molecules (Fig. 3). We also detected this 80 kd/18 kd complex in the immunoprecipitates from B16 culture supernatants (Fig. 4B). To analyze the topological relationship of the two molecules, we used the membrane-impermeable cross-linker DTSSP, because we could not exclude the possibility that the 18 kd molecule was linked to the 80 kd antigen intracytoplasmically in the experiment using the membrane-permeable reagent DSP. The results in Fig. 3B clearly demonstrate that the 18 kd molecule we detected is in fact expressed on the cell surface. The above data indicate that the 18 kd molecule is noncovalently associated with the 80 kd glycoprotein on the cell surface.

The association of the 18 kd protein with the 80 kd melanoma antigen is influenced by the glycosylation of the 80 kd molecule. The experiments involving treatment of B16 cells with tunicamycin (Fig. 5) supported this notion that the M622 antibody detected the 69 kd molecule of the nonglycosylated form of the 80 kd melanoma antigen but not the 18 kd molecule even after the cross-linking treatment. This shows that the N-linked glycosylation is indispensable for the association of the two molecules on the cell surface.

The secretion of melanoma antigen is observed when the N-linked glycosylation of the 80 kd molecule is intact. Especially, the physical linkage of the 80 kd antigen to the 18 kd molecule is believed to be essential for the secretory process, although we can not formally exclude the possibility that the loss of N-linked glycosylation of 80 kd itself is responsible for the secretion defect. Since the 18 kd protein seems to be unglycosylated, tunicamycin treatment affects only the 80 kd molecule. Therefore, it is the nonglycosylated 69 kd molecule of 80 kd origin that has lost the binding site. Thus, the conformational change of the 80 kd melanoma antigen, due to the loss of glycosylation, is crucial for the organization of the 80 kd/18 kd complex as a secretory form.

We have not precisely analyzed the 40 kd molecule which was specifically precipitated from cell lysates with the M622 antibody. It might be a degradation product of the 80 kd molecule, or an intermediate form during the biosynthesis. However, it seems more likely that the 40 kd is an entirely unrelated molecule with a cross-reactive epitope recognized by the M622 but not the M562 antibody.

Our previous studies have demonstrated that the culture supernatants of B16 melanoma cells are able to induce suppressor T cells. These suppressor T cells act on the precursor cells of anti-melanoma CTL and inhibit the generation of mature CTL.[9] In fact, we could not observe any biological activities of the supernatants to induce the anti-melanoma CTL. Moreover, the supernatants have been shown to inhibit specifically anti-melanoma CTL activity in the effector phase of the CTL response.[5] These results strongly suggest that the soluble form of the melanoma antigen in culture supernatants rather than the antigen on the cell membrane possesses the potent activity to stimulate negative immune responses, resulting in the escape of tumor cells from the host immune surveillance system.[1,9] The data in Fig. 6 clearly demonstrate that the affinity-purified soluble form of the 80 kd melanoma antigen indeed induced anti-melanoma suppressor cells for melanoma-specific CTL. Therefore, the 80 kd molecule itself seems to be responsible for the tolerance of anti-melanoma immune responses.

Our recent data have suggested that the melanoma antigen defined by blocking experiments of CTL activity with the M562 and M2590 antibodies is composed of GM3 in association with protein molecules.[5] Since GM3 itself is one of the epitopes for anti-melanoma suppressor T cells,[5] it is important to determine whether GM3 ganglioside is associated with the 80 kd in our preparation. According to preliminary examinations, GM3 was undetectable in this purified material by enzyme-linked immunosorbent assay, although quantitative data have not yet been obtained. It is, therefore, possible that the 80 kd glycoprotein or some components other than GM3 ganglioside associated with the 80 kd molecule are responsible for the induction of suppressor cells observed in this experiment. Since we can not formally rule out the possibility of the presence of small amounts of GM3 in our preparation, the precise determination of the quantity of GM3 and that of the particular epitopes associated with the 80 kd molecular complex for suppressor cell induction should be examined next.

In any event, the establishment of suppressor T cell clones and the molecular cloning of the 80 kd melanoma antigen provide further information for analyzing the functional epitopes of the melanoma antigen involved in tumor escape mechanisms. Further studies are in progress.

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