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Human Uveal Melanoma Cells Produce Macrophage Migration-Inhibitory Factor to Prevent Lysis by NK Cells¹

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Human uveal melanoma arises in an immune privileged ocular environment in which both adaptive and innate immune effector mechanisms are suppressed. Uveal melanoma is the most common intraocular tumor in adults and is derived from tissues in the eye that produce macrophage migration-inhibitory factor (MIF), a cytokine that has recently been demonstrated to produce immediate inhibition of NK cell-mediated lytic activity. Although NK cell-mediated lysis of uveal melanomas is inhibited in the eye, melanoma cells that disseminate from the eye are at risk for surveillance by NK cells. Moreover, uveal melanoma cells demonstrate a propensity to metastasize to the liver, an organ with one of the highest levels of NK activity in the body. Therefore, we speculated that uveal melanomas produced MIF as a means of escaping NK cell-mediated lysis. Accordingly, seven primary uveal melanoma cell lines and two cell lines derived from uveal melanoma metastases were examined for their production of MIF. MIF was detected in melanoma culture supernatants by both ELISA and the classical bioassay of macrophage migration inhibition. Melanoma-derived MIF inhibited NK cell-mediated lysis of YAC-1 and uveal melanoma cells. Cell lines derived from uveal melanoma metastases produced approximately twice as much biologically active MIF as cultures from primary uveal melanomas. Inhibition of NK cell-mediated killing by uveal melanoma-derived MIF was specifically inhibited in a dose-dependent manner by anti-MIF Ab. The results suggest that human uveal melanoma cells maintain a microenvironment of immune privilege by secreting active MIF that protects against NK cell-mediated killing. *The Journal of Immunology*, 2000, 165: 710–715.

Uveal melanoma is the most common and malignant intraocular tumor in adults (1). Uveal melanomas arise in the iris, ciliary body, and choroid of the eye and metastasize most frequently to the liver; >95% of the patients who die from uveal melanoma have liver metastases (1). Uveal melanomas reside in an immunologically privileged environment in which both the adaptive and innate immune systems are suppressed (2, 3). Although it has not been formally shown that the uveal tract enjoys immune privilege, the immune privilege of the juxtaposed subretinal space, vitreous cavity, and anterior chamber has been well established (2–6). Several factors present in aqueous humor contribute to adaptive immune privilege by suppressing the actions of T and B cells and APCs. These factors include α -melanocyte-stimulating hormone, glucocorticoids, and TGF- β (7–11). Fas ligand is expressed throughout the eye and is responsible for the induction of apoptosis in lymphocytes bearing its receptor, Fas (12). Recognition of intraocular target cells by T cells is impaired by low expression levels of MHC class I and II on ocular tissues (13). When Ag recognition does take place in the anterior chamber, a deviant immune response ensues, which down-regulates delayed-type hypersensitivity and preserves the integrity of delicate, nonregenerating ocular tissues (2, 3).

The innate response is also suppressed in the eye. We have recently demonstrated that the pleiotropic cytokine, macrophage migration-inhibitory factor (MIF),³ is present in the aqueous humor and produces an immediate inhibition of NK cell-mediated cytotoxicity in vitro (14, 15). The presence of MIF in the aqueous humor appears to protect intraocular tumors against NK cell-mediated surveillance, given that tumors that undergo NK cell-mediated rejection at extraocular sites grow progressively in the eye (16). Considerable evidence indicates that NK cells are important in controlling metastases of intraocular melanomas. In vivo depletion of NK cells with anti-asialo-G_{M1} Ab results in a sharp increase in metastases in mice bearing intraocular melanomas (17). Moreover, human uveal melanoma cells displaying high levels of MHC class I molecules are resistant to NK cell-mediated lysis in vitro and produce more extensive metastases than melanoma cells expressing low MHC class I (17).

MIF is produced by numerous cells in the eye including the lens, iris, ciliary body, retinal pigment epithelial cells, Müller cells, and corneal endothelial cells (18–21). Moreover, uveal melanomas arise from ocular tissues that produce MIF (19) and are often directly exposed to the aqueous humor, which contains biologically relevant concentrations of MIF (14, 15). Within the eye, one would expect uveal melanomas to be protected from NK cell-mediated immune surveillance. However, once uveal melanomas metastasize to the liver, an organ with exceptionally high levels of inherent NK activity (22, 23), the melanomas would be vulnerable to NK cell-mediated elimination. Accordingly, we suspected that uveal melanomas might produce MIF as a means of protecting themselves against NK cells following metastasis from the eye.

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³ Abbreviations used in this paper: MIF, macrophage migration-inhibitory factor; LAK, lymphokine-activated NK cell.

Materials and Methods

Animals

C57BL/6 mice were obtained from the mouse colony at the University of Texas Southwestern Medical Center, Dallas, TX. All animals were treated in accordance with the guidelines of the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research.

Tumor cell lines

Uveal melanoma cell lines Mel 202, Mel 270, Mel 290, and OMM 2.3 were generously provided by Dr. Bruce Ksander, Schepens Eye Research Institute, Boston, MA (24). OMM2.3 is derived from a liver metastasis that originated from the primary tumor represented by the Mel 270 cell line. We thank Dr. June Kan-Mitchell, University of California, San Diego, CA, for OCM1, OCM3, and OCM8 uveal melanoma cells and Dr. Gregorius P. Luyten, University Hospital Rotterdam, Rotterdam, The Netherlands, for OMM1 cells which were isolated from a s.c. metastasis originating from a uveal melanoma (25–27). Dr. Martine Jager, Leiden University Hospital, Leiden, The Netherlands, kindly provided 92-1 cells (28). YAC-1 murine lymphoma cells were a gift from Dr. Michael Bennett (University of Texas Southwestern Medical Center). OCM1 and OCM3 cells were maintained in Ham's F-12 medium (BioWhittaker, Walkersville, MD) containing 10% heat-inactivated FBS (HyClone Laboratories, Logan, UT), 2 mM L-glutamine (JRH Biosciences, Lenexa, KS), 10 mM HEPES buffer solution (JRH Biosciences), 1% nonessential amino acids solution (BioWhittaker), 1% penicillin-streptomycin-Fungizone solution (BioWhittaker), and 1 mM sodium pyruvate (JRH Biosciences). Mel 202, Mel 270, Mel 290, OMM 2.3, OCM8, OMM1, 92-1, and YAC-1 cells were maintained in complete RPMI 1640 (JRH Biosciences) containing the same additives as the Ham's F-12 with the addition of 5×10^{-5} M 2-ME (Sigma, St. Louis, MO).

MIF ELISA

Uveal melanoma supernatants were assayed for MIF by sandwich ELISA. Plates were coated overnight with a mouse IgG anti-human MIF capture Ab (R&D Systems, Minneapolis, MN) at $2 \mu\text{g/ml}$ and blocked for 3 h with PBS containing 1% BSA and 5% sucrose. Uveal melanoma supernatants were added for a 2-h incubation, followed by detection with a goat IgG anti-human MIF Ab (R&D Systems) at $0.2 \mu\text{g/ml}$ for 2 h. The detection reaction was completed by addition of streptavidin-HRP (Jackson ImmunoResearch Laboratories, West Grove, PA) followed by 1 mg/ml 2,2-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (Sigma) with 0.003% H_2O_2 . Plates were read at 405 nm, and readings from uveal melanoma supernatants were compared with a standard curve of recombinant MIF readings to give concentrations of MIF.

Inhibition of macrophage migration

Macrophage migration assays were performed as previously described (29). Briefly, spleen cells from C57BL/6 mice were washed, overlaid on Histopaque 1083 (Sigma), and centrifuged for 20 min at 3000 rpm. The buffy layer cells were resuspended to 1×10^8 cells/ml and $40 \mu\text{l}$ of this suspension were drawn into glass capillary tubes capped at one end. Tubes were centrifuged at 1500 rpm for 5 min, cut at the interface of the pellet and supernatant, and placed horizontally in 24-well plates. Wells were filled with $2 \times$ complete RPMI, $2 \times$ uveal melanoma supernatant, or aqueous humor, and macrophages were incubated for 24 h at 37°C , 5% CO_2 to allow macrophage migration from the capillary tube into the well. The migration area was calculated after 24 h.

NK cell cytotoxicity assay

NK cell-mediated cytotoxicity of tumor targets was assessed using a conventional 4-h ^{51}Cr release assay previously described (30). Lymphokine-activated killer (LAK) cells used as effectors were obtained from C57BL/6 mice. LAK cells have been previously used in place of resting NK cells for a variety of in vitro and in vivo experiments because they give higher and more consistent levels of cytotoxicity than resting NK cells. Moreover, LAK cells demonstrate the same in vivo effector function as NK cells (31). Briefly, spleens were collected, erythrocytes were lysed, and splenocytes were washed with HBSS (BioWhittaker) before 4 days of culture at 37°C , 10% CO_2 in complete DMEM medium (JRH Biosciences) containing 10% heat-inactivated FBS (HyClone Laboratories), 2 mM L-glutamine (JRH Biosciences), 1 mM sodium pyruvate (JRH Biosciences), 2 mM MEM vitamins (JRH Biosciences), 1% penicillin-streptomycin-Fungizone solution (BioWhittaker), 5×10^{-5} M 2-ME (Sigma), and 1000 U/ml recombinant human IL-2 (Hoffman-La Roche, Nutley, NJ). This procedure has previously been shown to enrich for NK cells (32). After 4 days of culture,

LAK cells were collected and centrifuged over Histopaque 1083 (Sigma) at 3000 rpm for 20 min. The buffy layer was collected, washed, and used as a source of effector cells in cytotoxicity assays where either YAC-1 cells or human uveal melanoma cells were used as targets.

Inhibition of NK cell-mediated killing

NK-mediated lysis was inhibited by incubating LAK cells with rabbit aqueous humor, or uveal melanoma supernatants for 30 min before combining the LAK effector cells with target cells. Aqueous humor was harvested from the anterior chamber of rabbit eyeballs (Pel-Freez Biologicals, Rogers, AR) by paracentesis under sterile conditions as previously described (14). Uveal melanoma supernatants were obtained from 3-day cultures of 5×10^6 uveal melanoma cells in 10 ml medium.

Neutralization of MIF

To demonstrate that the observed inhibition of NK lysis was mediated by MIF, we pre-treated four uveal melanoma supernatants with a goat anti-human MIF Ab (R&D Systems) or a control goat IgG Ab (Sigma) at a concentration of $67 \mu\text{g/ml}$ for 15 min. After this Ab treatment, supernatants were used to treat LAK cells, which were then used as effectors as described above. Dose dependency of the anti-MIF Ab was shown by treating OMM2.3 supernatant with anti-MIF Ab concentrations ranging from 600 to $7 \mu\text{g/ml}$ for 15 min and then proceeding with LAK treatment and target cell lysis as above.

Statistics

Differences between groups were analyzed by Student's *t* test; *p* values < 0.05 were considered significant.

Results

To demonstrate that human uveal melanoma cells constitutively produced MIF protein, cell supernatants were tested using a sandwich ELISA. Although MIF expression has been documented in both intracellular and secreted forms, secreted MIF has been shown to have biological activities such as inhibition of macrophage migration and mediation of endotoxemia. For this reason and because secreted MIF seemed more likely to have an effect on neighboring NK cells before they recognized and lysed uveal melanoma cells, we chose to focus on the expression of secreted MIF by uveal melanoma cells. A panel of human uveal melanoma cell supernatants harvested from a uniform number of cells was tested for MIF content. As a positive control, recombinant murine MIF was included. To demonstrate that the MIF detected in cell supernatants was not a component of the FCS used in our RPMI growth medium, complete RPMI was included as a negative control and was found not to contain detectable MIF (Fig. 1). As an additional negative control to demonstrate that the MIF Ab was not binding nonspecifically to the plate, we tested blank wells containing no MIF Ag. All cell lines expressed MIF, and levels of MIF expression were highest in cell lines OMM1 and OMM2.3, both of which were isolated from uveal melanoma metastases (Fig. 1). Elevated MIF secretion by both metastatic cell lines supports the hypothesis that a uveal melanoma can create its own microenvironment of immune privilege once it leaves the immune privileged ocular site.

Inhibition of macrophage migration by uveal melanoma supernatants

Our ELISA results confirmed the presence of secreted MIF in that the anti-MIF Ab bound a specific MIF epitope, but an ELISA assay cannot show a protein to be active. To determine whether the MIF detected by ELISA was biologically active, uveal melanoma supernatants were tested in a bioassay. MIF was originally named for its ability to inhibit the migration of macrophages, and the standard bioassay for MIF activity remains the most sensitive one for detecting this cytokine (29). Accordingly, the biological activity of the MIF-containing uveal melanoma supernatants was tested in a macrophage migration inhibition assay using rabbit aqueous humor as a positive control (15) (Fig. 2). Aqueous humor containing

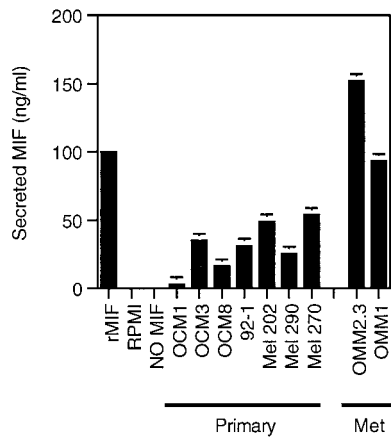


FIGURE 1. MIF secretion by human uveal melanoma cells. Uveal melanoma supernatants were assayed for MIF content by sandwich ELISA. Recombinant MIF was used as a positive control and to establish a standard curve (data not shown). RPMI was used as a negative control, as were empty wells. Uveal melanoma cell lines are divided into two groups, primary and metastatic. Data are displayed as the mean \pm SE.

MIF inhibited macrophage migration by $\sim 70\%$. The negative control used was $2\times$ RPMI, relevant because all melanoma supernatants were concentrated 2-fold before being used in this assay. Uveal melanoma supernatants from Mel 270 and OMM2.3 cell lines significantly inhibited the migration of macrophages out of capillary tubes as compared with the medium control (Fig. 2, A–D). Supernatant from Mel 270 cells caused $\sim 40\%$ inhibition of migration, and OMM2.3 supernatant slowed migration $\sim 50\%$, as shown in Fig. 2E.

Inhibition of NK cell-mediated cytotoxicity by uveal melanoma supernatants

Previously, it has been shown that recombinant MIF and aqueous humor-derived MIF inhibit NK cell-mediated lysis of YAC-1 lymphoma target cells (15). Therefore, we hypothesized that the biologically active MIF found in uveal melanoma supernatants would inhibit NK-mediated cytotoxicity of YAC-1 cells. The ability of MIF-containing cell supernatants to inhibit YAC-1 cell lysis by LAK cells was tested in a 4-h ^{51}Cr release assay. LAK cells were incubated in supernatants before adding YAC-1 target cells to allow MIF in the supernatants to have an effect on the LAK cells, and supernatants continued to be included throughout the 4-h incubation (Fig. 3). Killing of YAC-1 cell targets was $\sim 70\%$ in untreated wells containing RPMI and did not change significantly when $2\times$ RPMI was included, demonstrating that a 2-fold concentration of medium did not impact the ability of LAK cells to kill YAC-1 targets. This negative control was necessary because all uveal melanoma supernatants were concentrated 2-fold before their use in this assay. As a positive control, LAK cells were incubated in aqueous humor, which resulted in a decreased killing level of $\sim 50\%$ (15). All uveal melanoma cell lines tested significantly inhibited killing of YAC-1 cells by LAK cells compared with the $2\times$ RPMI control; levels of killing in the presence of uveal melanoma supernatants ranged from ~ 30 to 70% .

The cell lines in this experiment were then categorized into three groups based on their MIF secretion and NK-inhibitory effects (Fig. 3). The first group included cell lines that secreted high amounts of MIF by ELISA and, as expected, strongly inhibited NK killing. The second group contained cell lines that secreted little MIF and produced only weak inhibition of killing. The third was

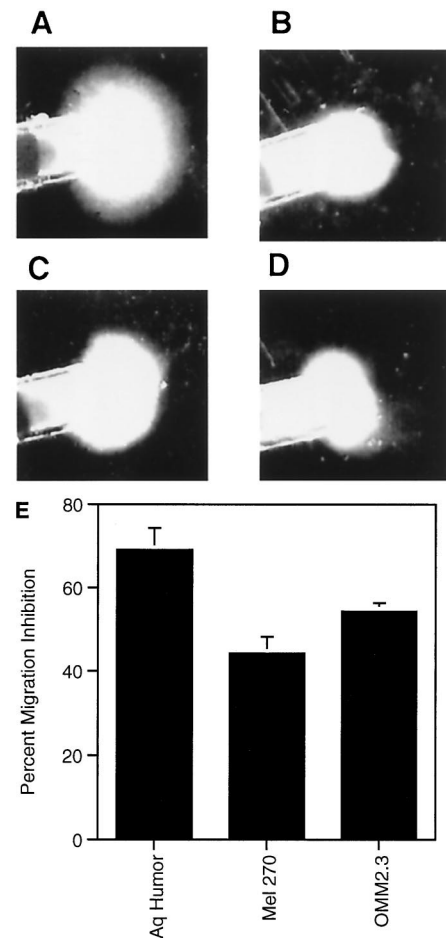


FIGURE 2. Inhibition of macrophage migration by uveal melanoma supernatants. To determine the bioactivity of MIF, macrophages were pelleted in capillary tubes and incubated in concentrated uveal melanoma supernatants to determine the degree of macrophage migration inhibition. MIF-containing aqueous (Aq) humor was included as a positive inhibition control, and RPMI was used as a negative control. Representative wells are shown: A, RPMI; B, aqueous humor; C, Mel 270 supernatant; D, OMM2.3 supernatant. Inhibition of macrophage migration is summarized in E as compared with the RPMI control. Significant migration inhibition, compared with the RPMI control, was observed with AH ($p < 0.0001$), Mel 270 supernatant ($p < 0.0001$), and OMM2.3 supernatant ($p < 0.0001$).

composed of cell lines that secreted low amounts of MIF yet still inhibited NK killing.

Abrogation of NK inhibition by anti-MIF Ab

To determine whether the observed NK inhibition was due to the action of MIF, uveal melanoma supernatants were incubated with anti-MIF Ab before the supernatants were added to LAK cells (Fig. 4A). Supernatants from four uveal melanoma cell lines were treated with either anti-MIF Ab or an isotype control goat IgG Ab. The inhibitory activity of OMM2.3 and OMM1 supernatants, both containing high amounts of MIF, was significantly neutralized by anti-MIF Ab but was unaffected by the control Ab. By contrast, Mel 270 and Mel 290 supernatants, both of which contain little MIF, were not significantly affected by anti-MIF or control Ab.

Anti-MIF Ab neutralized the NK inhibitory effect of melanoma supernatants containing high amounts of MIF, and this effect was dose dependent, as shown by an experiment in which OMM2.3 supernatant was treated with different concentrations of anti-MIF

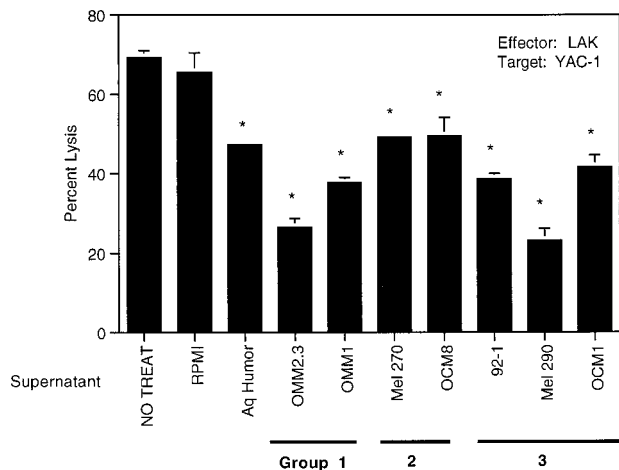


FIGURE 3. NK-inhibitory effect of uveal melanoma supernatants. Lysis of YAC-1 cells by LAK cells was assayed in a ^{51}Cr release assay in which LAK cells were preincubated in uveal melanoma supernatants to determine whether melanoma-derived MIF inhibited killing. Aqueous (Aq) humor was included as a positive control for preincubation of LAK cells, and RPMI was used as a negative control. As an additional control, $2\times$ RPMI was included to show that concentration does not affect killing of targets. Data are shown as mean \pm SE. Asterisks indicate $p < 0.05$ for supernatant vs $2\times$ RPMI control.

Ab (Fig. 4B). Untreated supernatant from OMM2.3 cells was included as a negative control in which MIF in OMM2.3 supernatant protected YAC-1 target cells and very little killing took place. Similarly, OMM2.3 supernatant treated with control Ab showed minimal lysis. A maximal dose of $200\ \mu\text{g/ml}$ anti-MIF Ab neutralized MIF in the OMM2.3 supernatant and produced $\sim 40\%$ YAC-1 killing. As the dose of anti-MIF Ab decreased from 200 to $7\ \mu\text{g/ml}$, the abrogation of NK inhibition lessened accordingly.

MIF-containing supernatant protects uveal melanomas from lysis

As a control experiment to demonstrate that human uveal melanomas are indeed susceptible to NK-mediated cytotoxicity, human uveal melanoma cells in the absence of conditioned melanoma supernatant were exposed to LAK effectors in a 4-h ^{51}Cr release assay. Without the protection of secreted MIF found in conditioned supernatant, all 10 uveal melanoma cell lines tested were susceptible to killing, to varying degrees (Fig. 5). YAC-1 cells were included as highly NK-susceptible positive control targets and were lysed at a rate of $\sim 75\%$ in this experiment. Percent lysis for human uveal melanoma cell lines ranged from 28 to 57%, suggesting that uveal melanoma cells themselves are not inherently resistant to NK killing and might benefit from inhibition of NK killing by MIF.

Having shown that human uveal melanomas are susceptible targets for NK killing, it was important to determine whether human uveal melanomas were protected by their secreted MIF. These experiments examined Mel 270, a primary uveal melanoma that secretes low amounts of MIF, and OMM2.3, a liver metastasis from Mel 270 that produces larger amounts of MIF. Both of these cell lines and their supernatants were used in assays in which NK effectors were pretreated with control medium, Mel 270 supernatant, or OMM2.3 supernatant before combining NK cells with Mel 270 or OMM2.3 tumor targets (Fig. 6). Target cells and supernatants were tested in various combinations to determine whether the level of secreted MIF correlated with the level of NK protection afforded. The results show that Mel 270 and OMM2.3 supernatant

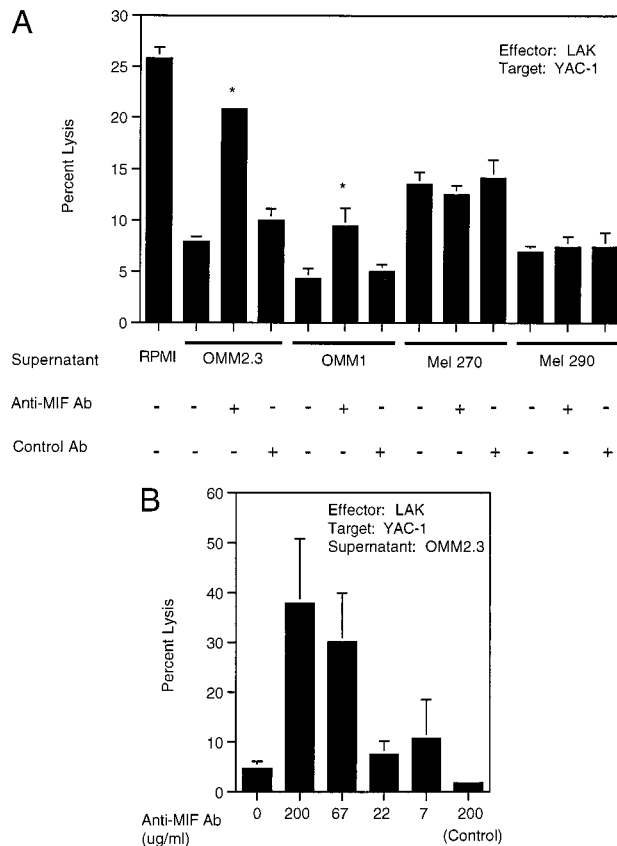


FIGURE 4. Abrogation of NK inhibition by anti-MIF Ab. Uveal melanoma supernatants were pretreated with anti-MIF Ab to neutralize MIF before incubation with LAK cells. An isotype control Ab was included as a negative control. Data are represented as mean \pm SE. Asterisks indicate $p < 0.05$ for anti-MIF treated supernatant vs untreated supernatant. *A*, Four uveal melanoma supernatants were treated with $67\ \mu\text{g/ml}$ anti-MIF Ab or goat IgG control Ab. *B*, Dose dependency of anti-MIF Ab was demonstrated by pre-treating OMM2.3 supernatants with a range of Ab concentrations.

furnished a significant amount of protection against NK killing for both Mel 270 and OMM2.3 target cells. Although OMM2.3 secreted ~ 2 -fold more MIF in ELISA assays, no significant difference was found between levels of NK protection furnished by OMM2.3 and Mel 270 supernatants.

Discussion

We and others have shown that NK cells are important for the elimination of uveal melanomas in vivo (17, 24, 33). Illustrating the role of NK cells in limiting the metastatic process is the observation that when NK cell function in nude mice is depleted by treatment with anti-asialo- G_{M1} Ab, human uveal melanomas injected intraocularly metastasize to the liver with greater incidence and severity (17). In patients, it is widely held that metastasis is accelerated when NK cell-mediated lysis of uveal melanomas is stymied by the expression of high levels of MHC class I on the tumor cells, making them resistant to lysis by NK cells. Human uveal melanomas expressing elevated amounts of class I correlate with a poor patient prognosis (33). Class I expression may be an important factor in determining the viability of a metastasis, as evidenced by an analysis of class I expression on the primary uveal melanoma cell line, Mel 270, and four of its metastatic cell lines. Flow cytometry revealed that the density of class I expression was strongly elevated on metastases, suggesting that during the process

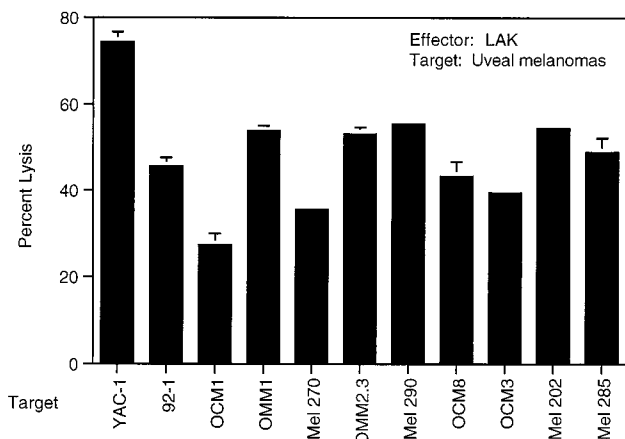


FIGURE 5. Susceptibility of uveal melanoma cells to NK lysis in the absence of supernatant. Ten uveal melanoma cell lines were tested for susceptibility to NK killing in a ^{51}Cr release assay, and all were lysed to varying degrees by the LAK effector cells. Highly NK-susceptible YAC-1 cells were used as a positive control target.

of metastasis NK cells may have selected for tumor cells bearing high class I expression levels (24).

If NK cells are an integral part of antitumor defense, an unexplained issue was how NK-susceptible uveal melanomas manage to evade NK-mediated destruction outside of the NK-quenching ocular environment to form metastases in the liver and in other sites. Lysis by NK cells is down-regulated within the eye, because MIF found in aqueous humor has been shown to inhibit NK killing of YAC-1 murine lymphoma, RMA-S, and uveal melanoma target cells (14–16). The present results show that the NK-inhibiting immune privilege of aqueous humor is perpetuated by uveal melanoma cells, which produce bioactive MIF that protects them from NK cytotoxicity.

MIF is produced in several extraocular tissues including brain, kidney, skin, lung, uterus, and liver (34–38), but it is not known whether it abrogates NK function in those sites. Despite its ex-

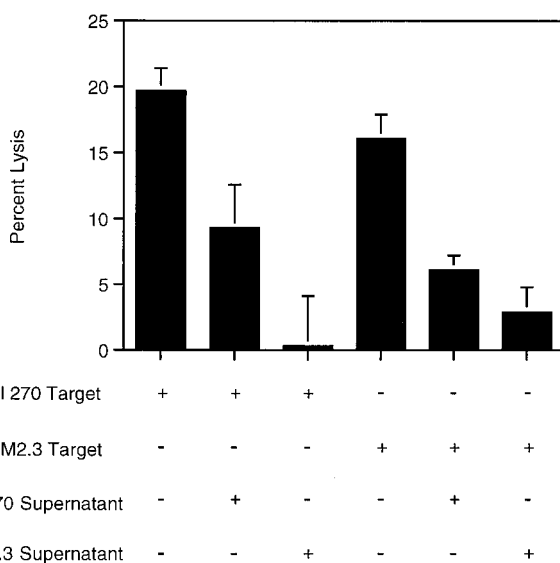


FIGURE 6. Protection of uveal melanoma cells by MIF-containing supernatants. Inhibition of uveal melanoma cell lysis by LAK cells was determined after preincubation in uveal melanoma supernatants. LAK cells were preincubated in Mel 270 or OMM2.3 supernatant before incubation with Mel 270 or OMM2.3 cells. Data are represented as mean \pm SE.

pression of MIF, the liver is known as a site of strong NK activity (22, 23). Uveal melanoma metastasizes preferentially to the liver, where its ability to secrete MIF may protect it from killing by NK cells. We hypothesize that uveal melanoma-produced MIF is involved in creating a microenvironment surrounding the melanoma cells in which MIF is found at a higher concentration than that found throughout the liver. This environment of concentrated MIF may provide immune privilege and protect the tumor cells from NK-mediated lysis.

Two groups of uveal melanoma cell lines provided supportive evidence for our hypothesis that tumor-secreted MIF protected targets from NK killing (Fig. 3). The first group included metastatic cell lines that produced high levels of secreted MIF and strongly inhibited LAK effectors from lysing YAC-1 targets. A second group of primary uveal melanoma cell lines secreted low amounts of MIF and only weakly limited the lysis of YAC-1 cells. A third group of uveal melanoma supernatants inhibited NK killing despite secreting very little MIF. Additionally, although anti-MIF Ab significantly decreased the protective effect of supernatants from the first group containing MIF, it did not inhibit completely (Fig. 4A). These two points suggest that another NK-inhibiting factor may be secreted by uveal melanoma cells in addition to MIF. One candidate factor is TGF- β , which is known to down-regulate NK activity. However, TGF- β is not likely to be at work in these 4 h assays because it requires a much longer, 20-h incubation period to exert NK-inhibiting effects (39). IL-10 and PGE $_2$ may be responsible for the NK inhibition not attributable to MIF, or it may be that a novel NK-inhibiting factor is secreted by uveal melanoma cells (39, 40).

Our results showing that metastases express elevated levels of MIF correlate with the work of Meyer-Siegler and Hudson (41), who showed by differential display PCR that MIF expression increases during the progression of prostate adenocarcinoma. Their work showed elevated MIF expression in focal carcinoma lesions as compared with normal prostate tissue, and a further augmentation of expression in metastatic prostate tissue harvested from lymph nodes. Our work, in addition to showing that MIF protein is secreted at a greater level by uveal melanoma metastases as compared with primary tumors, assigns a function to MIF as a protector of tumor cells from NK elimination. Whether the increased production of MIF by metastases is the result of selective pressure exerted by NK cells or is an intrinsic property of melanocytes as they undergo malignant transformation remains unknown. However, the recent findings of Shimizu et al. (42) are particularly illuminating. They found that normal skin melanocytes express MIF mRNA, however MIF expression was much higher in melanoma cells and correlated with tumor progression. Moreover, MIF expression correlated with increased melanoma cell proliferation, migration, and tumor-induced angiogenesis. Our findings and those of Shimizu et al. (42) suggest that the pleiotropic properties of MIF may contribute to the growth and metastasis of skin and ocular melanomas by affecting multiple parameters, both immunological and nonimmunological.

These data provide evidence for a mechanism by which NK-susceptible uveal melanoma tumors survive outside of the immune-privileged environment of the eye. The results suggest that uveal melanomas create their own NK-inhibiting immune privilege by secreting bioactive MIF, which inhibits NK-mediated cytotoxicity of uveal melanoma target cells. Because uveal melanoma preferentially metastasizes to the liver where NK function is particularly active, secretion of MIF by uveal melanoma cells may provide an immune privilege crucial for metastatic growth.

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