Expression and Possible Function of IL-2 and IL-15 Receptors on Human Uveal Melanoma Cells

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PURPOSE. Interleukin-2 (IL-2) and IL-15 receptors have been detected on some murine neoplasms. Accordingly, the expression of these receptors on human uveal melanoma cell lines was examined, and the effect of exogenous IL-2 and -15 on melanoma cell proliferation, susceptibility to natural killer (NK) cell-mediated cytolysis, and sensitivity to apoptosis were assessed.

METHODS. Nine human uveal melanoma cell lines and three cell lines from uveal melanoma metastases were tested by flow cytometry for the expression of human IL-2R and -15R. Melanoma cells were cultured, with or without recombinant human IL-2 or -15, cell proliferation was determined by tritiated thymidine incorporation, and IL-2 and -15 receptor expression was assessed by flow cytometry. The effect of these cytokines on NK activity was evaluated with a standard 51Cr-release assay.

RESULTS. All the melanoma cell lines expressed IL-2R and -15R. IL-2 induced a three- to eightfold upregulation of IL-2R expression in all the melanoma cell lines. Although IL-2 did not affect the proliferation of six of the seven uveal melanoma cell lines, it induced a 32% and 57% increase in the proliferation of both metastatic cell lines. IL-15 induced proliferation on all tested cell lines (4%–68%). Both IL-2 and -15 reduced melanoma cell sensitivity to NK-cell-mediated cytolysis and cisplatin-induced apoptosis.

CONCLUSIONS. The results suggest that IL-2 and -15 elaborated by tumor-infiltrating lymphocytes and macrophages may affect the malignant behavior of human uveal melanoma by stimulating proliferation and reducing uveal melanoma cell susceptibility to NK-cell-mediated cytolysis and cisplatin-induced apoptosis. (Invest Ophthalmol Vis Sci. 2004;45:4240–4246) DOI:10.1167/iovs.04-0599

Uveal melanoma is the most common primary intraocular malignancy of adults. Approximately 1500 new cases are diagnosed in the United States annually. The 5-year survival rate in uveal melanoma is 75% and is comparable to that of cutaneous melanoma. Unlike cutaneous melanoma, which can metastasize to a wide variety of organs, uveal melanoma demonstrates a strong propensity to spread to the liver, and some studies have indicated that liver metastases are present in >85% of the patients who die of uveal melanoma.

The immunobiology of uveal melanoma is a curious paradox. The tumors arise in an environment in which both the innate and adaptive immune systems are actively suppressed, thereby creating a condition that might handicap immune surveillance. Moreover, immune cells, such as lymphocytes and macrophages that infiltrate uveal melanomas, do not just fail to eliminate the tumors; there is evidence suggesting that their presence exacerbates the malignancy of uveal melanomas. One potential explanation for this paradox is the possibility that tumor-infiltrating lymphocytes (TILs) and tumor-associated macrophages (TAMs) secrete growth factors or cytokines that may (1) stimulate the proliferation of melanoma cells in situ; (2) reduce the melanoma cells’ susceptibility to apoptosis; or (3) protect melanoma cells from immune-mediated killing once they depart from the immune-privileged environment of the eye.

IL-2 is a T-cell–derived cytokine that may be produced by TILs and is a potent growth factor for T lymphocytes bearing its receptor. It is noteworthy that IL-2 receptors (IL-2R) are expressed by some nonhematopoietic tumor cells, including human and murine cutaneous melanoma cells. Moreover, murine B16 melanoma cells exposed to IL-2 demonstrate (1) increased proliferation, (2) decreased susceptibility to natural killer (NK) cell-mediated lysis, and (3) increased formation of liver metastases.

Numerous clinical studies have demonstrated a correlation between TAMs and poor prognosis in various tumors, including uveal melanoma. TAMs elaborate a variety of cytokines, proteases, and growth factors that can affect tumor growth and metastasis. IL-15 is one of the cytokines secreted by macrophages and shares many biological activities with IL-2. IL-15 signals through the β and γ subunits of the IL-2R and protects some tumor cells from Fas-induced apoptosis. Moreover, cutaneous melanomas have been shown to express IL-15 and -15R mRNA, and the production of IL-15 has been correlated with increased malignancy.

The present study examined IL-2 and -15 receptor expression on nine uveal melanoma cell lines and three cell lines derived from uveal melanoma metastases. The effect of exogenous IL-2 and -15 on susceptibility to NK-cell–mediated lysis and cisplatin-induced apoptosis was also evaluated.

METHODS

Uveal Melanoma Cell Lines

Nine primary uveal melanoma cell lines designated OCM1, OCM3, OCM8, MEL202, MEL270, MEL285, MEL290, 92.1, and OM431 and three cell lines isolated from metastatic lesions, designated OMM1, OMM1.5, and OMM2.3, were used. OCM1, OCM3, OCM8, MEL202, MEL270, MEL285, MEL290, 92.1, and OM431 were kindly provided by June Kan-Mitchell (University of California, San Diego, CA). MEL290, OMM1.5, and OMM2.3 were kindly provided by Bruce Ksander (Schepens Eye Research Institute, Boston, MA). The 92.1 line was provided by Martine Jager (Leiden University Hospital, Leiden, The Netherlands). OM431 was graciously given by Daniel Albert (University of Wisconsin, Madison, WI). OMM1 cells were isolated from a skin metastasis arising in a patient with uveal melanoma and was a gift from Gregorium Luyten (University Hospital Rotterdam, Rotterdam, The Netherlands). OCM1, OCM3, and OM431 cells were
cultured in Ham’s F12 medium (BioWhittaker, Walkersville, MD), as previously described.\textsuperscript{25} MEL202, MEL270, MEL290, OMM2.3, OCM8, OMM1, and 92.1 cells were maintained in complete RPMI 1640 (JRH Biosciences, Lenexa, KS).\textsuperscript{25} The establishment of the human uveal melanoma cell lines adhered to the tenets of the Declaration of Helsinki.

**Flow Cytometric Analysis**

Human uveal melanoma cells were tested to determine the effect of IL-2 on the expression of IL-2R, and the effect of IL-15 on the expression of IL-15R. Melanoma cells were trypsinized, washed twice in Hank's balanced salt solution (HBSS; BioWhittaker), and resuspended to $1 \times 10^5$ cells/mL in complete RPMI 1640 medium (RPMI; BioWhittaker), complete RPMI containing 100 U recombinant human IL-2 (rhIL-2; Hoffman-LaRoche, Inc., Nutley, NJ), or RPMI containing 5 ng/mL recombinant human IL-15 (rhIL-15; R&D Systems, Minneapolis, MN). One milliliter of each cell solution was added to 6 wells of a 24-well plate (Costar; Corning, Inc., Corning, NY), and cells were incubated for 24, 48, or 72 hours at 37°C in 5% CO\textsubscript{2}. Cells were processed for flow cytometry, as previously described, with anti-human IL-2R (Genzyme, Cambridge, MA) or 5 μg/mL goat anti-human IL-15R (R&D Systems).\textsuperscript{26} Normal rat IgG (BD PharMingen, Palo Alto, CA) served as an isotype control. Specific fluorescence was analyzed by flow cytometry (FACSCalibur; BD Biosciences, Palo Alto, CA).

**Cell Proliferation Assays**

Tritiated thymidine uptake assays were performed to determine the effects of IL-2 and -15 on cultured human uveal melanoma cell proliferation. Briefly, human uveal melanoma cells were trypsinized, washed twice in HBSS, and resuspended to $1 \times 10^5$ cells/mL in complete RPMI medium, RPMI containing 100 or 500 U/mL rhIL-2, or RPMI containing 1, 5, or 10 ng/mL rhIL-15. One milliliter of each cell solution was placed into wells of 24-well microplates (Corning Costar, Corning, NY) in triplicate and incubated for 24°C, 48°C, or 72°C hours at 37°C in 5% CO\textsubscript{2}. Twelve hours before cell harvest for each time point, 0.1 μCi of tritiated thymidine (ICN Biomedicals, Irvine, CA) was added to each well. At each time point, supernatant was aspirated from the wells, wells were washed twice with PBS, and cells were solubilized by the addition of 0.25 mL cell lysis solution (Zap-Ogloblin II; Beckman Coulter, Miami, FL). Cells were incubated at 37°C for an additional 15 minutes, 0.5 mL PBS was added to each well, the contents of each well were put into a glass scintillation vial, mixed with 10 mL scintillation fluid (Budget-Solve; Research Products International Corp., Mount Prospect, IL), and counted on a liquid scintillation counter (Beckman LS8001; Beckman Instruments, Inc., Fullerton, CA).

**NK Cell Assay**

The effects of rhIL-2 and -15 on human NK cell activity was assessed using a chromium release assay. Human lymphocytes were isolated from peripheral blood samples using density gradient centrifugation. Mononuclear cells were placed into culture dishes (Primaria; Falcon number 35-3803; BD Bioscience) and incubated for 2 hours at 37°C in 5% CO\textsubscript{2}. The nonadherent cells were collected, washed once in HBSS, and incubated for 24 and 48 hours in 100 U/mL rhIL-2 or 5 ng/mL rhIL-15. Cells were then washed in HBSS and tested for NK cell activity in a conventional 4-hour \textsuperscript{51}Cr-release assay.\textsuperscript{27,28} A human chronic rhIL-15. Cells were then washed in HBSS and tested for NK cell activity and incubated for 24 and 48 hours in 100 U/mL rhIL-2 or 5 ng/mL rhIL-15. rhTRAIL, or RPMI with protein G + anti-human Fas (R&D Systems), RPMI with protein G + anti-human Fas + 5 ng/mL rhIL-15. RPMI containing protein G + 3 μg/mL staurosporine (Sigma-Aldrich) served as a positive control. Plates were then incubated for 48 hours at 37°C in 5% CO\textsubscript{2}. Cells were trypsinized, placed into 12 × 75 mm plastic culture tubes, washed once in PBS, resuspended to 0.2 mL in 1× binding buffer (TACS Annexin V FITC Apoptosis Detection Kit; R&D Systems), and 1.0 μL annexin V and 10 μL propidium iodide (both from the TACS Kit; R&D Systems) were added to each tube. After incubating tubes in the dark at room temperature for 15 minutes, 0.5 mL 1× binding buffer was added to each tube, and cells were read on the flow cytometer (FACSCalibur; BD Biosciences). Cells staining positive for annexin V (FL1), but negative for propidium iodide (FL3), were considered apoptotic.

The effect of rhIL-2 and -15 on TRAIL-induced apoptosis was also assessed on uveal melanoma cells. As stated previously, cells were plated and allowed to attach for 6 hours; medium was aspired and replaced with complete RPMI, RPMI + 300 ng/mL rhTRAIL (R&D Systems); RPMI + rhTRAIL + 100 U/mL rhIL-2, or RPMI + rhTRAIL + 5 ng/mL rhIL-15. RPMI containing 3 μg/mL staurosporine served as a control. Plates were incubated for 36 hours at 37°C in 5% CO\textsubscript{2}, and cells were stained for annexin V binding, as just described.

Melanoma cells were also treated with a chemotherapeutic anti-cancer drug, cisplatin that induces apoptosis. Melanoma cells were cultured for 24 hours in either IL-2 (100 units/mL) or IL-15 (5 ng/mL) for 24 hours before the addition of 10 μM cis-platinum (II)diimminodichloride (cisplatin; Sigma-Aldrich). Cells were incubated an additional 24 hours, and apoptosis was evaluated using the aforementioned annexin V binding assay.

**Results**

**IL-2 and -15R Expression on Uveal Melanoma Cells and Metastases of Uveal Melanomas**

Nine uveal melanoma cell lines and three cell lines derived from metastases of primary uveal melanomas were examined by flow cytometry for their expression of IL-2R. All 12 cell lines expressed various levels of IL-2R, which increased sharply after cultivation in rIL-2 (Fig. 1). Flow cytometric analysis of the same cell lines revealed that all the cell lines examined expressed IL-15R, which increased after cultivation in rIL-15 (Fig. 1).

Previous studies have demonstrated that murine cutaneous melanoma cells expressed IL-2R and proliferated when exposed to IL-2 in vitro. Accordingly, we examined the effect of recombinant human IL-2 (rhIL-2) on the proliferation of uveal melanoma cells and metastases of uveal melanoma. The results demonstrating maximum proliferation of each cell line are shown in Figure 2 and indicated that IL-2 induced cell proliferation in two of the three metastatic cell lines, but did not stimulate proliferation above the medium control in any of the primary uveal melanoma cell lines tested.

The effect of rhIL-15 on melanoma cell proliferation was also tested. Maximum proliferation is shown based on the optimal incubation time and optimal dose of IL-15 for each cell line (Fig. 2). Unlike IL-2, IL-15 stimulated significant proliferation in all but one of the uveal melanoma cell lines. However, combining IL-2 and -15 did not produce synergistic or even additive effects in melanoma cell proliferation (data not shown).
Effect of IL-2 and -15 on Uveal Melanoma Cell Sensitivity to NK-Cell–Mediated Cytotoxicity

In addition to its effects as a growth factor, IL-2 has been reported to reduce tumor cell susceptibility to NK-cell–mediated cytolysis. Accordingly, three of the uveal melanoma cell lines and one of the metastatic cell lines were incubated in the presence or absence of IL-2, and their susceptibility to NK-cell–mediated killing was tested in a conventional 4 hr 51Cr-release assay. Exposure to IL-2 significantly \( P < 0.001 \) reduced the susceptibility to NK-cell–mediated killing in one of the uveal melanoma cell lines (92.1) and in the metastatic cell line (Fig. 3A).

IL-15 shares many activities with IL-2, including the use of two of the subunits of the IL-2R. Because IL-15 protects against Fas-induced apoptosis and NK cells can kill tumor cells via FasL-induced apoptosis, we examined the effect of IL-15 on the susceptibility of three uveal melanoma cell lines and a metastatic cell line to NK-cell–mediated cytolysis. Melanoma cells were incubated for 48 hours in IL-15 and tested for their susceptibility to NK-cell–mediated cytolysis in a conventional 51Cr-release assay. Although the metastatic cell line (OMM1) was not affected by IL-15, two of the three uveal melanoma cell lines displayed significantly reduced susceptibility to NK-cell–mediated cytolysis after exposure to IL-15 (Fig. 3B).

**Figure 1.** IL-2R and -15R expression on uveal melanoma cell lines and cell lines derived from uveal melanoma metastases. Melanoma cells were cultured in the presence of either recombinant human IL-2 (A) or recombinant human IL-15 (B) and assessed by flow cytometry for the expression of IL-2R and -15R. The results are expressed as the maximum expression of IL-2R and -15R after incubation in medium alone or after incubation in the optimal concentration and incubation period for IL-2 and -15 for each respective cell line.

**Figure 2.** Induction of melanoma cell proliferation by IL-2 and -15. Melanoma cells were cultured in the presence or absence of either rhIL-2 (A) or -15 (B). Results represent maximum cell proliferation for each cell line based on optimal incubation period and optimal quantity of each cytokine for each respective cell line. Cell proliferation was normalized and reported as a percentage of optimal cell proliferation for each cell line in the absence of IL-2. Dashed line: maximum (100%) proliferation for cells cultured in the absence of IL-2.
Effect of IL-2 and -15 on Melanoma Cell Susceptibility to Apoptosis

As stated earlier, IL-2 and -15 confer limited protection against apoptosis in some tumor cells. We have recently shown that the uveal melanomas used in this study express TRAIL receptors and are vulnerable to TRAIL-induced apoptosis.26 Accordingly, experiments were performed to determine whether exposure to either IL-2 or -15 would affect the susceptibility of uveal melanoma cells to TRAIL-induced apoptosis. The MEL285 uveal melanoma cells and cells of the OMM1 metastatic cell line were incubated with either IL-2 or -15 and assessed for apoptosis. Although rhTRAIL induced apoptosis in both cell lines (24.05% and 21.28% respectively), neither IL-2 nor IL-15 significantly affected TRAIL-induced apoptosis in either cell line (data not shown).

The uveal melanoma cell lines used in this study also express Fas and are susceptible to Fas-induced apoptosis (data not shown). Therefore, we examined the effect of IL-2 and -15 on the susceptibility of two uveal melanoma cell lines (OCM3 and MEL270) and one metastatic cell line (OMM1) to Fas-induced apoptosis. Melanoma cells were cultured in the presence of rhIL-2 and then incubated in an agonistic anti-Fas antibody. The result show that anti-Fas antibody induced 14% to 37% apoptosis; however, preconditioning cells in either IL-2 or -15 failed to affect Fas-induced apoptosis of either uveal melanoma cell line or the metastatic cell line (data not shown).

Many agents used for the management of neoplasms induce apoptosis of cancer cells, which is believed to be critical for their chemotherapeutic effect.31-33 In a retrospective study of 201 patients with uveal melanoma, Bedikian et al.31 reported that of the chemotherapeutic agents used for the treatment of liver metastases in patients with uveal melanoma, cisplatin was the only examined that produced meaningful responses. Therefore, we examined the effect of IL-2 and -15 in protecting uveal melanoma cells (92.1 and OCM3) and a cell line derived from a uveal melanoma cell line metastasis (OMM1) from cisplatin-induced apoptosis in vitro. The cell lines used in this experiment were selected based on their relatively high susceptibility to cisplatin-induced apoptosis (data not shown). Melanoma cells were cultured in either IL-2 or -15 before incubation in cisplatin. Apoptosis was measured using an ELISA that detects active caspase-3.33 The results of a typical experiment are shown in Figure 4 and indicate that neither IL-2 nor -15 affected the sensitivity of 92.1 or OCM3 uveal melanoma cells to cisplatin-induced apoptosis. By contrast, exposure to IL-2 resulted in a 30% reduction in cisplatin-induced apoptosis of the OMM1 uveal melanoma metastatic cell line.

DISCUSSION

On first blush, one might predict that the presence of TILs or TAMs would carry a beneficial prognosis in patients with uveal melanoma, as both T cells and macrophages are capable of killing a wide variety of tumor cells in vitro. However, unlike the condition in cutaneous melanoma, the presence of TILs is associated with a poor prognosis in uveal melanoma.9,10 Likewise, the appearance of TAMs in many tumors, including uveal melanomas, is correlated with increased malignancy.11,16,35 One of the potential ways that TILs and TAMs may influence the malignancy of uveal melanoma is through their elaboration of cytokines that stimulate tumor cell proliferation. The results reported herein indicate that all the uveal melanoma cell lines and metastatic cell lines tested expressed both IL-2R and -15R. Moreover, both receptors were upregulated after exposure to the respective cytokines. Although IL-2 did not stimulate proliferation of uveal melanoma cells, it did induce significant proliferation of two of the three metastatic cell lines (32% and 57% increase in cell proliferation). By contrast, IL-15 induced a significant increase in the proliferation in all the cell lines, with only one exception (MEL290).

In addition to promoting tumor cell proliferation, TILs may exacerbate malignancy by secreting cytokines that reduce melanoma cell susceptibility to immune elimination. It has been reported that B16 murine melanoma cells have reduced susceptibility to NK-cell-mediated killing after exposure to the T-cell cytokine, IL-2.15 Moreover, IL-2-treated B16 melanoma cells produce an increased number of liver metastases compared with untreated melanoma cells. This is especially interesting, as uveal melanoma displays a propensity to metastasize to the liver, which possesses one of the highest NK cell activities of any organ in the body.36 We propose the following scenario to explain the putative exacerbation of malignancy associated with TILs and TAMs. It is well-recognized that malignancy is closely correlated with the heterogeneity of the cell
populations that make up a tumor. The present results suggest that primary uveal melanomas contain subpopulations of cells that express IL-2 and -15 receptors. It is possible that TILs and TAMs that enter a primary uveal produce IL-2 and -15, respectively. Macrophages constitutively produce IL-15, whereas T cells require engagement of the T-cell receptor (TCR) before they synthesize IL-2. It is noteworthy that there is at least one report indicating that TILs in human uveal melanomas demonstrate a dominant expression of the vβ7 T-cell receptor.37 This finding is entirely consistent with the notion that TILs may be responding to and interacting with tumor-specific antigens expressed on primary uveal melanomas, and as a result, may produce IL-2 within the tumor.

The putative relationship between TILs and poor prognosis may be unrelated to the aforementioned IL-2 stimulation of uveal melanoma cell proliferation. It is possible that TILs are indicators, rather than causes, of a poor prognosis. That is, uveal melanoma metastases may occur early in the course of the ocular malignancy and induce the generation of melanoma-specific lymphocytes that are ineffectual in controlling the metastases, but are capable of recognizing and entering the ocular melanomas. In this case, TILs would be indicators of progressive metastatic disease, which ultimately is the leading cause of death in patients with uveal melanoma.

It is also possible that TILs indirectly exacerbate the malignancy of uveal melanomas by subverting the immune response. As mentioned earlier, at least one study has reported a higher than expected frequency of vβ7 TCR-positive cells in uveal melanomas,37 and anecdotal findings have reported an association between the vβ7 TCR with IL-10 production and suppressor cell activity. Thus, the TILs in uveal melanomas may represent an accumulation of regulatory cells that inhibit protective immune responses.

Many chemotherapeutic agents used for the management of neoplasms act by inducing tumor cell apoptosis. We examined the effect of IL-2 and -15 on apoptosis induced by a chemotherapeutic agent, cisplatin, that has been used for the treatment of liver metastases in patients with uveal melanoma.31 The results indicate that IL-2 produces a significant reduction in cisplatin-induced apoptosis of at least some uveal melanoma metastases. IL-2 has been used as an experimental treatment for various malignancies, but the results to date have been disappointing. To our knowledge, no studies have examined the effect of combining IL-2 and cisplatin for the treatment of uveal melanoma, but the data reported herein raise the possibility that the use of IL-2 could reduce the efficacy of cisplatin and other chemotherapeutic agents that act by inducing apoptosis. However, any antagonistic effect that IL-2 has on the efficacy of cisplatin may be moot, as none of the currently used chemotherapeutic agents, including cisplatin, has improved the 5-year survival rate of patients with uveal melanoma.41

Collectively, the results indicate that cytokines elaborated by TILs and TAMs affect the behavior of uveal melanoma cells and their metastases in a manner that is consistent with increased malignant potential. TIL and TAM cytokines stimulate melanoma cell proliferation and resistance to NK-cell-mediated cytolysis and apoptosis induced by a potent chemothera-

![Figure 4](https://iovs.arvojournals.org/)
peutic agent. It is entirely feasible that TILs and TAMs also influence the malignancy of uveal melanoma by additional mechanisms that are independent of either IL-2 or -15. Nonetheless, the expression of IL-2R and -15R on uveal melanomas and their metastases provides insights into the pathogenesis of uveal melanoma and may also be important in the design of therapeutic strategies for the management of uveal melanomas. For example, the IL-2 receptor has been an effective target for the treatment of patients with uveitis42,43 and for the prevention of corneal graft rejection in experimental animals.44 Long-term (>4 years) administration of the anti-IL-2 antibody daclizumab has been successfully used in the treatment of patients with uveitis, while producing only minimal secondary effects.42 Immunotoxin therapy is another potential avenue for targeting the IL-2R on uveal melanomas. IL-2 has been fused to a modified Pseudomonas exotoxin and administered systemically as a means of purging IL-2R-bearing T lymphocytes and preventing corneal allograft rejection in the rat.39 Although highly theoretical and subject to numerous caveats, it may be feasible to consider a similar form of immunotoxin therapy in the management of uveal melanoma.

References

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