Restoration by IL-15 of MHC Class I Antigen-Processing Machinery in Human Dendritic Cells Inhibited by Tumor-Derived Gangliosides

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We have recently reported that MHC class I Ag-processing machinery (APM) component expression in dendritic cells (DC) might be down-regulated by tumor cells. However, the tumor-derived factors responsible for inhibition of the APM component expression in DC generated in the tumor microenvironment as well as potential protective mechanism have not yet been investigated. In this article, we demonstrate that expression of several MHC class I APM components, including MB1 (β5), LMP2, LMP7, LMP10, and Erp57, is significantly down-regulated in human DC generated in the presence of primary oral squamous cell carcinoma cell lines or coincubated with purified gangliosides. Suppression of MHC class I APM component expression in DC generated in the presence of tumor cells was significantly attenuated by the inhibition of glucosyl transferase in tumor cells, suggesting that tumor-induced MHC class I APM component down-regulation in DC was mediated in part by oral squamous cell carcinoma-derived gangliosides. Furthermore, rIL-15 restored both tumor cell-induced and ganglioside-induced MHC class I APM component expression in DC, as well as their ability to present Ags to autologous Ag-specific T cells. These results demonstrate that IL-15 restores MHC class I APM component expression in DC down-regulated by tumor-derived gangliosides. The Journal of Immunology, 2005, 175: 3045–3052.

Dendritic cells (DC) are the most efficient APCs, capable of priming naïve T lymphocytes. DC play a crucial role in the generation of tumor Ag (TA)-specific immune response by activating Ag-specific naïve MHC class II-restricted CD4+ T cells as well as MHC class I-restricted CD8+ T cells (1, 2). DC are also capable of cross-presenting exogenous Ags via the MHC class I Ag-processing pathway (3, 4). To escape from immune recognition, tumor cells have evolved mechanisms that induce dysfunction of the DC system. Suppressed DC functions have been reported in various in vitro models simulating the tumor microenvironment, and in tumor-bearing animals, as well as in cancer patients (5–7). Several reports have described inhibitory effects of soluble tumor-derived factors such as IL-6, IL-10, TGF-β, vascular endothelial growth factor, and gangliosides on DC generation and function (8–11). Many tumor cells express membrane-associated gangliosides, which may be shed into the tumor microenvironment (12, 13). Purified or tumor-derived gangliosides inhibit the ability of human monocytes and DC to initiate MHC class II Ag-restricted T cell responses, by down-regulation of costimulatory and MHC class II molecules on DC. Gangliosides also reduce IL-12 and TNF-α production by DC (14–17). However, it is unknown whether gangliosides might have effects on Ag-processing machinery (APM) component expression. We have recently reported that MHC class I APM components in DC is down-regulated by tumor cells (18). However, the molecular mechanisms underlying these phenotypic and functional changes in DC have not yet been investigated.

APM components play a crucial role in the assembly of the trimeric MHC class I complex, which consists of the MHC class I H chain, β2-microglobulin, and an Ag-derived peptide (19). APM components include the following: constitutive β subunits of the proteolytic delta (δ) (or β1) and MB1 (β5); inducible proteasome (immunoproteasome) β-type subunits LMP2, LMP7, and LMP10; peptide transporters TAP1 and TAP2; and endoplasmic reticulum chaperones calnexin, calreticulin, Erp57, and tapasin. Acting in concert, these components are responsible for generation of antigenic peptides, their translocation into endoplasmic reticulum, and loading of β2-microglobulin-associated MHC class I H chains with peptides (20). Defects in MHC class I APM component expression are likely to affect the cell surface expression of the MHC class I trimeric complex and/or the repertoire of Ag-derived peptides presented by MHC class I Ags as well as DC interaction with T lymphocytes necessary for Ag presentation. Although the components of MHC class I Ag-processing and Ag presentation pathways have been well characterized in human DC (3, 21), it is not known how tumor-derived factors affect Ag-processing pathways in DC. Also, the ability of cytokines to interact with the components of these pathways in DC has not been evaluated to date.

IL-15 is produced by a variety of cells in response to environmental stimuli and has a broad spectrum of biological activities in

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various types of cells, including T and B lymphocytes, NK cells, mast cells, granulocytes, monocytes/macrophages, and DC (22–25). Although IL-15 uses the common β and γ subunits of the IL-2R, it exerts different biological effects than IL-2 on most cell populations (26). IL-15 itself is able to activate DC in vivo and in vitro, and the exposure of splenic DC to IL-15 up-regulates costimulatory molecules, markedly increases IFN-γ production, and enhances the ability of DC to stimulate Ag-specific CD8+ T cells (25, 27). Although the role of IL-15 in regulation of DC generation, function, and survival is well established, its effects on Ag-processing pathways in DC in the tumor microenvironment have not been characterized.

Using a unique set of APM component-specific mAb, we report that human DC generated in the presence of oral squamous cell carcinoma (SCC) cell lines down-regulate expression of several APM components, including MB1 (β5), LMP2, LMP7, LMP10, and ERP57. It appears that tumor-derived gangliosides are responsible for this effect and that incubation of DC with IL-15 reverses APM component down-regulation.

Materials and Methods

**Cells**

Primary oral SCC cell lines PCI-4B and PCI-38 established at the University of Pittsburgh Cancer Institute and described previously (28) were cultured in RPMI 1640 supplemented with 2 mM t-glutamine, 30 µg/ml gentamicin sulfate, 10 mM HEPES, 5% FBS, 10 mM nonessential amino acids, and 1 mM sodium pyruvate, (Invitrogen Life Technologies). This medium is referred to as complete medium (CM).

DC were generated from PBMC of healthy donors. After gradient separation on Histopaque-1077 (Sigma-Aldrich) and lysis of RBC, PBMC were resuspended in CM (5 × 10^6 cells/ml) and incubated for 1 h at 37°C. Nonadherent cells were removed, and adherent monocytes were cultured in CM with 1000 U/ml recombinant human (rh)GM-CSF and 1000 U/ml rhIL-4 (PeproTech). On day 7, nonadherent cells were collected and analyzed. Their morphology and phenotype corresponded to immature DC characterized previously (18, 29). Percentage ranges for various surface markers in DC obtained from 10 donors were 72–99% for HLA-DR, 60–87% for CD68, 16–25% for CD80, 12–30% for CD83, 54–88% for CD40, and 2–20% for CD14.

DC (0.7–1.5 × 10^6 cells/well) were coincubated with 40 µg/ml disialoganglioside GD1α (tested at a range of concentrations from 20 to 80 µg/ml) (Matreya) reconstituted in DMSO (Sigma-Aldrich) and added on a day for the first 3 days in culture. DC were also coincubated with SCC cell lines PCI-4B or PCI-38 (25 × 10^6 cells/well) in CM separated by membrane inserts with 0.4-µm pore size (Transwell system) for the first 3 days in culture. As a control, DC were treated with medium alone, medium with DMSO, or coincubated with immortalized human keratinocytes HET-1A (CRL-2692; American Type Culture Collection) in the same way. To test the ability of IL-15 to restore the integrity of APM in DC in the tumor microenvironment, we added rhIL-15 (50 ng/ml) to DC generated in the presence of SCC cell lines and to control DC once per day for the last three consecutive days in culture.

To block ganglioside synthesis, tumor cells were cultured in the presence of inhibitor of glucosyl transferase D-threo-PPMP (1-phenyl-2-hexanol) at room temperature (RT) for 16–18 h. Cultures were harvested on GF/C glass fiber filters (Whatman) and washed three times in PBS/1% BSA, fixed with 2% paraformaldehyde (PFA) at room temperature (RT) for 20 min, washed three times in PBS/1% BSA, resuspended in 10 ml of PBS containing 0.5% BSA, transferred to glass flasks, and treated in a microwave. Cells were then immediately

**Western blot**

The protein expression of APM components in nucleofected and control DC was assessed using a Western blot technique. Cells were collected, washed, in HBSS, and lysed in 100 µl of extraction buffer (1% Triton X-100, 0.1% SDS, 50 mM Tris, 150 mM NaCl, 0.6 mM PMSF, and 5 µg/ml leupeptin) for 30 min on ice. After centrifugation (9,000 × g, 15 min, at 4°C), the protein concentration was determined in the supernatants by the Bradford method using the BioRad protein kit (Bio-Rad). Fifty micrograms of total proteins were dissolved in electrophoresis sample buffer, separated by 4–12% PAGE, and transferred to a nitrocellulose membrane (NOVEX). The membrane was blocked with 0.5% nonfat milk, 0.1% Tween 20 (Fisher Scientific) in 20 mM Tris-HCl buffer (pH 7.2). LMP10 and MB1 were detected using the anti-LMP10 mAb TO-7 and anti-MB1 mAb SJ9-3 in a final concentration of 100 µg/ml and goat anti-mouse secondary mAb (IgG (H+L), 1:25,000 dilution; Pierce). Expression of β-actin was evaluated as a housekeeper control protein using mouse anti-human mAb (IgG1, 1:100,000 dilution; Sigma-Aldrich). Secondary Abs were the same as for APM components detection. The membrane was processed and treated with chemiluminescent reagents (Pierce), and the bands were visualized on a Kodak film (Eastman Kodak).

**OVA-specific presentation by DC**

To evaluate the capacity of DC to present Ags, 6-day-old DC, which had been generated for the first 3 days with tumor cells, and control DC incubated with immortalized keratinocytes were pulsed overnight with OVA (Sigma-Aldrich) (500 µg/ml). Next, DC were washed twice and cultured for 96 h at 37°C, 5% CO2 in 96-well plates in CM with autologous OVA-specific CD8 T cells (1 × 10^6 cells/well) at different DC:T ratios (1:100 to 1:1). At the end of incubation, T cell proliferation was measured by uptake of [3H]thymidine (1 µCi/well, 5 Ci/mmol; PerkinElmer Life Science) added for the last 16–18 h. Cultures were harvested on GF/C glass fiber filter paper (Whatman) using a MACH III microwell harvester (Tomtec). [3H]Thymidine incorporation was determined in a MicroBeta Trilux liquid scintillation counter (Wallac). Counts are expressed as cpm and presented as stimulation index (SI). SI was calculated as stimulated cpm divided by spontaneous cpm.

To generate autologous OVA-specific T cells, CD8+ T cells were isolated from PBMC using MACS paramagnetic purification system (Miltenyi Biotec) and cultured with weekly addition of irradiated autologous PBMC (1:1), OVA (Sigma-Aldrich; 500 µg/ml), and IL-2 (10 UI/ml) for 4 wk.

**Flow cytometry analysis of DC and SCC cells**

Intracellular staining for detection of APM protein expression in DC was performed with modifications, as described earlier (30). Briefly, DC were cultured for 48 h with 100 ng/ml IL-15, fixed with 2% paraformaldehyde (PFA) at room temperature (RT) for 20 min, washed three times in PBS/1% BSA, resuspended in 10 ml of PBS containing 0.5% BSA, transferred to glass flasks, and treated in a microwave. Cells were then immediately
The differences between groups were determined using the Student’s t test or the nonparametric Mann-Whitney U test after evaluation for normality. For multiple group comparison, ANOVA was used. For all statistical analyses, a p value of 0.05 was considered significant. Data are presented as mean ± SEM. All of the experiments were repeated at least three times.

Results

Down-regulation by oral SCC cells and restoration by IL-15 of APM component expression in DC

Previously, we observed that the expression of some APM components was down-regulated in DC incubated with SCC cells. To confirm these findings, we evaluated the expression of MB1 (β5), LMP2, LMP7, LMP10, calnexin, calreticulin, ERp57, and tapasin in DC, which had been coincubated with SCC cells in a Transwell system, intracytoplasmically stained with APM component-specific mAb, and analyzed by flow cytometry. As a control, DC were treated with medium alone or coincubated with immortalized keratinocytes. In 10 independent experiments with DC from 10 donors, expression of MB1, LMP2, LMP7, LMP10, and ERp57 was significantly down-regulated in SCC-treated DC (p < 0.05) Data represent the percentage of positive cells from 10 independent donors.

<table>
<thead>
<tr>
<th>APM Components</th>
<th>DC</th>
<th>DC + IL-15</th>
<th>DC + SCC</th>
<th>DC + SCC + IL-15</th>
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<td>Calnexin</td>
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<td>25</td>
<td>25</td>
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<tr>
<td>Calreticulin</td>
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</table>

Table 1. Restoration by IL-15 of HLA class I APM component expression in DC preincubated with oral SCC cells

DC were cocultured with SCC (PCI-38 and PCI-4B) cells in a Transwell system for 72 h. Then, recombinant human IL-15 (50 ng/ml) was added once per day for the last three consecutive days in culture. Expression of APM components in control and tumor-treated DC was determined by flow cytometry. The results of one representative donor among seven studied are shown.

Discussion

The results of previous studies have shown that SCC cells can induce suppression of DC function by inhibiting DC maturation, thus impairing the T cell response to SCC antigens. In the current study, we investigated HLA and APM component expression in DC coincubated with SCC cells in vitro. We found that the expression of MB1, LMP2, LMP7, LMP10, and ERp57 was significantly down-regulated in SCC-treated DC (p < 0.05). These results suggest that SCC cells may down-regulate the expression of these APM components, which are known to be involved in antigen presentation and T cell activation. This down-regulation may impair the DC ability to present SCC antigens to T cells, thereby reducing the immune response to SCC antigens.

Restoration by IL-15

To restore the expression of down-regulated APM components, we added IL-15 (50 ng/ml) to the coculture system. We found that IL-15 significantly restored the expression of MB1, LMP2, LMP7, LMP10, and ERp57 in SCC-treated DC (p < 0.05). These results suggest that IL-15 can reverse the down-regulation of APM components induced by SCC cells, thereby restoring the ability of DC to present SCC antigens to T cells.

Conclusion

In summary, our results indicate that SCC cells can down-regulate the expression of MB1, LMP2, LMP7, LMP10, and ERp57 in DC, which can be restored by IL-15. These findings suggest that IL-15 may be a potential therapeutic target for the treatment of oral SCC.
6.2% for LMP7, 36.6 ± 6.7% for LMP10, and 39.8 ± 9.8% for ERp57. Similar results were obtained by analyzing the corresponding MFI values (Table I). In contrast, expression of the calnexin, calreticulin, and tapasin was not significantly altered in the tumor cell-treated human DC.

Because IL-15 has been shown to promote DC activation (25) and increased DC function and survival in the tumor microenvironment (33), we hypothesized that IL-15 might restore the integrity of APM in DC generated in the presence of tumor cells. To test this possibility, we added rIL-15 (50 ng/ml) to DC generated in the presence of SCC cell lines and to control DC once per day for the last 3 consecutive days in cultures and measured the level of APM component expression in tumor cell-treated DC. Treatment with IL-15 restored expression of MB1, LMP2, LMP7, LMP10, and ERp57 in tumor cell-treated DC in the range from 53 to 98%, depending on the donor (Table I). These results demonstrate that not all APM components but only specific components of the constitutive proteasome and immunoproteasome are selectively decreased in expression in DC coincubated with tumor cells. Restoration by IL-15 of APM component expression suggests the ability of this cytokine to regulate APM in DC.

Depletion of APM component mRNA in DC correlates with significant reduction of APM protein expression in DC and with reduced expression of surface MHC class I molecules

To prove that anti-APM component Abs specifically detect up- or down-regulation of Ag-processing pathway components in DC, using commercially available siRNA for LMP10 and MB1, we assessed expression of LMP10 and MB1 in DC nucleofected with specific APM component siRNA, control RNA encoding GFP and empty vector. Our results revealed that the level of DC transfection using DC protocol developed by the manufacturer (Amaxis) was between 40 and 60% based on GFP expression assessed by flow cytometry. Next, we have demonstrated that depletion of LMP10 mRNA in DC correlates with significant reduction of LMP10 protein expression in the same cells, assessed by Western blot with specific anti-APM Abs (Fig. 2A). Importantly, down-regulation of LMP10 protein in DC induced by siRNA was associated with reduced expression of surface MHC class I molecules (Fig. 2B). Similar results were obtained for DC nucleofected with MB1 siRNA.

 Restoration by IL-15 of suppressed OVA-specific Ag presentation by DC generated in the presence of SCC cells

To test whether APM component down-regulation in DC generated in the presence of tumor cells has functional consequences of poor Ag presentation, we evaluated the ability of DC to present OVA to OVA-specific autologous CD8+ T cells. Six-day-old tumor cell-treated and control DC were pulsed with OVA (500 μg/ml) overnight, washed, and coincubated with OVA-specific autologous CD8+ T cells, and T cell proliferation was measured by uptake of [3H]thymidine. The ability of DC to induce proliferation of CD8+ T cells was significantly decreased when DC had been coinduced with PCL-4B cells, in comparison to DC coinduced with keratinocytes or in medium alone (Fig. 3). For instance, at the E:T ratio 1:10, [3H]thymidine uptake was reduced by 76 ± 12% (p < 0.05) and at the ratio 1:1 × 60 ± 8% (p < 0.05). The ability of DC to induce proliferation of autologous CD8+ T cells was HLA class I restricted, because it was inhibited by the addition of anti-HLA class I mAb W6/32. Thus, coinducation of DC with SCC cells impairs DC ability to present OVA-derived peptides to autologous OVA-specific CD8+ T cells. Because it has been shown that DC generated in the presence of GM-CSF plus IL-15 prime potent CD8+ Tc1 responses in vivo (27), and because of the ability of IL-15 to reverse tumor cell-induced down-regulation of APM component expression in DC, we hypothesized that IL-15 might also restore DC Ag-presenting function. To test this possibility, IL-15 (50 ng/ml) was added to DC generated in the presence of SCC cell lines and to control DC once per day for the last 3 consecutive days in culture, and the ability of DC to present OVA Ag to OVA-specific autologous CD8+ T cells was measured. The addition of IL-15 caused a complete recovery of suppressed DC Ag-presenting activity (Fig. 3). These results suggest that IL-15 is able to restore the SCC cell-induced inhibition of APM component expression and Ag-presenting function of DC.

Down-regulation by gangliosides and restoration by IL-15 of APM component expression in DC

It has been previously shown that gangliosides down-regulate maturation and function of APC (13–17). To test whether gangliosides are responsible for modifying APM component expression in DC, disialoganglioside GD3 (40 μg/ml) was added once daily for the first 3 days in culture. As a control, DC were treated with DMSO,

FIGURE 2. Down-regulation of expression of APM components in DC nucleofected with siRNA correlates with reduction of the corresponding protein expression in DC and reduced expression of surface MHC class I molecules. The transfection efficiencies of DC nucleofection ranged between 40 and 60% based on GFP expression. A, The protein expression of APM components in DC nucleofected with LMP10 siRNA, control RNA encoding GFP, and empty vector was assessed using Western blot technique as described in Materials and Methods. B, LMP10 protein expression and expression of surface MHC class I molecules in nontreated DC, control transfected DC, and DC nucleofected with LMP10 siRNA was determined by flow cytometry as described in Fig. 1 legend. Results are expressed as MFI. The results of one representative of three independent experiments are shown.
Discussion

Tumor cell-induced suppression of DC function is an important mechanism of tumor escape from immune recognition. It has been shown that the expression of HLA-DR, CD40, and CD80 molecules on DC obtained from cancer patients as well as DC ability to stimulate T lymphocytes were markedly suppressed explaining, in part, why tumor-infiltrating T cells fail to eliminate tumor cells (5, 34, 35). Troy et al. (36) have demonstrated that DC are not recruited in large numbers into renal cell carcinoma lesions and that DC extracted from these tumors are minimally activated and have reduced allostimulatory activity. Similar data were obtained with DC infiltrating prostate cancer (36) and basal cell carcinoma (37) lesions. Furthermore, tumor cells may also inhibit other functions of DC in patients with cancer and in tumor-bearing animals. For instance, Tas et al. (38) noticed a decreased ability of peripheral blood DC to form clusters with T cells in patients with head and neck cancers. Using neuroblastoma and colon adenocarcinoma as models, we have recently shown that DC obtained from tumor-bearing mice had a significantly decreased ability to produce IL-12 upon CD40 ligation (10). Thurber et al. (39) observed that DC obtained from renal cell carcinoma lesions had reduced potential to capture soluble Ag, as shown by the exclusion of FITC-labeled

Table II. Restoration by IL-15 of HLA class I APM component expression in DC pretreated with gangliosides

<table>
<thead>
<tr>
<th>APM Components</th>
<th>DC</th>
<th>DC + IL-15</th>
<th>DC + GD₃</th>
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<td>HLA ABC</td>
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GD₃ ganglioside expression by SCC cell lines PCI-4B and PCI-38

To test the hypothesis that tumor cell-induced APM component down-regulation in DC may be mediated by tumor cell-derived gangliosides, we first measured GD₃ ganglioside expression in the SCC cell lines PCI-4B and PCI-38. Neuroblastoma cell lines were used as a positive control (14). Flow cytometry and HPLC analysis showed that both cell lines express GD₃ ganglioside (Figs. 4 and 5).

Role of tumor cell-derived gangliosides in APM component down-regulation in DC

To prove that tumor cell-derived gangliosides are indeed responsible for APM component down-regulation in DC coincubated with SCC cells, we blocked synthesis of gangliosides in tumor cells using enzymatic inhibition. Tumor cells were pretreated with an inhibitor of glucosyl transferase β-threo-PPMP for 3 days to block glycosphingolipid synthesis, including ganglioside synthesis (14). APM component down-regulation in DC coincubated with SCC cells pretreated with PPMP was significantly (p < 0.05) less than down-regulation in DC coincubated with nontreated SCC cells (Fig. 6). Similar data were obtained for DC coincubated with both SCC cell lines PCI-4B and PCI-38. Thus, inhibition of ganglioside synthesis in tumor cells significantly decreased their inhibitory effect on APM component expression in DC, suggesting that the observed down-regulation of APM components is, at least in part, mediated by tumor cell-derived gangliosides.

FIGURE 3. IL-15 restores DC Ag-presenting capacity suppressed by oral SCC cells. DC were cocultured with SCC cell line PCI-4B (▼) or keratinocytes (▲) in Transwell system for 72 h. Then rhIL-15 (50 ng/ml) was added to DC generated in the presence of SCC cell lines (▼) and control DC (□) once per day for the last 3 consecutive days in culture. Tumor-treated and keratinocyte-treated 6-day-old DC were loaded with OVA overnight, then washed and cultured in 96-well plates in CM with autologous OVA-specific CD8⁺ T cells (1 × 10⁶ cells/well) for 96 h. Proliferation of T cells was measured by uptake of [³H]thymidine added for the last 16–18 h. Inhibition of the proliferation with anti-HLA class I W6/32 mAb indicates that DC-induced activation of CD8⁺ T cells was HLA class I restricted. The counts are presented as SI. Similar results were obtained with PCI-38 oral SCC cell line. Data represent the mean ± SEM of triplicate measurements from three independent experiments. * p < 0.05.

used as a solvent for gangliosides. Synthetic gangliosides significantly (p < 0.05) suppressed MB1, LMP2, LMP7, LMP10, and ERp57 expression in DC (Table II). For instance, MB1 was down-regulated by 37 ± 7%, LMP2 × 53 ± 10%, LMP7 × 60 ± 14%, LMP10 × 41 ± 8%, and ERp57 × 21 ± 6% in DC treated with gangliosides vs control DC. Other tested APM components were not significantly inhibited. Furthermore, the treatment of DC with IL-15 after coincubation with gangliosides reversed the inhibitory effect of gangliosides on MB1, LMP2, LMP7, LMP10, and ERp57 expression in the range from 40 to 100%, depending on the donor and specific APM components (Table II). Because synthetic gangliosides down-regulated similar APM components in DC as the SCC cell lines, and because IL-15 can restore these components expression in DC coincubated both with tumor cells or gangliosides, we hypothesized that tumor cell-induced APM component down-regulation in DC could be due to tumor cell-derived gangliosides.

Disialoganglioside GD3 (40 μg/ml) was added to DC once daily for the first 3 days in cultures. As a control, DC were treated with DMSO used as a solvent for gangliosides. Then, rhIL-15 (50 ng/ml) was added once per day for the last three consecutive days in cultures. APM component expression in ganglioside-treated and control DC was determined by flow cytometry. The results of one from four independent experiments are shown.
dextran. Down-regulation of DC functional activity in the presence of tumor cells has been also reproduced using tumor cell lines in vitro. This provides a model to investigate the identity of inhibitory factors and mechanisms responsible for suppression of DC in cancer. We used this in vitro model to investigate the effect of SCC cells on APM in human DC. In our previous observations, we have demonstrated down-regulation of several APM components in human DC coincubated with primary oral SCC cell lines (18). In this study, we have evaluated different APM component expression in DC and have found that SCC cells induced significant down-regulation of constitutive proteasome subunit MB1, immunoproteasome subunits LMP2, LMP7, and LMP10, and thiol oxidoreductase ERp57 in DC. These data might explain reduced ability of DC to present Ag(s) to T cells in cancer. However, the specific role of each APM component in DC failure to present TA(s) to T cells in cancer remains to be characterized.

Tumor cell-induced down-regulation of APM components might be in part associated with DC maturation status. It has been shown that cross-presentation in DC is developmentally regulated (40, 41). However, the data concerning the level of proteasome expression in immature and mature DC are not consistent. For instance, it has been reported that expression of immunoproteasome subunits LMP2, LMP7, and LMP10 is down-regulated, whereas expression of their activators PA28α and PA28β, as well as of TAP, tapasin, and MHC class I molecules is up-regulated during maturation of human DC (41). Other investigators have...
demonstrated up-regulation of the immunoproteasomes in mature DC (40). The observed LMP2, LMP7, and LMP10 down-regulation in tumor cell-treated DC could be interpreted as induction of DC maturation in the presence of the tumor. However, this speculation conflicts with the reports demonstrating down-regulation of the costimulatory molecules CD80 and CD86, CD40 and MHC class I and class II molecules in DC coincubated with tumor cell lines (42). It is also possible, that the blunted Ag processing, and low APM component expression in tumor cell-treated DC may simply reflect the immature status of treated DC. However, whereas immature DC display high endocytotic activity, DC co-incubated with tumor cells display also a reduced endocytotic activity (M. R. Shurin, unpublished data). Thus, it is unlikely that DC are simply immature in the tumor microenvironment. Moreover, our data reveal down-regulation of the constitutive proteasome molecules MB1, as well as of the immunoproteasome subunits LMP2, LMP7, and LMP10 in tumor cell-treated DC. Suppression of both proteasome types in DC coincubated with tumor cells is likely to be associated with a profound dysregulation of APM components and cannot be explained by the effect on DC maturation status. Furthermore, tumor cell-induced down-regulation of APM component expression in DC has been accompanied by impaired Ag-presenting function. Thus, our results suggest that tumor cells do not simply delay or prevent DC maturation, but actively suppress DC activity and function, including Ag processing and presentation.

One of the mechanisms used by tumor cells to induce alteration in APM components in DC and impaired function could be the release of gangliosides (13–16). In fact, Ladisch and his coworkers (43) showed that purified FBL-3 erythroleukemia cell-derived gangliosides inhibited synergistic TA-specific immune response in mu-

References


Disclosures

The authors have no financial conflict of interest.

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