Melanoma-Educated CD14⁺ Cells Acquire a Myeloid-Derived Suppressor Cell Phenotype through COX-2-Dependent Mechanisms

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Abstract

Tumors can suppress the host immune system by employing a variety of cellular immune modulators, such as regulatory T cells, tumor-associated macrophages, and myeloid-derived suppressor cells (MDSC). In the peripheral blood of patients with advanced stage melanoma, there is an accumulation of CD14⁺ HLA-DRlo/⁻ MDSC that suppress autologous T cells ex vivo in a STAT-3-dependent manner. However, a precise mechanistic basis underlying this effect is unclear, particularly with regard to whether the MDSC induction mechanism relies on cell–cell contact of melanoma cells with CD14⁺ cells. Here, we show that early-passage human melanoma cells induce phenotypic changes in CD14⁺ monocytes, leading them to resemble MDSCs characterized in patients with advanced stage melanoma. These MDSC-like cells potently suppress autologous T-cell proliferation and IFN-γ production. Notably, induction of myeloid-suppressive functions requires contact or close proximity between monocytes and tumor cells. Further, this induction is largely dependent on production of cyclooxygenase-2 (COX-2) because its inhibition in these MDSC-like cells limits their ability to suppress T-cell function. We confirmed our findings with CD14⁺ cells isolated from patients with advanced stage melanoma, which inhibited autologous T cells in a manner relying up prostaglandin E2 (PGE2), STAT-3, and superoxide. Indeed, PGE2 was sufficient to confer to monocytes the ability to suppress proliferation and IFN-γ production by autologous T cells ex vivo. In summary, our results reveal how immune suppression by MDSC can be initiated in the tumor microenvironment of human melanoma. Cancer Res; 73(13); 3877–87. ©2013 AACR.

Introduction

Myeloid-derived suppressor cells (MDSC) are present in a low frequency in healthy individuals, but are increased in sepsis and trauma to facilitate wound healing (1, 2). Recently, they have been recognized as cellular immune modulators that tumors employ to suppress both innate and adaptive immune responses in a variety of human solid and hematologic malignancies (1, 3, 4). MDSCs consist of immature myeloid cells and contain progenitors of granulocytes, dendritic cells, and macrophages. In mice, coexpression of CD11b and Gr1 was used to define MDSCs, which can be subdivided into CD11b⁺ Ly6-G⁻ Ly6-Clo monocytic and CD11b⁻ Ly6-G⁻ Ly6-Clo granulocytic MDSCs (5, 6). In humans, different MDSC phenotypes have been reported to be associated with different cancer types. In patients with melanoma, we and others have reported that CD14⁺ HLA-DRlo/⁻ monocyte MDSCs accumulate in the peripheral blood (7, 8). MDSCs can directly suppress T cells via a variety of factors, including reactive oxygen species (ROS), arginase activity, TGFβ, and indoleamine 2,3-dioxygenase (IDO; refs. 5, 9, 10).

Tumor cells, together with tumor-associated fibroblast and other stromal cells, release a number of factors, such as TGF-β, interleukin (IL)-10, PGE2, GM-CSF, VEGF, and IL-6, to facilitate immune escape and tumor progression (11, 12). Recent studies have shown that a combination of different cytokines, that is, GM-CSF and IL-6 (13, 14), or coculture with tumor cell lines (15, 16), can induce human MDSC-like cells in vitro from healthy monocytes or bone marrow aspirates to conduct potent immunosuppression.

Notably, Obermajer and colleagues have elegantly shown the importance of PGE2 in the induction of MDSCs (17). This is in accordance with previous observations that COX-2 and its direct product PGE2 are expressed at high levels in malignant melanomas and often correlate with poor prognosis (18, 19). Interruption of the COX-2/PGE2 pathway has been shown to be effective in blocking MDSCs induced by a mixture of cytokines in vitro (17, 20). Similar effects were also observed in a mouse model (21).

Yet, the importance of these factors versus that of a direct interplay between tumor cells and MDSCs in the tumor...
microenvironment remains to be elucidated. MDSCs in multiple myeloma microenvironment potently promote tumor growth and suppress T-cell functions (22). In contrast, Gros and colleagues reported the absence of T-cell suppressing capacity of those MDSCs that are retrieved from melanoma tumor tissues (23). In this study, we show that human melanoma cells can induce CD14+ monocytes from healthy individuals to acquire an MDSC-like phenotype and suppressive properties mediated by COX-2/PGE2 and STAT-3 signaling. We also present data supporting PGE2 as a T-cell–suppressive mediator employed by monocytes from patients with advanced stage melanoma.

Materials and Methods

Cell isolation and patient materials

Peripheral blood mononuclear cells (PBMC) were isolated from healthy donor buffy coats (Karolinska University Hospital, ethical permit: #20010305,01-50) by gradient centrifugation (Ficoll-Paque Plus, GE Healthcare), followed by monocyte isolation using a CD14+ monocyte isolation kit and MACS LS column (Miltenyi Biotech). Autologous T cells were purified from the negative fraction of the monocyte isolation by a Pan T-cell Isolation Kit (Miltenyi Biotech), according to the manufacturer’s instructions.

Five patients with stage IV melanoma were accrued with informed donor consent according to the Declaration of Helsinki and approved by the Karolinska Institutet review board (ethical permit: #2011/143-32/1). PBMCs were isolated from patient peripheral blood and CD14+ cells were purified as stated above. Monocyte-depleted autologous lymphocytes were collected for functional studies.

Tumor coculture and MDSC isolation

The EST human melanoma cell lines were from the European Searchable Tumor Line Database (ESTDAB, http://www.ebi.ac.uk/ipd/estdab) and maintained as described (24). Cell line authenticity was carried out by an STR identifi

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Monoclonal antibodies and flow cytometry

Information on the antibodies used in this study is provided in Supplementary Table S2. A panel of myeloid cell surface markers was measured on monocytes when they were freshly isolated (0 hours), and/or after 14, 40, and 64 hours of coculture with EST cell lines. For the monocytes cocultured with early-passage melanoma cell lines, the myeloid markers were evaluated at 0 hours and 64 hours. In brief, cells were harvested by gentle scraping and stained in PBS supplemented with 1% human serum albumin (HSA) at 4°C for 30 minutes. For the pSTAT-3 staining, cells were fixed with BD Phosflow Lyse/Fix Buffer (BD Biosciences), followed by permeabilization with BD Phosflow Perm Wash III (BD Biosciences). Cells were stained with antibodies for detecting phosphorylation of Serine 727 (Ser727) or Tyrosine 705 (Tyr705) on STAT-3 protein as mentioned above. All data were acquired on an LSRII flow cytometer (BD Biosciences) using FACS Diva software and were analyzed using FlowJo software (Treestar).

T-cell proliferation and cytokine production assays

To evaluate the suppressive functions of melanoma-educated monocytes, 5 million T cells were labeled in 1 mL PBS containing 1.4 μmol/L Carboxyfluorescein succinimidyl ester (CFSE, Invitrogen) for 5 minutes at room temperature and seeded at 100,000 cells per well in a 96-well U bottom plate in 100 μL IMDM medium containing 10% human AB serum. CD11b+ monocytes were purified from cocultures with or without tumor cells or from transwells at 64 hours and added to T cells at ratios of 1:2 (50,000 cells) or 1:4 (25,000 cells). Next, T cells were activated by addition of 1.5 μL anti-CD3/CD28 mAb-coated beads per well for 4 days. Subsequently, T-cell proliferation and CD62L expression were measured by flow cytometry and IFNy concentrations secreted to the supernatants were determined by ELISA (MabTech). Nonactivated T cells or activated T cells without monocyte coculture were used as controls. For the patient samples, monocyte-depleted lymphocytes were labeled with CFSE and seeded at 100,000 cells per well. Purified CD11b+ monocytes were added at ratios of 2:1, 1:1, and 1:2 to each well and the parameters described above were measured. Blocking assays for monocyte-suppressive functions were conducted at 1:2 monocyte:T-cell ratio for melanoma-educated monocytes and 1:1 ratio for ex vivo patient-derived monocytes. Briefly, monocytes were treated for 2 hours with 20 μmol/L AG490 (Sigma-Aldrich), 40 μmol/L celecoxib...
(Biovision), 400 IU/mL superoxide dismutase (Sigma-Aldrich), 200 IU/mL catalase (Sigma-Aldrich), 1 mmol/L N(ω)-hydroxy-nor-ω-arginine (nor-NOHA, Calbiochem), 20 μg/mL anti-TGFβ mAb (R&D Systems), and 20 μg/mL anti-CD80 mAb (Biolegend) in 100 μL medium. To minimize the effects on T cells, AG490 and celecoxib were removed from the wells by replacing with fresh medium, followed by adding T cells in 100 μL medium to all wells.

In addition, 50 μmol/L α,β-methylene ADP (Sigma-Aldrich), 20 nmol/L SCH 58261 (Sigma-Aldrich), 20 μg/mL anti-PGE2 mAb (Cayman Chemical), or 10 μmol/L PGE2 (Sigma-Aldrich) were added to the ex vivo isolated patient monocytes in the T-cell proliferation assay.

Statistical analysis

All data were summarized by Prism Version 6 software (GraphPad) and differences were analyzed by nonparametric, Mann–Whitney U test. Unless otherwise stated, the data from multiple experimental and control groups are presented as means and SD from the number of donors that are indicated in the figure legends. All results were confirmed in at least 3 individual experiments and the representative figures were chosen according to the average values.

Results

Melanoma-educated monocytes phenotypically resemble myeloid-derived suppressor cells

We previously described that CD14⁺HLA-DRlo/C0 MDSCs overexpress CD80, CD83, and DC-Sign in patients with advanced stage melanoma (7). To study if a similar MDSC population could be induced in vitro, we established an MDSC induction model by coculturing monocytes enriched from healthy individuals with melanoma cell lines.

When cocultured with established melanoma cell lines (EST025 and EST094), the upregulation of HLA-DR was markedly blocked compared with control monocytes.

![Figure 1](https://example.com/figure1.png)

Figure 1. Melanoma-educated monocytes phenotypically resemble MDSCs. Mean fluorescence intensity (MFI; A) and representative histograms (B) depicting HLA-DR, CD14, and CD1a expression kinetics at 0, 14, 40, and 64 hours on healthy donor monocytes (n = 4) cultured with or without EST025 (top) or EST094 (bottom) melanoma cell lines. Data are presented as means ± SD. *, P < 0.05.
tumor cell lines induced significantly increased over time on tumor cocultured monocytes (P < 0.05, Fig. 1A and B). Although not significant, expression of CD80 and DC-Sign were upregulated in monocyte–tumor cell cocultures compared with controls (Supplementary Fig. S1). Neither of the tumor cell lines induced significant changes of other myeloid and/or immunosuppressive surface molecules, such as, CD33, CD11b, CD11c, CD83, CD137, CD137L, PD-L1, and CD86 (data not shown).

**Early-passage melanoma cells can induce MDSC-like cells in a proximity-dependent fashion**

To exclude that alterations induced by long-term *in vitro* culture of the established melanoma lines could account for the observed effects, short-term (≤ 7 passages) cultured melanoma cells were cocultured with freshly isolated healthy donor monocytes.

In line with our observations from long-term established melanoma cell lines, the short-term cultured cell line also impaired the expression of HLA-DR and CD86 on monocytes, and induced significant upregulation of CD14 and CD1a (Fig. 2A).

Because separation of melanoma cells and monocytes in a transwell system did not result in the upregulated expression of CD14 and CD1a, as compared with levels observed on control cultured monocytes (Fig. 2A), we conclude that the effect was dependent on cell–cell contact or mechanisms that require close proximity to tumor cells. This argues against the possibility that the observed phenotypic changes were induced by nonspecific factors, that is, nutrient deprivation or pH value fluctuation due to the fast-growing tumor cells in the coculture.

In contrast, expression levels of HLA-DR and CD86 decreased even when cultured in the transwell system, suggesting that contact/proximity independent mechanisms may

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**Figure 2.** Early-passage melanoma cells can induce MDSC-like cells in a proximity-dependent fashion. Expression levels (A) and representative histograms (B) of HLA-DR, CD86, CD14, CD1a, CD80, DC-Sign, CD33, CD15, and PD-L1 on healthy donor monocytes (n = 6) cultured for 64 hours without tumor cells (empty columns), directly cocultured (black columns) with, or separated by a transwell setup (gray columns) from the early-passage GEWA melanoma tumor cell line. C, expression levels of CXCR2, 3, and 4, as well as the representative histograms, of healthy monocytes (n = 5) cultured for 64 hours without (empty columns) or cocultured with (black columns) early-passage GEWA tumor cell line. Data are presented as relative fold changes in MFI to freshly isolated CD14⁺ cells (dashed horizontal line), means ± SD. *, P < 0.05; ***, P < 0.01.
be involved in regulating tumor-induced changes for these markers (Fig. 2A and B).

Notably, the early-passage cell line was capable of inducing phenotypic changes that we did not observe by using long-term cultured melanoma cell lines but that were similar to those observed in patient-isolated MDSCs (7). Expression of CD80 (P < 0.05) and DC-Sign (P < 0.01) were significantly increased in melanoma-educated monocytes, but normalized by using transwells (Fig. 2A and B). Melanoma coculture also increased the expression of the myeloid/granulocytic cell markers CD33 and CD15 (P < 0.01), as well as PD-L1 (P < 0.05), all of which were normalized by using the transwell system. Moreover, expression of chemokine receptor CXCR4 on these monocytes were significantly increased when cocultured with tumor cells (P < 0.01). Due to the phenotypic similarities of these melanoma-educated monocytes to the MDSCs in patients with melanoma, we hereafter refer them as “MDSC-like” cells.

**Tumor-educated MDSC-like cells are potent suppressors of autologous T cells**

Since CD11b expression was unaffected by the presence of tumor cells (data not shown), this marker was used to purify CD14+ monocytes after 64 hours of coculture with the early-passage melanoma cell line. CD11b+ cells were then mixed with CFSE-labeled autologous T cells at monocyte:T-cell ratios of 1:2 and 1:4, followed by T-cell activation using anti-CD3/CD28 mAb-coated beads. T-cell proliferation and IFNγ production were evaluated after 4 days.

Of note, tumor education not only induced MDSC-like phenotype, but also resulted in potent suppressive activity of these healthy donor-derived monocytes. At a 1:2 MDSC-like cell:T-cell ratio, proliferation of T cells was significantly inhibited (73.6 ± 13.4% with MDSC-like cells versus 20.5 ± 26.1% with controls, P < 0.05) and the IFNγ production (2351.8 ± 934.1 pg/mL with MDSC-like cells versus 139.1 ± 136.8 pg/mL with controls, P < 0.05) was completely abrogated. T-cell suppression mediated by these cells was highly dose dependent and clearly alleviated by reducing the number of MDSC-like cells (Fig. 3A). In accordance with the functional assay, CD62L, a surface molecule that is down-regulated upon T-cell activation, was maintained on the surface at higher levels if T cells were stimulated in presence of MDSC-like cells (Supplementary Fig. S2). Remarkably, monocytes cultured in transwells during the tumor education phase failed to suppress T-cell proliferation and IFNγ production (Fig. 3A) and did not prevent CD62L down-regulation (Supplementary Fig. S2).

To ensure that these results were not dependent on the allogenicity between healthy donor monocytes and patient tumor cells, CD14+ cells were isolated from the patient, from whom the melanoma cell line was established. Identical experiments were conducted in this completely HLA-matched setting. Indeed, melanoma cells induced autologous CD14+ cells to potently suppress both autologous T-cell proliferation and IFNγ production, whereas transwell separation abolished the MDSC induction (Fig. 3B).

**MDSC-like cells suppress T-cell function through COX-2 production**

To explore the mechanisms of T-cell suppression, MDSC-like cells induced by the early-passage human melanoma cell line were used in the T-cell suppression assays. Cells were treated with a panel of inhibitors, including the ROS-inactivating enzymes superoxide dismutase and catalase, iNOS and arginase inhibitors NG-monomethyl-L-arginine-acetate (L-NMMA), and N(ω)-hydroxy-nor-arginine (nor-NOHA), as well as a TGFβ neutralizing antibody. These reagents only showed inconsistent or marginal improvements of T-cell proliferation (data not shown).

Obermajer and colleagues have recently shown that a PGE2 -COX-2-mediated positive feedback loop is essential for the induction of suppressive factors by human MDSCs (17). Thus, we pretreated the MDSC-like cells with the COX-2 inhibitor celecoxib. Strikingly, COX-2 blocking significantly reduced their ability to inhibit T-cell proliferation (P < 0.05, Fig. 4A and C), indicating the importance of COX-2 enzymatic activity in MDSC-mediated T-cell suppression. PGE2, a direct product of the COX-2 enzyme, has been reported to contribute to the activation of STAT-3 signaling (26). Therefore, MDSC-like cells were pretreated with the STAT-3 signaling inhibitor AG490. This resulted in reduced ability of these cells to inhibit T-cell proliferation and IFNγ production, although it did not reach statistical significance (Fig. 4).

**PGE2 production and STAT-3 signaling are involved in the T-cell suppression mediated by CD14+ monocytes from patients with advanced stage melanoma**

We next considered it of importance to confirm these observations in MDSCs derived from patients with melanoma. Similar to the *in vitro*-induced MDSC-like cells, CD14+ monocytes isolated from patients with melanoma were found to inhibit autologous T-cell proliferation and IFNγ production in a dose-dependent fashion (Fig. 5A). A similar trend was also observed for the induction of a decreased CD62L expression on CD3+ T cells (Fig. 5B). In accordance with previously published data (7), inhibition of STAT-3 signaling in patient-derived CD14+ cells significantly decreased their T-cell inhibitory functions (P < 0.05), resulting in improvements of T-cell proliferation (Fig. 5C). Blockade of PGE2 and superoxide production resulted in consistent improvements of T-cell proliferation in the cocultures with the CD14+ patient-derived monocytes (P < 0.05, Fig. 5C), confirming the important role of PGE2 and ROS in mediating T-cell suppression. Blocking COX-2, CD80, neutralizing TGFβ and arginase production, or interrupting adenosine-related pathways showed a nonsignificant trend toward alleviating T-cell suppression (Fig. 5C).

To further investigate its T-cell-suppressive effect, synthetic human PGE2 was added to the activated lymphocytes, with or without patient-derived monocytes. Strikingly, in the presence of PGE2, CD14+ monocytes almost completely abolished T-cell proliferation and IFNγ production (Fig. 5D). This suppression was predominantly mediated by the presence of CD14+ cells, because adding PGE2 without monocytes did not affect T-cell proliferation. However, a direct suppression of T-cell IFNγ production by PGE2 was observed (Fig. 5D).
Melanoma cells induce MDSC-like cells through COX-2/PGE2 production

Given that COX-2/PGE2 and STAT-3 were essential for mediating T-cell suppression by MDSCs, we investigated whether these 2 mechanisms also contributed to the induction of MDSC-like cells by melanoma cells.

Both celecoxib and AG490 markedly abolished the induction of MDSC-like cells from monocytes by early-passage melanoma tumor cells. Thus, T-cell proliferation and IFNγ production in cocultures of MDSC-like cells pre-treated with these reagents were comparable with the levels observed in control monocytes cultured without tumor cells (Fig. 6A–C). A similar trend was observed when measuring their ability to decrease CD62L expression on activated CD3+ T cells, although not as pronounced as in the functional assay (Supplementary Fig. S3).

Next, we tested the PGE2 concentrations in the culture medium collected from the in vitro MDSC induction model by ELISA. As shown in Fig. 6D, PGE2 was produced only at low levels by control monocytes. In contrast, high amount of PGE2 was secreted by early-passage melanoma tumor cells, similar to the levels observed in the tumor–monocyte coculture. Celecoxib, but not AG490, was found to efficiently reduce the PGE2 production to a nondetectable level in the tumor–monocyte coculture. We also observed that phosphorylation of STAT-3 protein was markedly increased on monocytes after being cocultured with tumor cells.

**Figure 3.** Tumor-educated MDSC-like cells are potent suppressors of autologous T cells. A, percentage of proliferated CD3+ T cells (top left), IFNγ production (top right), and representative histograms of T-cell proliferation (bottom) with the presence of control (empty columns), GEWA-educated (black columns), and GEWA transwell cocultured (gray columns) healthy donor monocytes (n = 4) after 4 days. Data are presented as means ± SD. *, P < 0.05. B, T-cell proliferation (top left) and IFNγ production (triplicates, top right) were investigated in setup as described in A, but with the autologous CD14+ monocytes from the GEWA melanoma patient.

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between PGE₂ to its EP3 receptor resulted in a marginal inhibition of this phosphorylation (Supplementary Fig. S4).

Collectively, our data provide strong evidence that COX-2/PGE₂ production and STAT-3 activation in melanoma tumor microenvironment play an essential role in converting normal CD14⁺ cells to display MDSC-like properties.

Discussion

We have developed an in vitro model to study how melanoma cells can induce monocytic MDSCs by coculturing monocytes from healthy donors with long- or short-term established melanoma tumor cell lines. The resulting MDSCs bear a striking resemblance to those isolated from patients with advanced stage melanoma, with significant upregulation of CD14 and downregulation of HLA-DR and CD86. Our data suggest that melanoma tumor cells can arrest monocytes from healthy individuals in an immature stage associated with a capacity to impair T-cell functions through novel mechanisms involving COX-2/PGE₂ production and STAT-3 signaling.

A variety of dendritic cell and/or macrophage markers, such as CD80, DC-Sign, PD-L1, and CD1a, were significantly upregulated on monocytes after being cocultured with early-passage melanoma tumor cells. This phenotype is similar to the overexpression of CD80 and DC-Sign that we described in MDSCs isolated from patients with advanced stage melanoma (7), even though the expression of PD-L1 was not evaluated in that study. The presence of these molecules may have functional implications for MDSCs via contact-dependent mechanisms, since CD80 has been proposed to play an immune regulatory role (27–29) and PD-L1 (B7-H1) is a B7 family member that transmits a negative signal to limit T-cell functions via PD1 (30, 31). This raises the possibility that the clinical effect of interfering with the PD-L1–PD1 interaction noted in a number of studies (32, 33) may involve blocking the negative influence of MDSCs on T cells.

Similar to what has been reported in patients with ovarian cancer (34), we observed that the presence of melanoma tumor cells is able to induce the upregulation of CXCR4 on monocytes, which may result in the modulation of homing capacity of these MDSC-like cells.

The induction of MDSCs by early-passage melanoma tumor cells was found to be dependent on COX-2 production and STAT-3 signaling, although inhibition of the latter did not reach statistical significance. This has also been confirmed by using tumor cells directly expanded from melanoma lymph node metastasis (data not shown). As expected (35), the early-passage melanoma lines utilized in this study produce significant amounts of PGE₂, which induced increased...
phosphorylation of STAT-3 on monocytes primarily via binding to the EP3 receptor. Although MDSC induction was completely abolished by the COX-2 inhibitor celecoxib, a direct involvement of tumor cell-derived COX-2 in the MDSC induction remains to be established.

In addition, we find that COX-2 production and STAT-3 signaling are also involved in T-cell suppression mediated by tumor-educated, MDSC-like cells. By blocking either inducers (13, 17, 40 – 26, 38, 39), all of which have been implicated as MDSCs suppressive mechanism employed by human patients with melanoma. Human synthetic PGE2 was added to monocyte-activated T-cell coculture (empty column) or activated T cells without monocytes (gray column), n = 4. Data are presented as means ± SD. *P < 0.05.

We have recently shown that MDSCs can induce a dose-dependent decrease in the maturation, ability to take up antigen, migrate, and induce T-cell IFNγ production of DC (43). Changes in DC characteristics were most notable when "pathologic" frequencies of more than 50% CD14+ HLA-DRlow cells were present in the starting culture. In light of the findings reported here and by Obermajer and colleagues (17), it is likely that this negative effect on DC maturation by MDSCs may be mediated via a COX-2/PGE2-dependent effect, where the presence of PGE2 at early stages of DC development suppresses the differentiation of human monocytes into functional DCs.

Our group has previously proposed STAT-3 signaling as a novel T-cell–suppressive mechanism employed by human MDSCs in patients with melanoma (7). By using CD14+ monocytes isolated from patients with advanced stage melanoma,
we here confirm that MDSCs mediate T-cell suppression in a STAT-3–dependent fashion \textit{ex vivo}. We also identified a novel mechanism showing that neutralization of PGE$_2$ secreted from these monocytes resulted in consistent and significant improvement of T-cell proliferation. Moreover, we confirmed the colocalization of COX-2$^+$ tumor cells and COX-2$^+$ infiltrating CD14$^+$ monocytes by evaluating the immunohistochemistry (IHC) staining on paraffin-embedded tumor tissues from patients with stage III melanoma (data not shown). However, blockade of COX-2 activity in the patient-derived monocytes did not yield consistent T-cell rescue, which suggests that PGE$_2$ from patient MDSCs are synthesized via an alternative pathway, that is, COX-1 activity (44).

Strikingly, we found that treatment of melanoma patient-derived monocytes with PGE$_2$ provided them with capacity to induce complete abrogation of T-cell proliferation and IFN$\gamma$ production. This effect of PGE$_2$-treated monocytes was significantly more pronounced than the suppressive effects exerted by PGE$_2$ alone on the activated T cells. Similar results were also observed by treating monocytes isolated from healthy donors (data not shown). In contrast to other MDSC induction models, in which healthy donor monocytes are treated by a
A combination of cytokines for as long as 7 days (13, 17), we here show PGE2, as a single reagent to induce potent immunosuppressive MDSCs within a short period of time. This model may offer a new perspective of the biologic characteristics of MDSC induction, which could facilitate the usage of MDSCs as a therapeutic approach in autoimmune diseases or transplantation (45).

Collectively, our findings show that once monocytes are exposed to the tumor microenvironment containing high concentration of cytokines and other inflammatory mediators, COX-2 production and STAT-3 signaling will be elevated and immune-suppressive MDSCs will be induced. During tumor progression, MDSCs may also migrate from the tumor microenvironment, explaining the enhanced levels of CD14+HLA-DRlow cells in the blood of patients with melanoma. Alternatively, MDSCs could be induced in the periphery by systemic release of inflammatory mediators, although our finding that direct tumor cell contact or a close proximity to the tumor is required for MDSCs is less compatible with this view.

A better understanding of how the interaction between tumor cells and the immune system can lead to immune suppression seems crucial for the development of strategies aimed at breaking tumor-induced immune tolerance. Our results may help to explain the mechanisms behind melanoma-induced impairment of T-cell functions, and suggest an important role for COX-2/PGE2 and STAT-3 activation in MDSC-mediated immune suppression. Consequently, methods where immunotherapy is combined with inhibiting COX-2 or STAT-3, might offer a better option to eliminate MDSCs in patients with advanced stage melanoma.

References
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doi:10.1158/0008-5472.CAN-12-4115

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