Tumor-Derived MUC1 Mucins Interact with Differentiating Monocytes and Induce IL-10 high IL-12low Regulatory Dendritic Cell

Paolo Monti, Biagio Eugenio Leone, Alessandro Zerbi, Gianpaolo Balzano, Silvia Cainarca, Valeria Sordi, Marina Pontillo, Alessia Mercalli, Valerio Di Carlo, Paola Allavena and Lorenzo Piemonti

*J Immunol* 2004; 172:7341-7349; doi: 10.4049/jimmunol.172.12.7341

http://www.jimmunol.org/content/172/12/7341

---

**References**

This article cites 64 articles, 20 of which you can access for free at: http://www.jimmunol.org/content/172/12/7341.full#ref-list-1

**Subscription**

Information about subscribing to *The Journal of Immunology* is online at: http://jimmunol.org/subscription

**Permissions**

Submit copyright permission requests at: http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**

Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/alerts
Tumor-Derived MUC1 Mucins Interact with Differentiating Monocytes and Induce IL-10$^{\text{high}}$IL-12$^{\text{low}}$ Regulatory Dendritic Cell$^1$

Paolo Monti,* Biagio Eugenio Leone,‡ Alessandro Zerbi,* Gianpaolo Balzano,* Silvia Cainarca,† Valeria Sordini,† Marina Pontillo,* Alessia Mercalli,† Valerio Di Carlo,* Paola Allavena,§ and Lorenzo Piemonti$^{*†}$

Dendritic cells (DC) initiate immunity by the activation of naive T cells and control immunity through their ability to induce unresponsiveness of lymphocytes by mechanisms that include deletion and induction of regulatory cells. An inadequate presentation to T cells by tumor-induced “regulatory” DC, among several mechanisms, can explain tolerance to tumor-associated Ags. In this study, we show that tumor-derived mucin profoundly affects the cytokine repertoire of monocyte-derived DC and switch them into IL-10$^{\text{high}}$IL-12$^{\text{low}}$ regulatory APCs with a limited capacity to trigger protective Th1 responses. In fact, DC cocultured with pancreatic tumor cell lines in a Transwell system did not reach full maturation, had low immunostimulatory functions, did not produce IL-12, and released high levels of IL-10. The involvement of known tumor-derived immune-suppressive factors (e.g., vascular endothelial growth factor, TGF-β, IL-6, and IL-10) was considered and excluded. We provide evidence that tumor-derived MUC1 mucins are responsible for the impaired DC maturation and function. DC obtained in the presence of tumor microenvironment preferentially polarized IL-4$^+$ response. Moreover, T cells primed by these regulatory DC became anergic and behaved as suppressor/regulatory cells. These findings identify mucin secretion as a novel mechanism of tumor escape from immune surveillance and provide the basis for the generation of potentially tolerogenic DC. The Journal of Immunology, 2004, 172: 7341–7349.

The immune system is potentially able to recognize Ags expressed on human and experimental tumors and to mount a protective immune response (1–4). However, malignant tumors are tolerated, progress, and ultimately kill their host. Among several mechanisms, tolerance can be explained by an inadequate presentation of tumor-associated Ags to T cells.

Ag presentation is professionally performed by dendritic cells (DC) (5–8) that themselves need to be activated to prime T cell responses. Consistent with DC involvement in the immune unresponsiveness found in tumors, tumor-associated DC usually express an immature phenotype and have defective Ag-presenting ability (9–13). Accordingly, T cell responses are also impaired in cancer patients. In particular, reports have suggested that Th1-type immunity is impaired and that Th2 responses predominate in some neoplastic diseases (14–19). The tumor microenvironment may influence the differentiation of DC as well as their capacity to prime T cells in a variety of ways. Tumors have been reported to produce factors including IL-10, vascular endothelial growth factor (VEGF), TGF-β, IL-6, and M-CSF, which inhibit or suppress the differentiation and activation of DC (20–28).

Mucins are large (>200 kDa) glycoproteins with a high carbohydrate content (50–90% by weight). They are expressed by a variety of normal and malignant epithelial cells (29, 30). MUC1 mucins are giant, complex glycoproteins, comprising a polypeptide core with multiple oligosaccharide side chains in O-linkage to serine or threonine residues. Although the mature molecule is anchored within the cell surface by a characteristic transmembrane domain, most of the mucin is expressed extracellularly in an elongated form extending far beyond most other cell surface-expressed macromolecules. Although the exact function(s) of MUC1 mucins in the normal cell are still a matter for debate, they have had considerable impact as markers of many human carcinomas. In malignant cells, the expression of MUC1 is elevated and its orientation within the tissue is no longer polarized to apical surfaces. Moreover, in many human cancer cells, MUC1 mucins display an altered glycosylation, resulting in tumor-specific exposure of immature carbohydrate structures and peptide epitopes within the tandem repeat (31, 32). In particular, the elongation of the O-linked saccharide chain does not occur, thus leading to the generation of antigenic determinants such as Tn (GalNAc-O-Ser/Thr), sialyl-Tn (NeuAc-GaINac-O-Ser/Thr), and T (Gal-GalNAc-O-Ser/Thr) (33).

These tumor-specific forms of MUC1 are cleaved off the tumor cells, drain to regional lymph nodes (LN), and enter the peripheral...
circulation, where they could potentially modulate immune responses, and indeed, an immunoregulatory role for tumor-derived mucins has been recently suggested (34).

In this study, we investigated the development of DC differentiated in a tumor microenvironment, mimicked by coculturing human monocytes and tumor cells in a Transwell system (Costar, New York, NY). We demonstrate that inhibition of DC differentiation and function is associated with an augmented production of IL-10 by DC cocultured with MUC1-expressing tumor cells. We propose that tumor-derived MUC1 mucins convert them into IL-10-producing IL-12-deficient APCs with a limited capacity to trigger protective Th1 responses and the ability to promote T cell anergy and regulatory activity.

Materials and Methods
Cell lines and reagents
Human pancreatic carcinoma cell lines ASPC-1, Capan-1, MiaPaca-2, Panc-1, HS766T (American Type Culture Collection, Manassas, VA), PT45, PC13, HPAF, CFPCAC, T3M4, and PaCa 44 (kindly provided by Prof. Scarpa, Department of Pathology, University of Verona, Verona, Italy) were grown in RPMI (Biochrom, Berlin, Germany) 10% FCS (HyClone Laboratories, Logan, UT). Human recombiant granulocyte macrophage CSF (GM-CSF; specific activity, 1 x 10⁶ U/mg) was obtained from Novartis Pharmaceuticals (Basel, Switzerland). Human rIL-4 (IL-4; specific activity > 2 x 10⁶ U/mg) and IL-12 were obtained from Peprotech EC (Rocky Hill, NJ). Macrophage-inflammator protein (MIP)-3β/C chemokine ligand (CCL19) was purchased from PepTech (Rocky Hill, NJ). Neutralizing Abs were used at 10 μg/ml and included anti-IL-10, anti-TGF-β, and anti-VEGF (R&D Systems, Minneapolis, MN). All of the neutralizing Abs were added from day 0 during all of the differentiation periods. Anti-mouse IgG was from Sigma-Aldrich (St. Louis, MO). Extractive MUC1 (CA15-3) was from Fujirebio Diagnostics (Malvern, PA).

In vitro model of DC differentiation and maturation in a pancreatic cancer microenvironment
Peripheral blood mononuclear cells were obtained by density gradients, as described in Refs. 35 and 36. To evaluate the effects of the tumor environment on DC, monocytes (10⁶/ml) were seeded with GM-CSF (50 ng/ml) and IL-4 (10 ng/ml) in the lower chamber of 6-well Transwell plates with permeable membrane pores (0.2 μm; Costar). Tumor cell lines or tumor cells from primary cultures (1 x 10⁷ cells) were seeded in the upper chamber. For differentiation and function, DC were induced to mature by addition of a transfected CD40 ligand (CD40L) cell line (J558LmCd40L) or with LPS (10 ng/ml). At day 8, DC were harvested and used in functional assays.

Phenotype analysis. Phenotype analysis was performed by flow cytometry with the following mouse Abs: L243 (IgG2a, anti-MHC class II (MHC II)), W6/32 (IgG2a, anti-MHC class I (MHC I)) from Sigma-Aldrich; SK9 (IgG2b, anti-CD1a) from BD Biosciences (San Jose, CA); PAM-1 (IgG1, anti-mannose receptor (MR) (37)); and 19-2 (IgG1, anti-MR) from BD PharMingen. FITC-conjugated, affinity-purified, isotype-specific, goat anti-mouse Abs (Ancell) were also used. Results are expressed as percentage of positive cells or as mean fluorescence intensity (MFI).

Endocytosis. MR-mediated endocytosis was measured as FITC-dextran uptake as previously described (35, 36) and quantified by flow cytometry.

Chemotaxis. Cell migration was evaluated in modified Boyden chambers for 1.5 h, as previously described (38).

Mixed leukocyte reaction. Priming of allogenic T cells was assessed by [³H]thymidine uptake as previously described (35, 36). Results are presented as cpm.

Polarization of naïve T lymphocytes. Cord blood from normal end-stage deliveries was obtained from the Department of Obstetrics and Gynecology (University Vita Salute San Raffaele, Milan, Italy) under protocols approved by the board of the local ethical committee. Cord blood naïve T cells were cultured with differentially treated DC (10:1) for 10 days. At the end of the incubation, cells were collected and stimulated with PMA and ionomycin (Sigma-Aldrich) for 6 h in the presence of 10 pg/ml brefeldin A for the last 2 h. Cytokine detection was assessed by in situ staining after cell permeabilization with the following mAbs: MP4-25D2 (rat IgG1, anti-IL-4) from Serotec (Oxford, U.K.) and G7-4 (mouse IgG, anti-IFN-γ) from Bender MedSystems (Vienna, Austria).

T cell anergy assay. Allogenic T cells were first primed with either control (Ctrl)-DC or ASPC-1-DC (10:1 ratio). Ten days later, T cells were collected, depleted of residual DC by anti-CD3 or anti-MR-coated beads, and rested for 5 days in medium alone. T cells were then cultured again with fully mature DC from the same donor as the first stimulation or DC from an unrelated donor. [³H]Thymidine incorporation was measured after 5 days of culture.

T cell suppressor assay. Allogenic CD4 T cells were purified and cocultured (1 x 10⁶/ml) for 7 days with irradiated Ctrl-DC or ASPC-1-DC at a 10:1 ratio. T cells were then mixed with freshly isolated T cells at ratios ranging from 1:5 to 1:100 (suppressor/responder ratio), and with Ctrl-DC (10:1). [³H]Thymidine incorporation was measured after 5 days of culture.

Preparation of tumor supernatants (TSNs)
TSNs were prepared by seeding 1 x 10⁷ tumor cells in 1 ml of complete medium, and collected after 24 h. The effects of TSNs on DC were examined by replacing 33% of the culture medium with TSN at the initiation of culture. DC were cultured with TSN in a 2:1 ratio. Allogenic T cells were then mixed with freshly isolated T cells at ratios ranging from 1:5 to 1:100 (suppressor/responder ratio), and with Ctrl-DC (10:1). [³H]Thymidine incorporation was measured after 5 days of culture.

Expression of MUC1 in Capan-1
The epitope-tagged (Flag) MUC1 (intact MUC1 mucin with 23 tandem repeats) was described previously (39, 40). Briefly, a double-stranded synthetic oligonucleotide was designed to encode the amino acid sequence DYKDDDDQILDMVA, where DYKDDDD is the Flag epitope recognized by the mAb M2. The remainder of the inserted amino acid sequence (QILDMVA) is a result of the addition of two unique restriction enzyme sites. This oligonucleotide was ligated into a Bsm1 I site at position 232 of the MUC1 cDNA. The MUC1/Flag was inserted between Hind III and Eco RI in pCR3 vector (Invitrogen, Carlsbad, CA). The vector was linearized with Not I and transformed into Capan-1 pancreatic cancer line by Fujirebio Diagnostics. The construct was introduced into Capan-1 pancreatic cancer line by Genegun (Roche, Basel, Switzerland) transfection according to the manufacturer’s instructions. The expression of MUC1 was evaluated by anti-Flag mAb M2 (Sigma-Aldrich), anti-TAG 72 mAb that recognizes the mucin-carried sialylated-Tn epitope, a Ctrl mAb (anti-CD20), or culture plastic without Abs coating was exposed to TSN from ASPC-1 and Capan-1. After 1 h, TSNs were removed and the plastic was washed. Monoclonal antibodies were subsequently cultured on this culture plastic in the presence of GM-CSF and IL-4.

Cytokine measurement by ELISA
Cytokine production by Ctrl-DC, tumor-DC, or tumor cells was measured at the indicated times and quantified by ELISA with the following commercial kits: IL-10, IL-12 p70, IL-1β, IL-6, VEGF (Endogen, Boston, MA); and TGF-β1 (R&D Systems).

Statistical analysis
Data are expressed as mean ± SD and compared by Student’s t test or Wilcoxon rank sign test. For all analyses, a two-tailed p value of 0.05 was considered significant. Statistical analyses were performed using the Statistical Package for Social Science (SPSS 11.0; SPSS, Chicago, IL).

Results
DC differentiation and maturation in a tumor microenvironment
To evaluate the effects of a tumor environment on DC, monocytes were cultured with GM-CSF and IL-4 in the presence of 11 different pancreatic cancer cell lines in a Transwell system with 0.2-μm porous membrane. After 6 days, DC were induced to maturation by CD40 ligation for 48 h. Fig. 1A shows that 9 of 11 pancreatic cancer cell lines inhibited DC maturation, evaluated as
ASPC-1 (ASPC-1-DC) showed lower expression of CD83, as well as other molecules involved in Ag presentation (MHC class I, MHC II, CD80, CD86, CD40), compared with Ctrl-DC. In contrast, DC cocultured with the pancreatic cancer cell line Capan-1 (Capan-1-DC) were not significantly affected. A similar inhibition of DC maturation by ASPC-1 was observed when LPS was used instead of CD40L as the maturation stimulus (data not shown). Interestingly, the only maturation marker that was not down-regulated by ASPC-1-DC was the chemokine receptor CCR7. In maturing DC, CCR7 up-regulation is instrumental in guiding Ag-carrying DC to LNs where primary immune responses take place (41, 42). In chemotaxis assay, ASPC-1-DC migrated in response to CCL19 (the CCR7 ligand) (Fig. 2B).

Functional activity and cytokine production of DC differentiated in the presence of pancreatic cancer cell lines

Immature DC display potent endocytic activity, which decreases upon maturation. To study the endocytic capacity of ASPC-1-DC, FITC-dextran uptake, a marker of MR-mediated endocytosis, and lucifer yellow uptake, a nonspecific marker of macropinocytosis, were examined (Fig. 2A). Immature ASPC-1-DC showed very low levels of FITC-dextran uptake (MFI at 120 min: 30 ± 12; n = 5) compared with immature Ctrl-DC (MFI at 120 min: 125 ± 18; p = 0.02 vs ASPC-1-DC; Student’s t test) and Capan-1-DC (MFI at 120 min: 140 ± 30; p = 0.03 vs ASPC-1-DC; Student’s t test). Lucifer yellow uptake was also decreased, although to a lesser extent (data not shown). Surface expression of MR was tested in immature ASPC-1-DC and, in line with defective endocytosis, was reduced compared with Ctrl-DC and Capan-1-DC (Ctrl-DC: 84 ± 12 MFI; Capan-1-DC: 88 ± 10 MFI; ASPC-1-DC: 54 ± 6 MFI; p = 0.004 ASPC-1 DC vs Ctrl-DC; n = 13).

We next tested whether ASPC-1-DC were able to stimulate allogenic T lymphocytes in MLR. The immunostimulatory capacity of ASPC-1-DC in MLR was very low compared with that of Ctrl-DC and Capan-1-DC and was not increased by LPS or CD40L stimulation, confirming an impaired capacity to reach maturation (Fig. 2C).

The immunostimulatory capacity of mature DC is associated with their relative amounts of IL-10 and IL-12 secretion (43). Consistent with their reduced immunostimulatory capacity, mature ASPC-1-DC produced on average 6.6 ± 0.7-fold higher levels of IL-10 and 6.7 ± 2.5-fold less IL-12 than Ctrl-DC (IL-10: p = 0.001; IL-12: p = 0.001; n = 5; Student’s t test) (Fig. 3). IL-10 and

---

Table 1. Phenotype analysis of DC differentiated in the presence or absence of pancreatic cancer cell lines

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Ctrl-DC</th>
<th>Capan-1-DC</th>
<th>ASPC-1-DC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% MFI</td>
<td>% MFI</td>
<td>% MFI</td>
</tr>
<tr>
<td>MHC II</td>
<td>98 ± 50</td>
<td>98 ± 47</td>
<td>98 ± 26</td>
</tr>
<tr>
<td>MHC I</td>
<td>98 ± 48</td>
<td>68 ± 26</td>
<td>60 ± 20</td>
</tr>
<tr>
<td>CD1a</td>
<td>69 ± 5</td>
<td>90 ± 59</td>
<td>46 ± 18</td>
</tr>
<tr>
<td>CD80</td>
<td>28 ± 5</td>
<td>190 ± 25</td>
<td>85 ± 90</td>
</tr>
<tr>
<td>CD86</td>
<td>25 ± 5</td>
<td>186 ± 31</td>
<td>60 ± 70</td>
</tr>
<tr>
<td>CD40</td>
<td>98 ± 18</td>
<td>53 ± 6</td>
<td>58 ± 3</td>
</tr>
<tr>
<td>MR</td>
<td>83 ± 4</td>
<td>85 ± 10</td>
<td>58 ± 13</td>
</tr>
<tr>
<td>CD83</td>
<td>42 ± 6</td>
<td>40 ± 6</td>
<td>58 ± 3</td>
</tr>
<tr>
<td>CCR5</td>
<td>10 ± 2</td>
<td>23 ± 5</td>
<td>22 ± 5</td>
</tr>
<tr>
<td>CCR7</td>
<td>82 ± 8</td>
<td>49 ± 8</td>
<td>90 ± 8</td>
</tr>
</tbody>
</table>

* DC were differentiated from monocytes with GM-CSF (50 ng/ml) and IL-4 (10 ng/ml) in absence (Ctrl-DC) or presence of pancreatic cancer cell lines Capan-1 (Capan-1-DC) and ASPC-1 (ASPC-1-DC) for 7 days. Maturation was induced by culture with CD40L-transfected cell line (J558LmCD40L) for 48 h. Results are expressed as percentage of positive cell and MFI. Data were expressed as mean ± SD (n = 13) and compared by Student’s t test; nt, not tested; *, p < 0.05 vs Ctrl-DC.
IL-12 production of Capan-1-DC was not significantly different from that of Ctrl-DC.

Addition of a blocking anti-IL-10 mAb (10 μg/ml) partially restored the expression of CD40, CD83, CD86, and IL-12 release (data not shown), indicating that the autocrine production of IL-10 negatively affected IL-12 synthesis. Both ASPC-1 and Capan-1 cell lines did not produce detectable IL-10 even after 7-day culture and exposure to GM-CSF/IL-4 or monocytes/DC, confirming that DC were the source of IL-10 in the coculture.

Identification of tumor-derived factor affecting DC differentiation and function

A number of tumor-derived factors, such as VEGF, TGF-β, IL-10, and IL-6, that have the capacity to alter differentiation or maturation of DC have been described (20–28). TGF-β, IL-6, and IL-10 were not produced or were produced at very low levels by the 11 tumor cell lines examined, and were undetectable in ASPC-1 cells (data not shown). Low amounts of VEGF were secreted by both ASPC-1 cells and by the noninhibitory cell line Capan-1, suggesting that VEGF did not mediate the inhibitory capacity of ASPC-1. Moreover, the inhibitory effect of ASPC-1 TSN on DC was not reversed by the addition of neutralizing Abs against VEGF and TGF-β (data not shown). Because the inhibitory capacity of ASPC-1 could not be ascribed to reported mediators of DC function, other factors were examined.

Engagement of C-type lectin receptors on DC were described to be able to modify DC function in a tolerogenic fashion (44). Recently we have described that MR (a C-type 1 lectin receptor) engagement of C-type lectin receptors on DC were described to be able to modify DC function in a tolerogenic fashion (44). Recently we have described that MR (a C-type 1 lectin receptor)
engagement on myeloid DC, with selected ligands or Abs, activates an alternative maturation program characterized by a profile of anti-inflammatory and tolerogenic cytokines that would prevent the generation of Th1-polarized responses (45). Because DC stimulated through the MR up-regulate IL-10 production and down-regulate IL-12 (45), we examined whether factor(s) released by tumor cell lines were able to bind MR on DC. Pretreatment of MR-expressing DC (immature DC) with TSN from the ASPC-1 cell line dose-dependently inhibited the binding of anti-MR mAbs, while TSN from the noninhibitory cell line Capan-1 did not (Fig. 5A). Binding of a different mAb (anti-CD40) to DC was not affected by TSN pretreatment (data not shown), ruling out a non-specific effect. Recent evidence shows that MUC1 mucins, expressed and secreted at a high level in pancreatic cancer, are able to engage MR (46). A soluble form of MUC1 mucins (also named CA15-3) is found in several tumors. Interestingly, the inhibitory

**FIGURE 4.** T cell polarization by DC cocultured with cancer cells and generation of anergic regulatory T cell. A, Intrastaining cytokine production by naïve T cells cocultured for 6 days with DC differentiated in the presence of ASPC-1, Capan-1, and MiaPaca-2 cancer cell lines. A representative experiment of six performed is shown with anti-IL-4 PE-mAb and anti-IFN-γ FITC-mAb. Numbers are percentages of positive cells. B, Induction of T cell anergy by ASPC-1-DC. Allogenic T cells were first primed with CD40L-stimulated ASPC-1-DC (●) or Ctrl-DC (○). T cell cultures from both groups were restimulated 1 wk later with CD40L-Ctrl-DC generated from the same donor (Auto) or a different unrelated donor (Allo). Shown is T cell proliferation after the second MLR as proliferation index. Data are from a representative experiment of two performed. C, Suppressor cell activity of T cells originally primed by ASPC-1-DC or Ctrl-DC. Allogenic CD4 T cells were purified and cocultured (1 × 10^6/ml) for 7 days with irradiated Ctrl-DC or ASPC-1-DC at a 10:1 ratio. T cells were then mixed with freshly isolated T cells at ratios ranging from 1:5 to 1:100 (suppressor/responder ratio) and with Ctrl-DC (10:1). [3H]Thymidine incorporation was measured after 5 days of culture. Shown is a representative experiment of two performed.

**FIGURE 5.** MUC1 mucins secretion and MR binding. A, Immature Ctrl-DC (day 6 of culture) were preincubated at 4°C with TSN from ASPC-1 and Capan-1 cancer cell lines. Binding to MR was evaluated as competition of binding of a specific anti-MR (PAM-1). ASPC-1 TSN dose-dependently inhibited anti-MR binding, while Capan-1 TSN did not. Results are from one experiment of four performed. B, Kinetics of MUC1 (CA15-3) release in culture medium during DC/tumor coculture with ASPC-1 and Capan-1 cancer cell lines (ELISA). ASPC-1 and Capan-1 lines analysis for MUC1 expression (TAG 72 epitope) in flow cytometry. Results are from one experiment of two performed. C, upper panel, MUC1 mucins expression on pancreatic cancer cell lines was detected by flow cytometry and expressed as MFI; n = 3. Sialyl Tn, TAG 72 mAb; core protein, CBL 204 mAb. C, lower two panels, Immature Ctrl-DC (day 6 of culture) were preincubated at 4°C with TSN from pancreatic cancer cell lines. Bindings to MR and DC-SIGN were evaluated as competition of binding of a specific anti-MR and anti-DC-SIGN mAb. Results are expressed as change in percentage of MFI vs Ctrl (DC incubated with medium alone); n = 2.
null
mucins produced by cancer cell lines, as well as CA15-3, dose-dependently increased IL-10 production in DC. To confirm the role of MUC1 mucins in inducing DC modification, we transfected the tumor cell line Capan-1 with MUC1. The transfection of Capan-1 with full-length MUC1 (mucin with 32 tandem repeats) resulted in the expression of a sialyl-Tn glycoform of this mucin, as demonstrated by TAG 72 positivity. MUC1 mucins expression by Capan-1 inhibited maturation, suppressed IL-12 production, and increased the release of IL-10 by DC. This experiment unambiguously identified MUC1 mucins as a factor responsible for the observed effects on DC.

The finding that some tumor-derived mucins deliver a negative signal on DC may be a novel strategy by which tumor cells escape immune surveillance. DC with an IL-12p70 low IL-10 high phenotype signal on DC may be a novel strategy by which tumor cells escape immune surveillance. DC with an IL-12p70 low IL-10 high phenotype do not promote a protective Th1-type anti-tumor immune response. Rather, as shown in this and other studies, DC producing high amounts of IL-10 induce T cell anergy (48–50). The fact that tumor-glycosylated mucins may play a relevant role in modulating DC function in cancer was already supported by indirect data. Anti-MUC1 immune responses in cancer patients are characterized by a low frequency of MHC-unrestricted CTL and a lower titer of IgM Abs. Tumor-derived MUC1 mucins glycosylated with short linear carbohydrates and C-type lectin receptors on DC, and, therefore, probably the critical interaction occurs between MUC1 mucin and may differ substantially from one cell type to another. Cancer cells express aberrant forms of MUC1 mucins. In fact, the expression of distinct oligosaccharide structures, together with differential glycosylation of mucin core proteins, confers on tumor cells an enormous range of potential mucin forms. In our study, a clear characterization of the glycans structure of MUC1 mucins able to modulate DC function was not performed. In fact, we decided to use MUC1-transfected cells as a source of MUC1 mucins, because only “naked” recombinant or some glycoforms of a tandem repeat have been described (57, 58). Our data suggest that probably the critical interaction occurs between MUC1 mucin carbohydrates and C-type lectin receptors on DC, and, therefore, we considered the tumoral form of MUC1 with its unique glycans as the best source. This obviously did not permit us to identify the active glycans structure of MUC1 mucins, but the result obtained using anti-TAG 72 mAb suggested that MUC1 carrying sialylated-Tn epitope is the relevant one. A recombinant glycoform (with sialylated core 1 glycans occupying 90% of potential O-glycosylation sites) was actually tested in our system, and showed similar suppressive activity on DC (data not shown).

We recently reported that DC stimulated through the MR up-regulate IL-10 production and down-regulate IL-12 (45). The glycosylated mucin of tumor cells was a likely candidate as ligand of a variety of cancer types (56). Elevated levels of serum MUC1 mucins in patients with metastatic breast, colorectal, and ovarian cancer following immunotherapy are associated with poor survival and a lower anti-tumor immune response (34, 56). Cumulatively, all these results are consistent with an immunoregulatory role for MUC1 mucins.

MUC1 mucins can exist in many different glycoforms. In fact, the pool of O-glycan structures produced by a single cell is the product of complex biosynthetic processes, which are not template-guided and require the ordered action of multiple glycosyltransferases. Accordingly, O-glycan patterns are often cell and tissue specific and may differ substantially from one cell type to another. Cancer cells express aberrant forms of MUC1 mucins. In fact, the expression of distinct oligosaccharide structures, together with differential glycosylation of mucin core proteins, confers on tumor cells an enormous range of potential mucin forms. In our study, a clear characterization of the glycans structure of MUC1 mucins able to modulate DC function was not performed. In fact, we decided to use MUC1-transfected cells as a source of MUC1 mucins, because only “naked” recombinant or some glycoforms of a tandem repeat have been described (57, 58). Our data suggest that probably the critical interaction occurs between MUC1 mucin carbohydrates and C-type lectin receptors on DC, and, therefore, we considered the tumoral form of MUC1 with its unique glycans as the best source. This obviously did not permit us to identify the active glycans structure of MUC1 mucins, but the result obtained using anti-TAG 72 mAb suggested that MUC1 carrying sialylated-Tn epitope is the relevant one. A recombinant glycoform (with sialylated core 1 glycans occupying 90% of potential O-glycosylation sites) was actually tested in our system, and showed similar suppressive activity on DC (data not shown).

We recently reported that DC stimulated through the MR up-regulate IL-10 production and down-regulate IL-12 (45). The glycosylated mucin of tumor cells was a likely candidate as ligand of
MR. MUC1 mucins have been shown to recognize MR (46). Mucins released by ASPC-1 cell line and other DC-modulating lines, but not those from the Ctrl cell lines Capan-1 and H766T, inhibited the binding of a specific mAb to MR. The same competition for MR-binding was observed when the extractive MUC1 mucins were used. A nonspecific hindrance is unlikely, as these mucins did not affect the recognition of a nonrelated molecule CD40 by a specific anti-CD40 mAb. Myeloid DC express several C-type lectin receptors specialized in the binding of carbohydrate-rich molecules (59). Therefore, we cannot exclude the possibility that tumor-derived mucins bind to receptors expressed on the surface of DC other than MR. However, the finding that the inhibitory activity of tumor cell lines is strongly associated with a MR-binding activity, together with the evidence that engagement of MR had similar effect on cytokine production (45), suggest that the inhibitory effect of tumor-derived mucins is at least in part mediated via interaction with the MR. Interestingly, competition experiments excluded an involvement of the C-type lectin receptor DC-SIGN.

In this study, DC cocultured with inhibitory tumor cell lines showed a decreased ability to polarize naive T cells into IFN-γ-producing effectors, while IL-4-producing T cells were higher than with Ctrl-DC. It is commonly recognized that an IL-12-induced Th1 phenotype is strongly associated with a Th1/Th2 balance. It should also be remembered that evidence has recently accumulated indicating that the carcinoma-associated Tn and sialyl-Tn Ags are also expressed by helminth and protozoan parasites (64). In rodents and in humans, parasite infections are strongly interacting with MR or other C-type lectin receptors on DC, these conditions the mechanism described in our study could be applicable to the binding of a specific molecule CD40 by a specific anti-CD40 mAb. Myeloid DC express several C-type lectin receptors specialized in the binding of carbohydrate-rich molecules (59). Therefore, we cannot exclude the possibility that tumor-derived mucins bind to receptors expressed on the surface of DC other than MR. However, the finding that the inhibitory activity of tumor cell lines is strongly associated with a MR-binding activity, together with the evidence that engagement of MR had similar effect on cytokine production (45), suggest that the inhibitory effect of tumor-derived mucins is at least in part mediated via interaction with the MR. Interestingly, competition experiments excluded an involvement of the C-type lectin receptor DC-SIGN.

In this study, DC cocultured with inhibitory tumor cell lines showed a decreased ability to polarize naive T cells into IFN-γ-producing effectors, while IL-4-producing T cells were higher than with Ctrl-DC. It is commonly recognized that an IL-12-induced Th1 phenotype is strongly associated with a Th1/Th2 balance. It should also be remembered that evidence has recently accumulated indicating that the carcinoma-associated Tn and sialyl-Tn Ags are also expressed by helminth and protozoan parasites (64). In rodents and in humans, parasite infections are strongly interacting with MR or other C-type lectin receptors on DC, these conditions the mechanism described in our study could be applicable to the binding of a specific molecule CD40 by a specific anti-CD40 mAb. Myeloid DC express several C-type lectin receptors specialized in the binding of carbohydrate-rich molecules (59). Therefore, we cannot exclude the possibility that tumor-derived mucins bind to receptors expressed on the surface of DC other than MR. However, the finding that the inhibitory activity of tumor cell lines is strongly associated with a MR-binding activity, together with the evidence that engagement of MR had similar effect on cytokine production (45), suggest that the inhibitory effect of tumor-derived mucins is at least in part mediated via interaction with the MR. Interestingly, competition experiments excluded an involvement of the C-type lectin receptor DC-SIGN.

In this study, DC cocultured with inhibitory tumor cell lines showed a decreased ability to polarize naive T cells into IFN-γ-producing effectors, while IL-4-producing T cells were higher than with Ctrl-DC. It is commonly recognized that an IL-12-induced Th1 phenotype is strongly associated with a Th1/Th2 balance. It should also be remembered that evidence has recently accumulated indicating that the carcinoma-associated Tn and sialyl-Tn Ags are also expressed by helminth and protozoan parasites (64). In rodents and in humans, parasite infections are strongly interacting with MR or other C-type lectin receptors on DC, these conditions the mechanism described in our study could be applicable to the binding of a specific molecule CD40 by a specific anti-CD40 mAb. Myeloid DC express several C-type lectin receptors specialized in the binding of carbohydrate-rich molecules (59). Therefore, we cannot exclude the possibility that tumor-derived mucins bind to receptors expressed on the surface of DC other than MR. However, the finding that the inhibitory activity of tumor cell lines is strongly associated with a MR-binding activity, together with the evidence that engagement of MR had similar effect on cytokine production (45), suggest that the inhibitory effect of tumor-derived mucins is at least in part mediated via interaction with the MR. Interestingly, competition experiments excluded an involvement of the C-type lectin receptor DC-SIGN.

In this study, DC cocultured with inhibitory tumor cell lines showed a decreased ability to polarize naive T cells into IFN-γ-producing effectors, while IL-4-producing T cells were higher than with Ctrl-DC. It is commonly recognized that an IL-12-induced Th1 phenotype is strongly associated with a Th1/Th2 balance. It should also be remembered that evidence has recently accumulated indicating that the carcinoma-associated Tn and sialyl-Tn Ags are also expressed by helminth and protozoan parasites (64). In rodents and in humans, parasite infections are strongly interacting with MR or other C-type lectin receptors on DC, these conditions the mechanism described in our study could be applicable to the binding of a specific molecule CD40 by a specific anti-CD40 mAb. Myeloid DC express several C-type lectin receptors specialized in the binding of carbohydrate-rich molecules (59). Therefore, we cannot exclude the possibility that tumor-derived mucins bind to receptors expressed on the surface of DC other than MR. However, the finding that the inhibitory activity of tumor cell lines is strongly associated with a MR-binding activity, together with the evidence that engagement of MR had similar effect on cytokine production (45), suggest that the inhibitory effect of tumor-derived mucins is at least in part mediated via interaction with the MR. Interestingly, competition experiments excluded an involvement of the C-type lectin receptor DC-SIGN.

In this study, DC cocultured with inhibitory tumor cell lines showed a decreased ability to polarize naive T cells into IFN-γ-producing effectors, while IL-4-producing T cells were higher than with Ctrl-DC. It is commonly recognized that an IL-12-induced Th1 phenotype is strongly associated with a Th1/Th2 balance. It should also be remembered that evidence has recently accumulated indicating that the carcinoma-associated Tn and sialyl-Tn Ags are also expressed by helminth and protozoan parasites (64). In rodents and in humans, parasite infections are strongly interacting with MR or other C-type lectin receptors on DC, these conditions the mechanism described in our study could be applicable to the binding of a specific molecule CD40 by a specific anti-CD40 mAb. Myeloid DC express several C-type lectin receptors specialized in the binding of carbohydrate-rich molecules (59). Therefore, we cannot exclude the possibility that tumor-derived mucins bind to receptors expressed on the surface of DC other than MR. However, the finding that the inhibitory activity of tumor cell lines is strongly associated with a MR-binding activity, together with the evidence that engagement of MR had similar effect on cytokine production (45), suggest that the inhibitory effect of tumor-derived mucins is at least in part mediated via interaction with the MR. Interestingly, competition experiments excluded an involvement of the C-type lectin receptor DC-SIGN.

In this study, DC cocultured with inhibitory tumor cell lines showed a decreased ability to polarize naive T cells into IFN-γ-producing effectors, while IL-4-producing T cells were higher than with Ctrl-DC. It is commonly recognized that an IL-12-induced Th1 phenotype is strongly associated with a Th1/Th2 balance. It should also be remembered that evidence has recently accumulated indicating that the carcinoma-associated Tn and sialyl-Tn Ags are also expressed by helminth and protozoan parasites (64). In rodents and in humans, parasite infections are strongly interacting with MR or other C-type lectin receptors on DC, these conditions the mechanism described in our study could be applicable to the binding of a specific molecule CD40 by a specific anti-CD40 mAb. Myeloid DC express several C-type lectin receptors specialized in the binding of carbohydrate-rich molecules (59). Therefore, we cannot exclude the possibility that tumor-derived mucins bind to receptors expressed on the surface of DC other than MR. However, the finding that the inhibitory activity of tumor cell lines is strongly associated with a MR-binding activity, together with the evidence that engagement of MR had similar effect on cytokine production (45), suggest that the inhibitory effect of tumor-derived mucins is at least in part mediated via interaction with the MR. Interestingly, competition experiments excluded an involvement of the C-type lectin receptor DC-SIGN.


