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Dendritic Cells Induce MUC1 Expression and Polarization on Human T Cells by an IL-7-Dependent Mechanism

Baldev Varsh, David Avigan, Zekui Wu, Keith Crawford, Shawn Turnquist, Jian Ren, and Donald Kufe

The MUC1 transmembrane mucin is expressed on the surface of activated human T cells; however, the physiologic signals responsible for the regulation of MUC1 in T cells are not known. The present studies demonstrate that IL-7, but not IL-2 or IL-4, markedly induces MUC1 expression on CD3 \(^+\) T cells. MUC1 was also up-regulated by IL-15, but to a lesser extent than that found with IL-7. The results show that IL-7 up-regulates MUC1 on CD4 \(^+\), CD8 \(^+\), CD25 \(^+\), CD69 \(^+\), and memory CD45RO \(^+\) T cells. In concert with induction of MUC1 expression by IL-7, activated dendritic cells (DC) that produce IL-7 up-regulate MUC1 on allogeneic CD3 \(^+\) T cells. DC also induce MUC1 expression on autologous CD3 \(^+\) T cells in the presence of recall Ag. Moreover, DC-induced MUC1 expression on T cells is blocked by a neutralizing anti-IL-7 Ab. The results also demonstrate that DC induce polarization of MUC1 on T cells at sites opposing the DC-T cell synapse. These findings indicate that DC-mediated activation of Ag-specific T cells is associated with induction and polarization of MUC1 expression by an IL-7-dependent mechanism. The Journal of Immunology, 2005, 174: 2376–2386.

A heterodimeric type I transmembrane glycoprotein, MUC1 (CD227), is expressed on the apical or luminal borders of normal ductal epithelial cells (1). MUC1 is aberrantly overexpressed in a nonpolarized pattern on the entire surface of cells derived from diverse carcinomas (1). MUC1 is also overexpressed by certain hematologic malignancies such as multiple myeloma (2, 3), non-Hodgkin’s lymphoma (4, 5), and myeloid leukemias (3, 6). The MUC1 N-terminal subunit (MUC1 N-ter) or ectodomain consists of a variable number of 20-aa tandem repeats that are modified by O-linked glycans and extends well beyond the cell glycocalyx as a predicted rod-like structure (7, 8). The MUC1 C-terminal subunit (MUC1 C-ter) consists of a 58-aa extracellular domain, a 28-aa transmembrane domain and a 72-aa cytoplasmic domain (CD) (9). MUC1-CD interacts with members of the catenin family (β-catenin, γ-catenin, and p120ctn) of cell adhesion and signaling proteins (10–15). The interaction between MUC1-CD and β-catenin is regulated by the epidermal growth factor receptor, c-Src (16, 17), and the Wnt effector, glycogen synthase kinase 3β (11). Other studies have demonstrated that MUC1 C-ter localizes to the nucleus (13, 14, 18, 19) and coactivates β-catenin/Tcf-mediated transcription of Wnt target genes (20). MUC1 also attenuates stress-induced apoptosis by a mechanism mediated in part by localization of MUC1 C-ter to mitochondria (21, 22).

A role for the involvement of MUC1 in immune regulation has been supported by the observation that MUC1 is expressed on the surface of PHA- and anti-CD3-stimulated T cells (23–25). Moreover, recent studies have shown that TCR activation is associated with phosphorylation of MUC1-CD by Lck and ZAP-70 (26). This interaction with Lck and ZAP-70 increases the binding of MUC1 and β-catenin (26). Other insights into the regulation of MUC1 in hematopoietic cells have been derived from the demonstration that IL-7, a cytokine produced by stromal and dendritic cells (DC), induces expression of MUC1 on multiple myeloma cells (18). IL-7 is required for the development and survival of T and B cells in the mouse (27–29). Engagement of the IL-7R stimulates the proliferation of developing thymocytes (27), confers resistance of pro- and pre-T cells to apoptosis (30), promotes progression of T cell development (31, 32), and induces the rearrangement and transcription of TCR γ-chain genes (33). Other work has shown that IL-7 is necessary for the homeostatic proliferation and survival of naive T cells to stably maintain T cell number in the periphery (34). IL-7 is also necessary for the maintenance and survival of memory CD4 \(^+\) and CD8 \(^+\) T cells (35). In concert with the multiple roles of IL-7, mice deficient for IL-7 or the IL-7R exhibit substantial decreases in T cell numbers and an absence of B cells (36, 37). By contrast, in humans, defects in the IL-7R are associated with a SCID and normal B development (38, 39). These findings have indicated that IL-7 is necessary for T, and not B, cell development in humans.

The present studies demonstrate that IL-7 induces MUC1 expression on human T cells. The results also show that DC stimulate MUC1 expression on Ag-specific T cells by an IL-7-dependent mechanism. Activation of T cells by DC is associated with polarization of MUC1 on T cells at sites that oppose the T cell-DC synapse.

Materials and Methods

Isolation of T cells

T cells were isolated from leucopacks from healthy donors (Kraft Blood Donor Center, Dana-Farber Cancer Institute) using the RosetteSep Lymphoid (CD3 \(^+\)) enrichment kit according to the manufacturer’s instructions (StemCell Technologies). The T cells were harvested from the Histopaque...

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interface and incubated in RBC-lysing buffer (Hybri-Max; Sigma-Aldrich). The isolated population was 95–97% CD3+ T cells as determined by FACS analysis. Naive CD45RA+ and memory CD45RO+ T cells were further isolated by negative selection using Miltenyi magnetic microbeads according to the manufacturer’s protocol (Miltenyi Biotec).

T cell culture
T cells were cultured in RPMI 1640 medium (Mediatech) containing 10% heat-inactivated human AB male serum (Sigma-Aldrich), 100 U/ml penicillin, 100 μg/ml streptomycin, and 2 mM l-glutamine (Mediatech). Cells were stimulated with recombinant human IL-7, IL-2, IL-4, IL-15 (R&D Systems), or PHA (Sigma-Aldrich).

Monoclonal Abs
FITC-conjugated mouse anti-human mAbs directed against CD3 (clone HIT3a, IgG1), CD4 (RPA-T4, IgG1), CD8 (RPA-T8, IgG1), CD19 (HB19, IgG1), CD45RA+ (H100, IgG1), CD45RO+ (UCHL1, IgG1), CD25 (A251, IgG1), and CD69 (FN50, IgG1) were purchased from BD Pharmingen. Mouse anti-human CD3 labeled with TRI-Color was purchased from Caltag Laboratories. mAb DF3 (anti-MUC1 N-ter) has been described (1). Anti-MUC1 C-ter (Abs) was purchased from Neomarkers. FITC- and PE-conjugated control IgG1, IgG2a, IgG2b Abs, and purified mouse monoclonal IgG1 (MOPC-21) were purchased from BD Pharmingen.

Assessment of MUC1 expression
Purified T cells (1 × 10^6) suspended in 100 μl of blocking buffer containing 10% human nonimmune IgGs (Sigma-Aldrich) with 10% human AB serum (Sigma-Aldrich) in PBS were incubated for 30 min at 4°C to block nonspecific binding to FcγR. T cells were incubated with mAb DF3 (anti-MUC1 N-ter) or purified mouse mAb IgG1 for 30 min followed by secondary labeling of the cells with PE-conjugated anti-mouse IgG for an additional 30 min at 4°C. After washing, the cells were incubated with FITC-conjugated anti-CD3, -CD4, or -CD8 mAbs for 30 min at 4°C, washed in PBS, and fixed in 2% paraformaldehyde. In certain experiments, cells were stained with FITC-conjugated anti-CD25, -CD69, -CD45RA, or -CD45RO. Stained T cells were analyzed by flow cytometry using FACS-Scan and CellQuest Pro software (BD Biosciences). Viable cells were gated based on forward- and side-scatter characteristics, and a minimum of 1 × 10^4 events was acquired for analysis. Standard unstained, isotype-stained, and positively stained control T cells were used for quadrant gate settings. Where indicated, results are expressed as an expression index (EI) (percentage positive population multiplied by the mean fluorescence intensity (MFI)) (40, 41), RT-PCR and immunoblot analysis for MUC1 and β-actin expression were performed as described (18).

Purification of CD19+ B cells
B cells were isolated from whole fresh blood using the RosetteSep B cell enrichment mixture of mAbs (StemCell Technologies) according to the manufacturer’s instructions. Purity of >90% was achieved as determined by FACS analysis.

Generation of monocyte-derived DC
DC were prepared from PBMC following separation by Histopaque centrifugation. Briefly, PBMC were suspended at 1 × 10^6/m in complete RPMI 1640 medium, plated in 5-ml aliquots in six-well tissue culture plates, and incubated for 2 h at 37°C. Monocytes were isolated by plastic adherence and cultured in complete RPMI 1640 medium containing GM-CSF (1000 U/ml; Immunex) and IL-4 (1000 U/ml; R&D Systems). On day 7, the loosely adherent cells were determined to be >95% CD14+, CD11c+, CD86+, and HLA-DR+. DC maturation was induced by adding 25 μg/ml TNF-α (R&D Systems) to the GM-CSF/IL-4 cultures on day 5 and was monitored by up-regulation of CD80 and CD83 on day 7.

Cocultures of DC and T cells
DC (1 × 10^5) were cultured with allogeneic or autologous T cells (1 × 10^6) in 200 μl of complete RPMI 1640 medium in 96-well U-bottom culture plates for 5 days at 37°C. In certain experiments, cells were cultured in the presence of neutralizing Abs against IL-7, IL-12, IL-15, IL-17, or IL-12/IL-23p40 (R&D Systems; Abs used at concentrations >10-fold higher than that recommended by the manufacturer) or purified isotype-matched goat or mouse IgG control Abs (R&D Systems) for 2 h before the addition of T cells. Autologous cocultures were pulsed with 10 μg/ml tetanus toxoid (List Biological Laboratories).

Assessment of T cell proliferation
T cell proliferation in the DC-T cell cocultures was determined by incorporation of [3H]thymidine (1 μCi/well; 37 kBq; NEN-DuPont) for 18 h before the end of the culture period. The cells were then harvested onto glass fiber filter paper (Wallac) using an automated TOMTEC harvester (Mach II), dried, and placed in Betaplate sample bags (Wallac) with 10 ml of ScintiVerse (Fisher Scientific). Cell-bound radioactivity was counted in a liquid scintillation counter (Wallac; 1205 Betaplate). Data are expressed as the stimulation index (SI) determined by the ratio of [3H]thymidine incorporation over that obtained for unstimulated DC-T cell cocultures.

Detection of IL-7 in culture supernatants
Levels of IL-7 in culture supernatants were determined by a human IL-7 ELISA Immunoassay kit (BioSource International). Briefly, IL-7 standards and culture supernatants (100 μl undiluted) were incubated in duplicate on microtiter strips coated with Ab specific for human IL-7. The ELISA based on a streptavidin-HRP detection method was performed according to the manufacturer’s protocol. The OD_{450} of each sample was measured after color development using a microplate reader (Molecular Devices Corporation). IL-7 concentrations were determined by Softmax Pro software (Molecular Devices Corporation). Sensitivity of the assay was <9 pg/ml.

Immunohistochemistry
Cells (4 × 10^5) were spun onto slides with a cytofilm (Cytospin; Shandon Lipshaw), dried, and fixed in acetone. The slides were incubated with anti-MUC1 (mAb DF3) or an isotype-matched negative control at room temperature for 1 h, washed, incubated with alkaline phosphatase-labeled anti-human IgG (Vectastain ABC-AP kit; Vector Laboratories) for 30 min, followed by an additional 30-min incubation at room temperature with ABC-AP reagent solution (Vector Laboratories). Specific binding was detected (blue stain) in alkaline phosphatase substrate solution according to the manufacturer’s protocol. In DC-T cell coculture experiments, DC were stained with anti-HLA-DR, and red biotin complexes were detected with 3-aminio-9-ethylcarbazole solution. Slides were fixed in 2% paraformaldehyde (Sigma-Aldrich) and analyzed by phase contrast light microscopy (Olympus AX70 microscope) using an oil immersion objective lens (×100). T cells engaged in tightly formed conjugates with DC were quantitated for polarization of MUC1 expression.

Statistical analysis
Results are expressed as mean ± SEM. Student’s t-test was used for comparisons.

Results
IL-7 activates MUC1 expression by primary human T cells
MUC1 expression on CD3+, CD4+, and CD8+ T cells was assessed after stimulation with PHA or IL-7 for 48 h. Bidimensional FACS analysis of resting T cells cultured in parallel without stimulation showed that 6% or less of the CD3+, CD4+, or CD8+ T cells express MUC1 (Fig. 1A, left). Following PHA stimulation, mean expression of MUC1 on PHA-activated CD3+ T cells in five separate experiments was 23.7% (SEM, ±6.3%), with no significant difference between the CD4+ and CD8+ subsets (Fig. 1A, left), By contrast, IL-7 stimulation was associated with a marked increase in MUC1 expression (Fig. 1A, left). Analysis of 25 separate T cell preparations demonstrated that a mean of 47.8% (SEM, ±2.5%; range, 23–76%) of the CD3+ T cells were MUC1 positive after IL-7 stimulation, representing an 11-fold increase in MUC1 expression (p < 0.001) compared with that found in CD3+ T cells cultured in parallel without cytokine stimulation (Fig. 1A, right). After IL-7 exposure, mean values of MUC1-positive CD4+ and CD8+ T cells in 12 separate experiments were 30% (SEM, ±2.7%) and 12.8% (SEM, ±1.6%), respectively. A time course of IL-7-stimulation showed that MUC1-expressing CD3+ T cells are detectable at 24 h and near maximal at 72 h (Fig. 1B, upper panels). Calculation of the EI of MUC1+ CD3+ T cells demonstrated a substantial increase at 72 and 96 h as compared with that obtained at 24 h (Fig. 1B, upper panels). As a control, IL-7-induced MUC1 expression was blocked by incubation of CD3+ T cells in the presence of a neutralizing anti-IL-7 Ab (Fig. 1B, lower panels).
To confirm these results, RT-PCR for MUC1 expression was performed on parallel cultures of IL-7-stimulated T cells. The results demonstrate a low but detectable level of MUC1 mRNA in resting T cells that was maximally increased at 96 h of IL-7 stimulation (Fig. 1C and data not shown). Immunoblot analysis of T cell lysates with an Ab against MUC1 C-ter demonstrated similar kinetics of IL-7-induced MUC1 expression (Fig. 1D). These findings indicate that IL-7 activates MUC1 expression in T cells.

Selectivity of IL-7-induced MUC1 expression by T cells

To further define the effects of IL-7 on MUC1 expression, we treated purified T cells with increasing (0.5–60 ng/ml) concentrations of IL-7 for 72 h. MUC1 expression was significantly increased by stimulation of CD3⁺ T cells with 5 ng/ml IL-7 and reached near-maximal levels at 30–40 ng/ml IL-7 (Fig. 2A). By contrast, IL-7 had no detectable effect on MUC1 expression on CD19⁺ B cells (Fig. 2A). The EI of CD3⁺ T cells expressing MUC1 increased >15-fold from baseline to a peak EI of >2500 at an IL-7 concentration of 60 ng/ml, whereas the EI of CD19⁺ B cells was <500 following stimulation at the different IL-7 concentrations (Fig. 2B). Unstimulated and IL-7-stimulated T cells were also analyzed for expression of MUC1 and the CD25 and CD69 activation markers. The results demonstrate that MUC1 expression is low on unstimulated CD25⁻ and CD69⁻ T cells (Fig. 2C). In concert with previous findings, IL-7 stimulation was associated with marked up-regulation of CD25 (42, 43). Moreover, after IL-7 stimulation, MUC1 was increased on CD25⁺ T cells to a much greater extent than on CD69⁺ T cells (Fig. 2C), indicating that up-regulation of MUC1 is associated with that of the IL-2R. IL-7 is a member of the family of cytokines that signal through the common γ-chain (γc). To further assess specificity of IL-7-induced MUC1 expression, T cells were stimulated with other γc cytokines. Exposure of purified T cells to 5–60 ng/ml IL-2 for 72 h had little effect on MUC1 expression (Fig. 2D). Similar results were obtained.
with IL-4 (Fig. 2D). In these experiments, CD3+ T cells expressing MUC1 did not exceed 9%, and they exhibited a low EI (Fig. 2D). However, exposure of the purified T cells to IL-15 was associated with a significant increase in MUC1 expression, although not as pronounced as that found for IL-7 (Fig. 2D). The EI of CD3+ T cells expressing MUC1 after stimulation with IL-15 for 72 h did not exceed 3700, compared with an EI of nearly 8000 for IL-7-stimulated T cells (Fig. 2D). These results indicate that the effects of IL-7 on MUC1 expression are selective for T, and not B, cells, and that similar, but less pronounced, effects are observed with IL-15.

**IL-7 stimulates MUC1 expression on naive CD45RA+ and memory CD45RO+ T cells**

IL-7 functions in the extrathymic expansion and maintenance of naive CD45RA+ T cells (44) and facilitates memory CD45RO+ T cell differentiation (35). To assess the effects of IL-7 on MUC1 expression by CD45RA+ T cells, highly purified (≥96%; Fig. 3A, left upper panel) negatively selected CD45RA+ cells were left untreated or stimulated with IL-7 for 72 h. Bidimensional FACS analysis using a FITC-conjugated anti-CD45RA Ab demonstrated that MUC1 expression is detectable on 13% of unstimulated CD45RA+ T cells (Fig. 3A, upper panel). Notably, compared with unstimulated CD3+ T cells, constitutive expression of MUC1 on CD45RA+ T cells was nonspecifically increased during the negative-selection procedure. Stimulation with IL-7 was associated with a 4-fold increase in MUC1 expression (Fig. 3A, upper panel). IL-7 also increased MUC1 expression on CD45RO+ T cells, although to a lesser extent than that found for CD45RA+ T cells (Fig. 3A). In six separate experiments, mean MUC1 expression on unstimulated CD45RA+ cells was 11% (SEM, ±2.8%; range, 4–23.5%) compared with 56.7% (SEM, ±3.2; range, 45–67%) on IL-7-stimulated CD45RA+ T cells (p < 0.001) (Fig. 3B). The results also demonstrated a mean of 16.9% (SEM, ±2.9%; range, 5.9–22.7%) for unstimulated MUC1-positive CD45RO+ cells and after IL-7 stimulation a mean of 36.3% (SEM, ±1.2%; range, 31.7–40.3%) (p < 0.001) (Fig. 3B). MUC1 expression on IL-7-stimulated CD45RA+ T cells was significantly up-regulated.
compared with similarly stimulated CD45RO+ T cells (p < 0.01) (Fig. 3B). Moreover, MUC1+CD45RA+ dual-positive T cells demonstrated a 5.6-fold higher mean MFI of 163 (SEM, ±22.3; range, 110 – 237) compared with a MFI of 29 (SEM, ±3.4; range, 19 – 44) for MUC1+CD45RO+ T cells. To further assess the pattern of MUC1 expression, cytospin preparations of CD45RA+ and CD45RO+ cells were subjected to immunohistochemical staining. Unstimulated CD45RA+ cells showed weak staining with anti-MUC1 over the cell surface. In contrast, IL-7-stimulated CD45RA+ cells exhibited substantially more intense staining (Fig. 3C). Similarly, IL-7-stimulated CD45RO+ cells demonstrated more intense staining for MUC1 expression compared with that seen for unstimulated cells (Fig. 3D).

**DC induce MUC1 expression on T cells by an IL-7-dependent mechanism**

Mature DC produce IL-7 and thereby contribute to T cell activation (45, 46). To determine whether the interaction between DC and T cells is associated with induction of MUC1 expression, we first cocultured CD3+ T cells with allogeneic DC. Bidimensional FACS analysis of the CD3+ T cells demonstrated that the allogeneic coculture stimulates MUC1 expression (Fig. 4A, left). From eight separate experiments, the mean percentage of MUC1+CD3+ cells in the allo-coculture was 49.5% (SEM, ±2.3%) (Fig. 4A, right). To assess the involvement of IL-7, the cocultures were incubated in the presence of a control goat IgG or a neutralizing anti-IL-7 Ab. Anti-IL-7 and not the control IgG substantially decreased the percentage of CD3+ T cells that express MUC1 (Fig. 4A, left and right). Incubation of CD3+ T cells with autologous DC was associated with an increase in MUC1 expression, although not to the extent observed with allogeneic DCs (Fig. 4B, upper panels). Moreover, anti-IL-7 and not a control IgG decreased induction of MUC1 expression in response to autologous DC and tetanus toxoid.
FIGURE 4. DC-induced MUC1 expression on T cells is mediated by an IL-7-dependent mechanism. A, Purified T cells were cocultured with mature allogeneic DC in the presence of 10 μg/ml control goat IgG1 or anti-IL-7 for 5 days. MUC1 expression on CD3⁺ T cells was determined by FACS analysis (left panels). The percentage (mean ± SEM) of dual MUC1⁺ CD3⁺ T cells was determined for eight separate experiments (right panel). B, MUC1 expression was assessed on CD3⁺ T cells cocultured with autologous immature or mature DC in the absence or presence of tetanus toxoid (TT), control goat IgG1, or anti-IL-7 for 5 days (upper panels). The percentage of MUC1⁺ CD3⁺ T cells stimulated by immature (■) or mature (□) DC was determined from six separate experiments (lower panel). The asterisk (*) denotes a p < 0.001 when compared with autoco-cultures pulsed with tetanus toxoid (TT). C and D, T cells were cultured in the presence of IL-7 (■), IL-2 (□), or PHA ( □) for 72 h and then cocultured with immature (C) or mature (D) DC and tetanus toxoid in the presence of the indicated concentrations of anti-MUC1. T cell proliferation was measured after 5 days by [³H]thymidine uptake. The SI (mean ± SEM) was determined from four separate experiments.
The IL-7-dependent induction of MUC1 in the response to tetanus toxoid was confirmed in six separate experiments (Fig. 4B, lower panel). To determine whether MUC1 expression affects T cell activation and subsequent proliferation, we incubated T cells in the presence of IL-7 and, as controls, IL-2 or PHA. Coculture of the IL-7-stimulated T cells with immature DC and tetanus toxoid was associated with a >10-fold stimulation of proliferation (Fig. 4C). The SI was significantly less when IL-2- or PHA-stimulated T cells were incubated with immature DC and tetanus toxoid (Fig. 4C). To further assess the role of MUC1, the IL-7-stimulated T cells were incubated with 10–200 μg/ml anti-MUC1. Blocking the MUC1 ectodomain with the anti-MUC1 Ab had no detectable effect on the SI (Fig. 4C). Anti-MUC1 also had little effect when IL-2- and PHA-stimulated T cells were cocultured with immature DC and tetanus toxoid (Fig. 4C). Similar results were obtained when the experiments were performed with mature DCs (Fig. 4D). These findings indicate that coculture of T cells with allogeneic DC or autologous DC and recall Ag results in up-regulation of MUC1 expression by an IL-7-dependent mechanism and that blocking the MUC1 ectodomain has no apparent effect on Ag-specific T cell activation.

Independent function of IL-7 in the induction of MUC1 expression on T cells

PCR analysis of activated DC has demonstrated the expression of IL-7 and other cytokines, including IL-12 and IL-15 (45). To assess the levels of IL-7 protein produced by DC, we assayed the culture supernatants by ELISA. The results demonstrate that IL-7 is undetectable in supernatants of immature and mature DC incubated in the absence of T cells (Fig. 5A). T cells cultured alone in parallel also had no detectable IL-7 in supernatants (data not shown). However, incubation of DC with allogeneic T cells was associated with the detection of IL-7 at levels of ~25 pg/ml (Fig. 5A). Moreover, whereas incubation of DC with autologous T cells had no apparent effect on IL-7 production, the addition of tetanus toxoid resulted in IL-7 concentrations of 15–25 pg/ml (Fig. 5A). As a control, the addition of anti-IL-7 to the coculture supernatants

**FIGURE 5.** Role of IL-7 as a key cytokine in the induction of MUC1 on T cells. A. Immature and mature DC were cultured alone (2 × 10⁴/well) or in cocultures with allogeneic or autologous T cells at a DC:T cell ratio of 1:10 for 5 days. Tetanus toxoid was added to the autologous DC-T cell cocultures as indicated. Culture supernatants were assayed for IL-7 by ELISA. The results are expressed as the IL-7 concentration (mean ± SEM) of four separate experiments.

B. DC cocultured with allogeneic T cells were incubated for 5 days in the absence (○) or presence of neutralizing Abs against IL-12, IL-15, IL-17, and IL-12/23 p40 at concentrations of 1 (△) and 5 (□) μg/ml. Nonspecific goat and mouse IgG were used as isotype controls.

C and D. Immature (C) and mature (D) DC cocultured with autologous T cells and tetanus toxoid were incubated for 5 days in the absence (□) or presence of the indicated Abs at 1 (△) and 5 (□) μg/ml. CD³⁺ T cells that express MUC1 were analyzed by FACS. The results are expressed as the percentage (mean ± SEM) of MUC1/CD³⁺ cells as determined from four separate experiments.
Figure 6. DC induce polarization of MUC1 on IL-7-stimulated CD3+ T cells. A, CD3+ T cells were stimulated with IL-7 for 72 h and then stained with the indicated Abs. B, IL-7-stimulated CD3+ T cells were cocultured with allogeneic DC for 5 days and then stained with anti-HLA-DR (red) and IgG1 or anti-MUC1 (blue). C, IL-7-stimulated CD3+ T cells were cocultured with autologous immature (left) and mature (right) DC for 5 days in the presence of tetanus toxoid and then stained with anti-HLA-DR (red) and anti-MUC1 (blue) (magnification, ×100).

Table I. Quantitation of T cells with polarized MUC1 expression in DC-T cell conjugates in relation to contact interface (immunological synapse)*

<table>
<thead>
<tr>
<th>DC-T Cell Coculture</th>
<th>No. of DC-T Cell Conjugates Analyzed</th>
<th>Total No. of T Cells Counted</th>
<th>No. (%) of T Cells in DC-T Cell Conjugates with MUC1 Expression</th>
<th>Absence of MUC1 on T Cells in DC-T Cell Conjugates (No. (%))</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) IL-7-stimulated T cells + allo-DC</td>
<td>42</td>
<td>265</td>
<td>206 (77.7)</td>
<td>59 (22.3)</td>
</tr>
<tr>
<td>(2) IL-7-stimulated T cells + auto-immature DC</td>
<td>24</td>
<td>131</td>
<td>107 (81.7)</td>
<td>24 (18.3)</td>
</tr>
<tr>
<td>(3) IL-7-stimulated T cells + auto-mature DC</td>
<td>36</td>
<td>355</td>
<td>292 (82.3)</td>
<td>63 (17.7)</td>
</tr>
<tr>
<td>(4) Unstimulated T cells + allo-DC</td>
<td>60</td>
<td>322</td>
<td>261 (81.1)</td>
<td>61 (18.9)</td>
</tr>
<tr>
<td>(5) Unstimulated T cells + auto-immature DC + TT</td>
<td>20</td>
<td>155</td>
<td>131 (84.5)</td>
<td>24 (15.5)</td>
</tr>
<tr>
<td>(6) Unstimulated T cells + auto-mature DC + TT</td>
<td>30</td>
<td>200</td>
<td>164 (82)</td>
<td>36 (18)</td>
</tr>
<tr>
<td>(7) Unstimulated T cells + auto-immature DC + goat IgG + TT</td>
<td>35</td>
<td>287</td>
<td>238 (82.9)</td>
<td>49 (17.1)</td>
</tr>
<tr>
<td>(8) Unstimulated T cells + auto-mature DC + goat IgG + TT</td>
<td>30</td>
<td>190</td>
<td>159 (83.7)</td>
<td>31 (16.35)</td>
</tr>
<tr>
<td>(9) Unstimulated T cells + auto-immature DC + TT + anti-IL-7</td>
<td>60</td>
<td>386</td>
<td>72 (18.7)</td>
<td>39 (10.1)</td>
</tr>
<tr>
<td>(10) Unstimulated T cells + auto-mature DC + TT + anti-IL-7</td>
<td>35</td>
<td>258</td>
<td>38 (14.7)</td>
<td>24 (9.3)</td>
</tr>
</tbody>
</table>

Median Values | 35 | 261.5 | 185 (82.2)* | 42.5 (18)* |

* DC-T cell cocultures were harvested on day 5, and cytospin slides were prepared for immunohistochemical staining. DC-T cell conjugates were analyzed as described in Materials and Methods. Allo-DC, Allogeneic DC; auto-immature DC, autologous immature DC; auto-mature DC, autologous mature DC; TT, tetanus toxoid.

* Median values for cocultures 1–8.
DC induce MUC1 expression on T cells by an IL-7-dependent mechanism

IL-7 is produced by stromal cells and DC (45, 46, 51). Consequently, we reasoned that interactions between T cells and stromal cells or DC might be associated with induction of MUC1. Indeed, incubation of unstimulated CD3+ T cells with allogeneic DC was associated with up-regulation of MUC1 expression. We also found that incubation of CD3+ T cells with autologous DC and tetanus toxoid as a recall Ag resulted in the induction of MUC1 expression. When CD3+ T cells were incubated with autologous DC in the absence of recall Ag, MUC1 expression was also increased, but to a limited extent, on those T cells that formed synapses with DC.

Assessment of IL-7 production by activated DC demonstrated picogram/milliliter concentrations in the coculture supernatants, levels 3 logs lower than that used to induce MUC1 expression on T cells with rIL-7. We propose that the concentrations of IL-7 found at the DC-T cell synapse would be substantially higher than that detected in the culture supernatants and that the local IL-7 concentrations produced by activated DC are sufficient to induce MUC1 expression on T cells in the DC-T cell complex. In support of this conclusion, treatment of the DC-T cell cocultures with anti-IL-7 neutralizing Ab substantially decreased MUC1 expression. These results suggest that induction of MUC1 may accompany CD25 (IL-2R) expression in response to DC-T cell interactions and TCR signaling (49, 50).

Discussion

MUC1 expression by T cells is induced by IL-7

Stimulation of resting T cells with PHA or anti-CD3 has been associated with induction of MUC1 expression (23–25). However, the signals responsible for the induction of MUC1 expression in T cells are not known. The present results demonstrate that MUC1 is induced by IL-7 stimulation of resting T cells. Compared with PHA- and anti-CD3-treated T cells, which express relatively low levels of MUC1 (25), IL-7 conferred a substantial induction of MUC1 expression. Our results also indicate that treatment of T cells with IL-2 or IL-4 has little effect on MUC1 expression. However, the demonstration that IL-15 induces MUC1, but to a lesser extent than IL-7, indicates that this response is not restricted to IL-7. As an additional control, thymic stromal lymphopoietin, a cytokine that interacts with a receptor complex that includes the IL-7Rα subunit (48), had no detectable effect on MUC1 expression by T cells (data not shown). IL-7 stimulates MUC1 expression in multiple myeloma cells (18). By contrast, exposure of CD19+ B cells to IL-7 had no apparent effect on MUC1 expression. The present studies further demonstrate that MUC1 is detectable at low levels on unstimulated CD3+ T cells. A low signal for MUC1 expression was also detected in unstimulated T cells by RT-PCR. Moreover, IL-7 stimulation was associated with a marked increase in MUC1 mRNA, indicating that IL-7 induces transcription of the MUC1 gene. In concert with these results, a low level of MUC1 is detectable in lysates from resting T cells, and in response to IL-7, expression of this MUC1 subunit paralleled the increases found for MUC1 transcripts and MUC1 N-ter. Analysis of different T cell subtypes demonstrated that IL-7 induces MUC1 expression on both CD4+ and CD8+ T cells. We also found that IL-7 induces MUC1 on activated CD25+ T cells and, to a lesser extent, on CD69+ T cells. These results suggest that induction of MUC1 may accompany CD25 (IL-2R) expression in response to DC-T cell interactions and TCR signaling (49, 50).

The present results further demonstrate that IL-7 induces MUC1 expression on both naive CD45RA+ and memory CD45RO+ T cells. These findings thus provide the first evidence that MUC1 is induced in diverse subsets of T cells by IL-7, a cytokine that regulates T cell development.

The CD3+ T cells were cultured with immature or mature allogeneic DC (Fig. 7C). Moreover, DC-induced up-regulation and polarization of MUC1 was blocked by anti-IL-7 (Fig. 7D). Visual phase contrast light microscopy assessment of multiple DC-T cell conjugates was performed to confirm the polarization of MUC1 under different experimental conditions. In cocultures of unstimulated and IL-7-stimulated T cells with DC (cocultures 1 to 8; Table I), >75% (range, 77–85%) of the T cells in tightly formed junctions with the DC expressed MUC1 in a polarized pattern that opposed the DC-T cell contact interface. By contrast, only 18% (median) of the T cells analyzed showed MUC1 that was not opposed to the contact interface (Table I). In autologous cocultures with both immature and mature DC in the presence of recall Ag (tetanus toxoid) and neutralizing Ab, <19% of the T cells expressed MUC1 compared with >80% in parallel cocultures pulsed with goat IgG (Table I). These findings indicate that DC-mediated Ag responses are associated with induction and polarization of MUC1 expression on T cells by an IL-7-dependent mechanism.

FIGURE 7. DC up-regulate and polarize MUC1 expression on unstimulated CD3+ T cells. A, Unstimulated CD3+ T cells were stained with the indicated Abs. B, Unstimulated CD3+ T cells were cocultured with allogeneic DC for 5 days and then stained with the indicated Abs. C, Unstimulated CD3+ T cells were cocultured with autologous immature (left) and mature (right) DC for 5 days in the presence of tetanus toxoid and then stained with the indicated Abs. D, Unstimulated CD3+ T cells were cocultured with mature DC and tetanus toxoid in the presence of goat IgG (left) or anti-IL-7 (right) for 5 days and then stained with anti-HLA-DR (red) and anti-MUC1 (blue) (magnification, ×100).
induction of MUC1 by T cells interacting with DC. To our knowledge, this is the first demonstration that T cells respond to DC with induction of MUC1 expression. Previous work had demonstrated that MUC1 can interact with ICAM-1 (52, 53) and that induction of PHA-stimulated T cells with an Ab that binds to the tandem repeats of the MUC1 ectodomain is associated with inhibition of the proliferative response (23). In the present studies, however, incubation of the DC-T cell cocultures with anti-MUC1 (mAb DF3), which reacts with the tandem repeats (1), had no effect on T cell proliferation. Anti-MUC1 also had no effect on T cell proliferation when added at increasing concentrations to auto-cocultures with DC and tetanus toxoid. Moreover, anti-MUC1 had no effect when IL-7, IL-2, or PHA-stimulated T cells were incubated with autologous DC (immature or mature) and tetanus toxoid. These findings indicate that DC induce MUC1 on T cells and that engagement of the MUC1 ectodomain with Ab has no apparent effect on the induction of T cell proliferation.

**Polarization of MUC1 expression on activated T cells**

The MUC1 ectodomain consists of 50–110 tandem repeats, each containing 20 aa, that are extensively modified by O-linked glycans (7, 8). As such, the MUC1 ectodomain has been predicted to extend as a rigid rod-like structure beyond the glycoalyx to ~200–500 nm from the plasma membrane (54). Of note, the interaction between T cells and APC occurs at a distance of ~15 nm to facilitate TCR-peptide MHC binding (55). Thus, MUC1 expression on the T cell surface would be expected to interfere with TCR engagement. The present results provide a mechanism by which MUC1+ T cells can interact with DC. Studies with IL-7-stimulated T cells demonstrated that MUC1 is expressed on the entire cell membrane. More importantly, we found that, when IL-7-stimulated T cells are incubated with DCs, MUC1 expression is positioned at sites opposed to the synapse. A similar pattern of MUC1 expression was observed when unstimulated T cells were cocultured with autologous DC and tetanus toxoid. Formation of the immunological synapse is initiated by adhesion between the T cell and APC, and is mediated by LFA-1-ICAM-1, CD2-CD58, and/or DC-specific ICAM-3-grabbing nonintegron (DC-SIGN)-ICAM-3 interactions (56). LFA-1 and ICAM-1 can interact at ~40 nm, a distance that would not be readily achieved with the MUC1 ectodomain extending several hundred nanometers beyond the glycoalyx. Thus, polarization of MUC1 to a site not involved in the synapse is conceivably necessary for the initiation of T cell-APC adhesion. In this model, IL-7-induced expression of MUC1 on activated T cells would need to be coupled with signals for MUC1 polarization. Formation of the synapse is initiated by both adhesion and chemokine receptor-mediated signals. Chemokines that contribute to T cell polarization include secondary lymphoid tissue chemokine (SLC) and EBI1-ligand chemokine (ELC), which bind to the CCR7, and stromal-derived factor-1 (SDF-1), which binds to CXCR4 (56). Further experimentation will thus be needed to determine whether IL-7 acts in concert with chemokines to induce both expression and polarization of MUC1 on the T cell surface.

**Why is MUC1 expressed on activated T cells?**

MUC1 is localized to the apical borders of secretory epithelial cells where it contributes to a protective mucous barrier against environmental stress. The available evidence indicates that, following shedding of the MUC1 ectodomain, the MUC1 C-terminal subunits functions in transducing stress signals to the interior of the cell. The MUC1 C-terminal localizes to the nucleus and coactivates β-catenin/Tcf-mediated transcription of Wnt target genes (20). In the stress-repair pathway of epithelial cells, MUC1 C-terminal is also targeted to mitochondria (22). In concert with these findings, MUC1 functions in blocking stress-induced apoptosis (21, 22). In the context of T cells, polarized expression of MUC1 may contribute to protection against cell-cell or cell-matrix interactions at sites opposing the immunological synapse. Recent studies have demonstrated that activation of T cells is associated with Lck- and ZAP70-mediated phosphorylation of the MUC1 cytoplasmic domain (26). Lck and ZAP70 increase the interaction between MUC1 and β-catenin, and thereby the potential for coactivation of Wnt target genes (26). The activation of transcriptional responses to extracellular Wnt signals is of importance to the survival of immature T cells (57). Moreover, IL-7 stimulation of T cells is associated with survival through up-regulation of the antiapoptotic Bcl-2 and Bcl-xL proteins (58, 59). In other cell types, MUC1 also contributes to survival by up-regulating Bcl-xL expression (60). Importantly, generation of a Muc1-deficient mouse that expresses low levels of Muc1 has shown that thymocytes fail to survive to the CD4+CD8+ stage (61). Thus, IL-7-induced expression of MUC1 could confer a survival function for T cells through coactivation of Wnt signals, localization to mitochondria, and/or up-regulation of Bcl-xL. In this regard, IL-7-induced MUC1 expression could thus contribute to the expansion and maintenance of Ag-specific T cells.

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**References**


