Expression of MUC1 Mucin on Activated Human T Cells: Implications for a Role of MUC1 in Normal Immune Regulation

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Abstract

MUC1 mucin is expressed by normal and malignant epithelial cells and is thought to function through cell-cell interactions and transmembrane signal transduction events. Secreted cancer-associated MUC1 is immunosuppressive and inhibits human T-cell proliferation. We report here that newly synthesized MUC1 is expressed on the surface of mitogen-activated human T cells and is also found in soluble form in the supernatants from cultures of mitogen-activated human T cells. After removal of the mitogenic stimulus from the T-cell cultures, MUC1 expression is down-regulated. The addition of anti-MUC1 monoclonal antibody to mitogen-activated cultures partially inhibits the T-cell proliferative response. These data suggest that MUC1 serves an immunomodulatory function for human T lymphocytes.

Introduction

Many functions have been proposed for MUC1 (1) that include steric hindrance by the large glycosylated extracellular domain of cell-cell or cell-substratum interactions, remodeling of the cytoskeletal network, and down-regulating the activities of other molecules such as catenins, cadherins, or integrins via signal transduction events (2). Its cytoplasmic tail is phosphorylated, consistent with a transmembrane signal transduction function for MUC1 (3, 4). Elevated levels of serum MUC1 are associated with poor survival and a lower anticancer immune response of cancer patients after immunotherapy (5, 6). Direct demonstration of an immunosuppressive role of MUC1 mucin came from recent work (7) showing that cancer-associated, MUC1 polypeptide core specifically inhibited human T-cell proliferation at 0.1-4 ng/ml MUC1 mucin and synthetic tandem repeats of the mucin came from recent work (7) showing that cancer-associated, (5, 6). Direct demonstration of an immunosuppressive role of MUC1 mucin that similar MUC1-mediated immunoregulatory mechanisms are operational in the normal functioning of the immune system.

Materials and Methods

Cell Surface Immunofluorescence Staining. For detection of cell surface antigens, normal human PBLs, cultured as indicated in each experiment, were stained essentially as described previously (8). Anti-MUC1 MAb B27.29 (2 μg/5 x 10^6 T cells; Ref. 9) or isotype control antibody B80.3 (2 μg/5 x 10^6 T cells) were used with indirect labeling with FITC or a Phycoerythrin-conjugated second antibody (Goat IgG1). Isotype control groups had <2% positive cells. The samples were analyzed by flow cytometry using FACSORT (Becton Dickinson). The percentage of positive cells was defined as the

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2 The abbreviations used are: PBL, peripheral blood lymphocyte; MAb, monoclonal antibody; RT-PCR, reverse transcription-PCR; PHA, phytohemagglutinin.

fraction of cells exhibiting fluorescence intensities beyond a region set to exclude at least 98% of the control isotype-matched antibody stained cells.

Proliferation Assay. PBLs were stimulated with PHA (1 μg/ml) for 3 days. T cells were then harvested and recultured in the presence or absence of OKT3, B27.29 MAb, isotype control MAb B80.3, and goat-antimouse in 96-well plates in quadruplicate. On the 3rd day, the wells were pulsed with 1 μCi/well 3H Thymidine (Amersham Corp.). The incorporation of 3H Thymidine into the DNA of proliferating T cells was measured after harvesting the plates after 18-24 h and counting in a liquid scintillation counter (Beckman LS 6000IC; Mississauga, Ontario, Canada).

Determination of mRNA for Human MUC1 by PCR. MUC1 mRNA in the lymphocytes was analyzed using RT-PCR. Total RNA was extracted from the T cells using Trizol according to the manufacturer’s instructions (Life Technologies) and was reverse transcribed into cDNA with M-MLV Reverse Transcriptase and oligo d(T; Perkin-Elmer Corp., Norwalk, CT). Subsequent DNA amplification was performed in the same tubes using AmpliTaq DNA polymerase (Perkin-Elmer Corp.) and MUC1-specific primers (5'-TC-TACTCTGTTGCAACACGG-3' and 5'-TTATATCGAGAGGCTTGCTCTCC-3'). These primers spanned a region within the genomic DNA that contained two introns and would result in the amplification of a 489-bp fragment from RNA and a 738-bp fragment from any contaminating genomic DNA. MCF-7 (a human breast cancer cell line obtained from American Type Culture Collection) RNA was used as a positive control, and mouse spleen RNA was used as a negative control. RNA-specific primers for human β actin (Stratagene, La Jolla, CA) were used as a positive control with each RNA sample. Amplified fragments were run on a 2% agarose gel.

Determination of Soluble MUC1 Mucin in Cell Supernatants. MUC1 mucin in cell culture supernatants was determined with a sandwich enzyme immunoassay using MAb B27.29 (9) as solid phase on polystyrene microwells (Nunc, Waco, Japan), horseradish peroxidase (Boehringer Mannheim), conjugated MAb B27.29 as tracer, and tetramethylbenzidine (Biomira Diagnostics Inc., Toronto, Ontario, Canada) as substrate.

Results

Expression of Cell Surface MUC1 Mucin and MUC1 mRNA by PHA-activated Human T Cells. We examined MUC1 expression on days 1, 3, and 6 after in vitro culture of human PBLs with or without PHA stimulation (Fig. 1 A). In cultures without added PHA there was a low (1-4%) number of MUC1 positive cells in the CD3+ T cell population (only day 6 is illustrated in Fig. 1A). In PHA-stimulated cultures there was an increase in the number of B27.29+ CD3+ cells to a peak of approximately 80% positive cells 3–6 days after culture initiation. As a control for MAB B27.29 binding specificity, we determined whether the presence of soluble MUC1 mucin inhibits MAB B27.29 binding to 3-day PHA-activated T cells. We observed a MUC1 mucin dose-dependent inhibition of staining of activated human T cells with B27.29: at 1 μg of MUC1 mucin, a 25% inhibition of binding was noted; at 10 μg of MUC1, a 45% inhibition of binding was noted; and at 50 μg of MUC1, a 65% inhibition of B27.29 binding to activated T-cells was noted. A negative control mucin (OSM) did not inhibit binding of B27.29 to PHA-activated T cells (0% inhibition of binding of MAB B27.29 at 50 μg of OSM).

To confirm that the appearance of cell surface MUC1 on activated T cells represents the presence of newly synthesized mucin, RT-PCR
A. FACS Analysis:

Fig. 1. Time course of MUC1 expression by activated T cells. PBLs were cultured for 1, 3, and 6 days in the absence or presence of mitogen PHA. At each time point, cells were collected and stained for CD3 and MUC1 expression. A. cell surface expression determined by fluorescence-activated cell sorting. The number in parentheses represents the percentage of MUC1-positive T cells. B. RT-PCR for mRNA for MUC1. RT-PCR was performed on T cells cultured in the presence or absence of PHA for 1, 3, or 6 days. mRNA for MUC1 was present in PHA-stimulated cells and correlated with surface expression of MUC1.

B. RT-PCR Analysis:

Fig. 2. Down-regulation of MUC1 mucin expression on activated T cells. PBLs were cultured in the presence of PHA for 6 days. At day 6, the cells were washed, harvested, and recultured in the absence of PHA (media alone) for an additional 3–6 days. CD3-Direct FITC staining was also performed in parallel cultures. All samples from lymphocytes that had been stimulated with PHA produced a fragment of approximately 489 bp, indicating the presence of human MUC1 mRNA. Fig. 1B demonstrates that MUC1-specific mRNA could be detected after 24 h of PHA stimulation with increased expression noted at days 3 and 6. Faint bands were also noted in the cultures not stimulated with PHA, which are consistent with the finding of 1–4% MUC1+ cells in nonstimulated T-cell cultures.

MUC1 mucin is coexpressed with other T-cell markers. Double staining with anti-CD4 or anti-CD8 MAbs and MAb B27.29 demonstrated that at days 5 and 7, after activation of PBLs with PHA, approximately 80% of the CD4+ T cells were MUC1 positive and approximately 65% of the CD8+ T cells were MUC1 positive.

Down-regulation of MUC1 Expression on Activated T Cells after Removal of the Mitogen. T cells were cultured in the presence of PHA for 1, 3, and 6 days, followed by washing and reculturing in media without PHA for an additional 3 and 6 days. Fig. 2 shows that after removing the PHA from the cultures, MUC1 expression was reduced with time. The reduction in the number of T cells positive for MUC1 expression does not seem to be due to selective death of MUC1+ T cells because, by using a trypan blue exclusion test, we observed a <20% cell death corresponding to an approximate 60% reduction in MUC1 expression on T cells after removal of the mitogenic stimulus. In parallel, T cells were cultured in the absence of PHA for 6 days and then stimulated with PHA. MUC1 expression on T cells was not observed up to 6 days in culture without PHA, but MUC1 mucin expression was detected after subsequent stimulation with PHA (Fig. 2).

Soluble MUC1 Mucin Is Found in Cell Supernatants of Activated Human T-Cell Cultures. We used an enzyme immunoassay specific for MUC1 mucin to test supernatants from PHA-activated T cells for the presence of soluble MUC1 mucin. Table 1 shows that...
supernatants from PHA-activated, but not nonactivated, cultures contained increasing amounts of soluble MUC1 mucin with a peak level of approximately 27 units/ml culture supernatant at day 6.

**Anti-MUC1 Mucin Antibody Partially Inhibits the T-cell Proliferative Response.** Human PBLs were stimulated with PHA for 3 days to induce the expression of MUC1 mucin. At this time the cells were harvested, washed, and recultured in the presence of anti-CD3 (OKT3, as a polyclonal stimulus), with or without anti-MUC1 MAb B27.29 or isotype control MAb B80.3 and goat-antimouse antibody. In three experiments, T cells stimulated in the presence of anti-MUC1 antibody showed inhibited proliferation responses (Table 2). The overall reduction in the T-cell proliferative response by anti-MUC1 MAb B27.29 was 32.5 ± 5.8% compared with 5 ± 3.7% by negative control MAb B80 (P = 0.0007).

**Discussion**

Our working hypothesis is that MUC1 mucin is involved in normal immune regulation. Evidence supporting this hypothesis includes the observation that (a) newly synthesized MUC1 mucin is rapidly induced and appears on the cell surface of the majority of activated human T cells; (b) there is down-regulation of MUC1 mucin expression after the mitogenic stimulus is removed; (c) cross-linking surface MUC1 by anti-MUC1 MAb B27.29 modulates the T-cell proliferative response; (d) MUC1 mucin is either shed or secreted into the supernatants of cultures of PHA-activated human T cells; and (e) soluble MUC1 mucin inhibits T-cell proliferation and induces an anergy-like state that is reversible by interleukin-2 or anti-CD28 antibody (7).

The exact role and the mechanism of immune regulation by MUC1 mucin is not clear yet. However, the observations that MUC1 mucin can present multiple functional domains e.g., antiadhesion, proadhesion as well as inhibition of T-cell proliferation (7, 10–16), support the hypothesis that MUC1 expression on T cells may play an important homeostatic function. It is possible that MUC1 mucin expressed by activated T cells helps in actively terminating T-cell immune responses by down-regulating their proliferative response and also serves a role in lymphocyte trafficking due to its adhesion and/or antiadhesion properties. We are presently investigating whether expression of MUC1 mucin by activated T cells is one of the negative feedback mechanisms to return the immune system to its basal resting state from an activated stage after antigenic stimulation.

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**References**

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