Expression of Tumor-Associated Differentiation Antigens, MUC1 Glycoforms and CEA, in Human Thymic Epithelial Cells: Implications for Self-Tolerance and Tumor Therapy

Silvie Cloosen, Janna Arnold, Marco Thio, Gerard M.J. Bos, Bruno Kyewski, and Wilfred T.V. Germeraad

Abstract

Expression of tissue-restricted self-antigens in the thymus, termed promiscuous gene expression, imposes T cell tolerance and protects from autoimmune diseases. This antigen pool also includes various types of tumor-associated antigens (TAA) previously thought to be secluded from the immune system. The scope of promiscuous gene expression has been defined by mRNA analysis at the global level of isolated medullary thymic epithelial cells (mTECs). Information at the protein level on the frequency of mTECs expressing a given antigen, on coexpression patterns, and post-translational modifications is largely missing. We report here promiscuous expression at the protein level of two TAA, MUC1 and CEA, in situ and in purified human mTECs. Both antigens are expressed in 1% to 3% of mTECs, either individually or coexpressed in the same cell. Using a panel of anti-MUC1 monoclonal antibodies recognizing different post-translational modifications, i.e., glycoforms of MUC1, we show that only fully glycosylated forms of MUC1 and the differentiation-dependent glycoforms were detected on mTECs, but not the cancer-associated glycoforms. Our findings imply that MUC1 and CEA are amenable to central tolerance induction, which might, however, be incomplete in case of tumor cell–restricted MUC1 glycoforms. Knowledge of these subtleties in promiscuous gene expression may, in the future, assist the selection of T cell tumor vaccines for clinical trials. [Cancer Res 2007;67(8):3919–26]

Introduction

Immunotherapy based on active immunization is an increasingly applied adjuvant therapy for the treatment of hemopoietic and epithelial tumors. Clinical immunization protocols vary widely with vaccine delivery ranging from naked DNA, antigen-pulsed dendritic cells, whole cell lysates to single T cell epitopes (1–3). Currently, there is no consensus as to which approach will yield the best clinical results. Irrespective of these considerations, success in eliciting an immune response against tumors will also depend on the nature of the targeted antigens. Genetic alterations unique to the particular tumor, e.g., point mutations, deletion, duplications, or chromosomal rearrangements, potentially generate new “foreign” T and/or B cell epitopes (4), which will induce immune responses directed against such tumor-specific antigens. The induction of immune responses directed against such tumor-specific antigens should, in principle, follow rules governing pathogen-driven responses. In contrast, antigens, which are induced or highly up-regulated in tumor cells, are classified as self-antigens. These self-antigens can potentially still be seen as foreign when post-translational mechanisms like glycosylation have been altered due to oncogenic transformation. It has been a controversial issue as to what extent these tumor-associated antigens (TAA) are subject to self-tolerance, a process which would quench an endogenous or a deliberately induced antitumor response. With the assumption that antigens with a spatially and/or temporally restricted expression pattern (i.e., oncofetal or cancer germ cell antigens) may be exempt from tolerance induction, such antigens have been preferentially selected for clinical trials (5). This assumption has been questioned recently with the demonstration that members of these types of TAA are also expressed in medullary thymic epithelial cells (mTECs), along with a host of other tissue-restricted self-antigens, a phenomenon termed “promiscuous gene expression” (6, 7). The demonstration of promiscuous expression of tissue-restricted antigens is largely based on mRNA expression analysis (7). Although in most cases thus far analyzed, expression of a given self-antigen at the mRNA level in mTECs correlates with tolerance (8), exceptions have been noted (9). The demonstration of promiscuous antigen expression at the protein level, therefore, supplies important complementary information, in particular in case of humans, where central tolerance cannot be assayed functionally. In addition, detection of protein expression by monoclonal antibodies (mAbs) may add information on post-translational modifications and coexpression patterns at the single cell level. Differences in post-translational modifications between thymic and peripheral (tissue or tumor-restricted) antigen expression could conceivably result in incomplete self-tolerance (10, 11).

Here, we report on the expression pattern of tumor-associated differentiation antigens [i.e., mucin-1 (MUC1) and carcinoembryonic antigen (CEA)] in situ and by purified human mTECs at the protein level. We also show that mTECs mimic the glycosylation pattern of normal epithelia, whereas cancer-associated glycosylation is absent in these cells. These results have implications for self-tolerance and the design of immunotherapeutic studies targeting these antigens.

Materials and Methods

Tissue. Human thymi were obtained from children (age range, 2 days to 5 years) undergoing corrective heart surgery (University Hospital of Cologne,...
and Department of Cardiac Surgery, Medical School, University of Heidelberg, Germany). Pieces of thymic tissue were either embedded in TissueTek (Sakura Finetek, Zoeterwoude, the Netherlands), snap frozen in liquid nitrogen, and stored at −80°C or further processed for the isolation of mTECs.

**Antibodies.** The MUC1 antibody was purified from the 214D4 cell line (ref. 12; kindly provided by Dr. J. Hilkens, the Netherlands Cancer Institute, Amsterdam, the Netherlands) and biotinylated. MAbs DF3 (purified; Centocor, Malvern, PA; ref. 13) and VU4H5 (supernatant; ref. 14) were kindly provided by Dr. S. von Mensdorff-Pouilly (Vrije Universiteit Medisch Centrum, Amsterdam, the Netherlands), and the mAb SM3 (supernatant; ref. 15) was kindly provided by Prof. Dr. J. Taylor-Papadimitriou (Guy’s Hospital, London, United Kingdom). MAbs 5E5 (purified) and 2D9 (purified) were kindly provided by Prof. Dr. H. Clausen (Department of Glycobiology, University of Copenhagen, Denmark; ref. 16). A detailed description of the specificities of the MUC1 mAbs has been provided by van Leeuwen et al. (17) and is summarized in Table 1. Briefly, in this study, we categorized the anti-MUC1 mAbs into three groups depending on their reactivity with the different glycoforms of MUC1. MAbs that recognize MUC1 irrespective of its glycosylation pattern, including the fully glycosylated forms as well as the differentiation-dependent glycoforms, were defined as group 1 (214D4, DF3), whereas mAbs that only recognize the differentiation-dependent glycoforms were defined as group 2 (VU4H5, SM3). Group 3 mAbs exclusively recognize cancer-associated glycoforms of MUC1 and do not react with healthy tissue (5E5, 2D9). Therefore, we categorized mAb SM3 into group 2 because although this mAb displays cancer-associated reactivity (15), it also stains healthy tissue as described in this study and has been shown before in the healthy human kidney (18).

Anti-CEA antibody Parlam-4 (19) was kindly supplied by Dr. H. Kawamoto and Dr. K. Kagawa (RIKEN, Yokohama, Japan). Anti-EpCAM antibody HEA-125 (20) was generously supplied by Dr. G. Moldenhauer (Deutsches Krebsforschungszentrum, Heidelberg, Germany). This mAb as well as the isotype control mouse immunoglobulin G1 (IgG1; purchased from BDBiosciences, Krebsforschungszentrum, Heidelberg, Germany). Specific staining of MUC1 was determined by incubation with primary anti-MUC1 mAbs (puriﬁed mAbs used at 5 µg/mL) for 60 min at room temperature (RT). Afterward, sections were rinsed with PBS/Tween 20 and incubated with Envision-HRP (DAKO, Glostrup, Denmark) for 30 min at RT. The color was developed by incubation for 5 min at RT using a substrate buffer containing 3,3′-diaminobenzidine (5 µg/mL; DAKO) and 0.15% H2O2 (Sigma, Zwijndrecht, the Netherlands) in PBS/Tween 20. Cytospins of the breast carcinoma cell line ZR75-1 (Department of Pathology, University Hospital of Maastricht, the Netherlands) were stained with all anti-MUC1 mAbs and served as intraexperimental positive controls for all antibodies.

For ﬂuorescent double staining, sections were incubated for 60 min with primary antibodies or matched isotype controls followed by incubation with goat–anti-mouse Alexa568 (Molecular Probes, Eugene, OR) for 30 min. After incubation, sections were rinsed and incubated with 2% normal mouse serum (DAKO) for 20 min. Alternatively, biotinylated primary antibodies or matched isotype controls were detected by streptavidin-Alexa488 (Molecular Probes). Sections were sealed with VectaShield (VectorLabs, Burlingame, CA). Confocal imaging was done using a Bio-Rad (München, Germany) laser scanning microscopy (LSM) in combination with computer-assisted analysis (Confocal Assistant 4.2).

**Isolation of human medullary thymic epithelial cells.** Isolation and puriﬁcation of human mTECs were done as described previously (6).

**Fluorescence-activated cell sorting analysis.** Sorted mTECs cells were incubated with primary antibodies for 90 min at 4°C. This was followed by incubation with the appropriate ﬂuorescent secondary antibody for 30 min at 4°C. In case of a combination with phenotypic markers, binding sites of rabbit anti-mouse IgG were blocked for 10 min by incubation with mouse serum (DAKO). Finally, cells were ﬁxed in 1% paraformaldehyde (Merck) and analyzed on a FACS-Calibur (BD Biosciences). Dead cells were excluded based on their forward/side-scatter properties, and mTECs were gated as CD45−; CD27−; EpCAM+ cells as described before (6).

**Cytosin staining.** Sorted mTECs (Supplementary Fig. S1) on cytopsins were stained with anti-MUC1 (groups 1, 2, and 3) or anti-CEA (Parlam-4) mAbs directly conjugated with Alexa488 (Alexa Fluor 488 Protein Labelling kit; Molecular Probes). After blocking with 5% mouse serum for 20 min, cytopsins were incubated with the primary antibody or matched isotype controls (Supplementary Fig. S2) for 1 h and washed with PBS, and

### Table 1. MUC1-specific antibodies

<table>
<thead>
<tr>
<th>MUC1 Antibodies</th>
<th>Specificity</th>
<th>Normal epithelial cells</th>
<th>Tumor cells</th>
</tr>
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<tbody>
<tr>
<td>Group 1: recognize the peptide backbone of MUC1 in its fully as well as the differentiation-dependent glycoforms</td>
<td>214D4</td>
<td>Tandem repeat, PDTR region</td>
<td>Secretory epithelial cells</td>
</tr>
<tr>
<td></td>
<td>DF3</td>
<td>Tandem repeat, RPAP region</td>
<td>Small intestine, colon, breast</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td>Secretory epithelial cells</td>
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<td></td>
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<td>Colon, breast</td>
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<tr>
<td>Group 2: recognize the peptide backbone of MUC1 with differentiation-dependent glycoforms</td>
<td>VU4H5</td>
<td>APDTRPAP; Thr not glycosylated</td>
<td>Corneal epithelium</td>
</tr>
<tr>
<td></td>
<td>SM3</td>
<td>PDTR, blocked by branched core 2 glycans</td>
<td>Kidney: distal tubules, collecting ducts</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Breast</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Colon, lung + ovarian carcinoma</td>
</tr>
<tr>
<td>Group 3: recognize defined cancer-associated glycoforms of MUC1</td>
<td>5E5</td>
<td>Tn and STn</td>
<td>Absent</td>
</tr>
<tr>
<td></td>
<td>2D9</td>
<td>Tn</td>
<td>Unknown</td>
</tr>
</tbody>
</table>
subsequently, cell nuclei were counterstained with Hoechst 33342. Additionally, mTECs were costained for both TAA either combining anti-MUC1–Alexa488 and anti-CEA–Alexa647 (Alexa Fluor 647 Protein Labelling kit, Molecular Probes) or anti-MUC1–Alexa647 and anti-MUC1–Alexa488 antibodies. Both combinations yielded similar staining patterns. The slides were analyzed and documented using either an Axioptipod 2 epifluorescence microscope (Zeiss, Jena, Germany) and Axiosvistion 3.1 software or a confocal microscope (LSM 510, Zeiss). The differences in the total number of cells counted were due to variable yields of mTECs in different isolates. Assuming a basal expression frequency for MUC1 and CEA of 2% in mTECs, the likelihood of coexpression of both antigens in one cell on a “random” base is 0.02 × 0.02 = 0.0004 or 0.04%.

**RNA isolation.** Human thymic epithelial cells were stained with either anti-MUC1 antibody (214D4) or CEA antibody (Parlam-4) and sorted on a FACs cellsorter. Sorted cells were lysed with buffer from the mRNA Capture kit (Roche Diagnostics GmbH, Mannheim, Germany). Isolation of mRNA, cDNA synthesis, and real-time PCR were further done as previously described (6). In short, mRNA was isolated and eluted in 50 µl H2O. Because of low cell numbers (<60,000), this total RNA was precipitated with ice-cold ethanol in the presence of NaCl and lipopolysaccharide as carrier. A desalted total RNA sample was dissolved in 8 µl cold ethanol in the presence of NaCl and lipopolysaccharide as carrier.

**Results**

MUC1 and CEA are expressed by mTECs. MUC1 protein expression in the thymus has been previously reported, yet the identity of the antigen-expressing cell type(s) remained unclear (21). Moreover, global gene expression analysis by gene arrays had shown that members of the mucin gene family were overexpressed in mTECs (6). Here, we show by double staining of thymic sections with anti-EpCAM and anti-MUC1 mAbs that MUC1 protein expression in the thymic medulla was confined to rare mTECs, whereas there was no detectable expression in the cortex (Fig. 1A). The same expression pattern with regard to location and frequency of positive cells was found for a second TAA, CEA (Fig. 1B). Both MUC1- and CEA-positive cells tended to cluster in the vicinity and the outer rim of Hassal’s corpuscles (HC), a site where terminally differentiated mTECs cluster (22). This staining pattern has been observed in four different thymi.

To confirm the identity of antigen-expressing cells and enumerate their frequency, mTECs were purified independently from two human thymi. MUC1 and CEA expressions were then analyzed on cytospins and by fluorescence-activated cell sorting (FACS) analysis. By visual counting of stained cytospins, 2.8% (30/1,080) of the sorted mTECs expressed MUC1, and 2.0% (23/1,119) expressed CEA. By visual counting of stained cytospins, 2.8% (30/1,080) of the sorted mTECs expressed MUC1, and 2.0% (23/1,119) expressed CEA (Fig. 2A and B). This frequency range was confirmed by FACS analysis on gated mTECs showing the expression of MUC1 on 2.8% and of CEA on 1.6% of these cells (Fig. 2C and D) and corroborated by further independent FACS measurements [MUC1: 2.6 ± 1.6% (n = 9) and CEA: 1.8 ± 0.8% (n = 6)]. These results are well in accordance with frequencies obtained for other promiscuously expressed tissue-restricted antigens in mouse or man (7, 23). The antibody specificity was confirmed by sorting mTECs in antigen-expressing and nonexpressing subsets and comparing mRNA transcription by qRT-PCR in both subsets. Specific mRNA was highly enriched in protein-expressing cells by a factor of 15- to 50-fold (Supplementary Fig. S3), thus documenting a clear correlation between mRNA and protein expression for these two TAA in mTECs.

mTECs coexpress MUC1 and CEA. The molecular mechanisms underlying promiscuous gene expression are largely unknown, and
this applies also to the pattern of gene (co)expression at the single cell level. The unequivocal detection of both antigens at the single cell level allowed us to assess whether expression of these functionally and genetically unrelated antigens were expressed in a mutually exclusive manner in individual mTECs, or whether there was partial or strict coexpression. Given the low frequency of MUC1 and CEA expressing mTECs, coexpression on a random base would be expected to occur in <0.1% of the cells (explanation in Materials and Methods). Double staining of thymic sections with both mAbs clearly identified coexpression of these two TAA, along with cells staining only with one mAb (Fig. 3A). To quantify the proportion of single versus double expressors, cytospins of purified mTECs were double stained and visually analyzed (Fig. 3B). In addition, double-stained cells were analyzed by flow cytometry (data not shown). A surprisingly high fraction of about 15% (or 0.7% of total mTECs) of MUC1- or CEA-positive mTECs coexpressed both antigens. Thus, the frequency of coexpressing cells was far above the value predicted if expression from both genes were independently regulated.

Expression of differentially glycosylated MUC1 isoforms by mTECs. The glycosylation pattern of MUC1 varies according to epithelial cell types; in addition, epithelial cell tumors generally

Figure 2. Expression of MUC1 and CEA on isolated human mTECs. Cytospins of purified human mTECs were stained for (A) MUC1 or (B) CEA expression. Infrequent cells showed cytoplasmic and surface expression (top, nuclei were counterstained with Hoechst 33342); bars, 20 μm. Purified human mTECs were stained for (A) MUC1 or (B) CEA surface expression to more precisely enumerate the number of TAA-positive cells (bottom). The percentage of gated positive cells is shown. Red histogram, control staining; blue histogram, TAA staining.

Figure 3. Coexpression of MUC1 and CEA in human thymic medulla and isolated mTECs. A, human thymus sections were costained for MUC1 (green) and CEA (red). Note that individual cells in the medulla staining either for MUC1 alone (yellow arrows) or both antigens (red arrow). Magnification, 400×. B, cytospins of purified human mTECs were costained for MUC1 (red) and CEA expression (green). Note the individual cells stain either for MUC1 alone or both antigens (yellow). Inset, cytoplasmic coexpression of both antigens. Bars, 20 μm.
display dysregulated and aberrant glycosylation profiles compared with their normal counterparts (24, 25). Because this differential glycosylation may include T cell epitopes and, thus, affect T cell recognition, i.e., tolerance, we characterized the glycosylation pattern of MUC1 expressed on human mTECs using a series of mAbs that recognize MUC1 with gradually lower degrees of glycosylation.

Few mTECs per section (Fig. 4A) or about 1% of purified mTECs expressed epitopes detected by mAbs from group 1 (15/1,012 were 214D4+ and 12/1,007 were DF3+; Fig. 5A and B). Epitopes recognized by mAbs from group 2 (VU4H5, SM3) were less frequently expressed, but still clearly detectable in thymic sections (Fig. 4B) and on <1% of mTECs on cytospins (4/997 were VU4H5+, 3/815 were SM3+; Fig. 5C and D). mTECs expressing epitopes recognized by mAbs from group 3 (5E5, 2D9; Fig. 4C) were neither detected in situ nor on cytospins (0/946 are 5E5+, and 0/933 are 2D9+; Fig. 5 and Table 1).

**Discussion**

Our results clearly ascribe promiscuous expression of MUC1 and CEA at the protein level to mTECs. This finding concurs with gene arrays analysis data, which revealed expression of MUC family members in human mTECs (6) and the detection of CEA in human

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**Figure 4.** Expression of different MUC1 glycoforms on human thymus sections. Thymic sections were stained with mAbs specific for different MUC1 glycoforms. Glycosylated MUC1 isoforms (as detected by mAbs from group 1: 214D4, DF3) were strongly expressed on rare epithelial cells in the medulla (A, arrows). Cells expressing shorter glycoforms of MUC1 (as detected by mAbs from group 2: VU4H5, SM3) were less frequent, but clearly detectable (B, arrows). No cells staining for cancer-specific glycoforms of MUC1 (as detected by mAbs from group 3: 5E5, 2D9) were observed (C). Staining with an isotype control reveals endogenous peroxidase-containing cells in the thymus (brown; D). Insets, positive control staining for the respective antibody done on cytospins from the ZR75-1 breast carcinoma cell line. These results have been observed in three different thymi. Magnification: 400x.
mTECs by reverse transcription-PCR (8). Moreover, this study shows that both proteins are stably inserted into the cell surface membrane of mTECs, allowing the enumeration of antigen-positive mTECs by flow cytometry. The frequencies of MUC1- and CEA-positive mTECs range between 1% and 3%, as being typical of promiscuously expressed genes (7). Both antigens seem to be preferentially expressed in mature mTECs subsets as revealed by their aggregation in the vicinity of HC (Fig. 3A) and by flow cytometry analysis of mTECs subsets, which were HLA-DR+ (data not shown), a feature shared by many tissue-restricted antigens (26). This expression of normal MUC1 and CEA in the thymic medulla includes them into the pool of self-antigens that are displayed to developing T cells. Further analysis of the MUC1 glycosylation pattern in thymus revealed that the cryptic epitopes that distinguish the TAAMUC1 from common epithelial MUC1 are not expressed in the thymus, indicating that thymocytes are not exposed to MUC1 cryptic glycopeptides.

Irrespective of this potentially tolerogenic expression pattern, bone marrow and blood of cancer patients do contain MUC1- and CEA-specific T cells that display a Th1 profile (27, 28) and are cytotoxic against autologous tumor cells in vitro (27). Moreover, Wierecky et al. (29) recently reported that several cancer patients showed tumor regression following treatment with MUC1-pulsed dendritic cells, which correlated with the presence of MUC1 peptide-specific T cells in their peripheral blood.

A likely explanation for this finding is that negative selection in the thymus is incomplete, and that T cells with intermediate to low avidity for self escape into the periphery (30). These potentially self-reactive T cells presumably are kept under the control of regulatory T cells. Repetitive immunization, however, has been shown to induce activation/proliferation of self-reactive T cells in most (27) and tumor regression in a portion of cancer patients without overt signs of autoimmunity (29), apparently overriding reversible suppression by regulatory T cells. It remains, thus far, conjectural whether or not the limited success of current clinical trials based on active vaccination against MUC1 (29), CEA (31), and other TAA relates to their ectopic expression in mTECs. Following this line of reasoning, holes in tolerance induction may be selectively exploited by immunizing against cryptic epitopes of MUC1, thus recruiting T cells with high(er) avidity. Clearly, T cells, which are able to recognize Tn- or T-glycosylated peptides processed and presented by MHC class II molecules on antigen-presenting cells (32, 33), have been shown, and glycopeptide-specific T cells can be induced in man (34–37) as well as in experimental animal models (38, 39). Differential self-tolerance to a glycosylated versus nonglycosylated T cell epitope has been well documented in the case of collagen II. Only T cells specific for a glycosylated epitope of collagen II accumulate in inflamed joints and are pathogenic upon experimental immunization, whereas T cells specific for the nonglycosylated epitope do not cause pathology (40).

On the basis of our findings and those of others (41–43), a thorough analysis of glycosylation patterns, splice variants, and interindividual variability in expression levels of nonmutated TAA in mTECs is warranted (44). Such an analysis may provide criteria to select suitable antigens/epitopes among the growing list of potential candidates for clinical trials.

Mice expressing human neo–self-antigens as transgenes, including TAA, serve as experimental models to study tumorigenesis and tolerance mechanisms. In the context of immunotherapy studies, it is important to know whether the transgene directed by its own promoter faithfully reflects the physiologic expression pattern found in humans, including ectopic expression in mTECs. Hence, we analyzed mice transgenic for MUC1 directed by its own promoter (44) for thymic MUC1 expression using an anti-MUC1

<table>
<thead>
<tr>
<th>MUC1-Antibodies</th>
<th>Absolute number of positive mTEC</th>
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<tbody>
<tr>
<td>214D4 (group 1)</td>
<td>15/1012</td>
</tr>
<tr>
<td>DF3 (group 1)</td>
<td>12/1007</td>
</tr>
<tr>
<td>VU4H5 (group 2)</td>
<td>4/997</td>
</tr>
<tr>
<td>SM3 (group 2)</td>
<td>3/815</td>
</tr>
<tr>
<td>5E5 (group 3)</td>
<td>0/946</td>
</tr>
<tr>
<td>2D9 (group 3)</td>
<td>0/933</td>
</tr>
</tbody>
</table>
mAb from each group that was used for the human thymus. MUC1 expression in these transgenic mice was comparable to that in human tissue (Supplementary Fig. S4), whereas wild-type control mice did not show any human MUC1 expression (Supplementary Fig. S4). Although previous studies agree that ectopic expression of MUC1 and CEA in these transgenic mice confers a state of tolerance, they differ with respect to the ease with which this tolerance can be broken (8, 45–47). In this respect, they might serve as a valid model for testing new experimental strategies of immunotherapy.

An unexpected finding of this study is the frequency of mTECs coexpressing both antigens (±15% or 0.7% of total mTECs), which is far above the value predicted, if both antigens were independently regulated (<0.1%). Given our current limited insight into the genetic and epigenetic regulation of promiscuous gene expression (26, 48), we can only speculate on this finding. Double expressing mTECs may represent a special subset mimicking the expression pattern of certain peripheral epithelial cells. Note, however, that MUC1 and CEA are rarely coexpressed in normal peripheral epithelial cells (49, 50), but frequently in cancerous cells, when antibodies to the highly glycosylated domain of MUC1 were used (51). Alternatively, molecular mechanisms specific to mTECs and unrelated to epithelial cell biology may favor the coactivation of the two gene loci on chromosome 1 and 19, which encode MUC1 and CEA, respectively. A more detailed analysis of the gene neighborhoods will be one route to further explore these issues.

In summary, our findings document that the two TAA, MUC1 and CEA, are expressed in human mTECs and are thus amenable to self-tolerance induction. However, the cryptic glycopeptides of MUC1 are not present in the thymus and, thus, presumably exempt from central tolerance. Such intricacies in the pattern of promiscuous gene expression may, in the future, help to guide the selection of T epitopes for tumor vaccines. 

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