Expression of MUC1 Mucin Increases Intracellular Calcium Levels Through a CAML-Dependent Mechanism

Wei Guang[1], K. Chul Kim[2], and Erik P. Lillehoj[1]
[1]University of Maryland School of Medicine, Baltimore, MD; [2]Temple University School of Medicine, Philadelphia, PA

Abstract

Rationale: MUC1 mucin is a type I transmembrane glycoprotein expressed on the surface of airway epithelial cells. Its cytoplasmic carboxyl-terminus (CT) is evolutionarily conserved and mediates intracellular signal transduction cascades that inhibit airway inflammatory responses (Kim and Lillehoj, 2008). However, the mechanism by which MUC1 inhibits inflammation remains to be elucidated. Our previous results showed that the MUC1 CT interacted with calcium-modulating cyclophilin ligand (CAML), a protein involved in Ca^{2+} signaling (Guang *et al*., 2008). The current study was designed to confirm MUC1-CAML protein binding and to determine the effects of this interaction on intracellular Ca^{2+} levels.

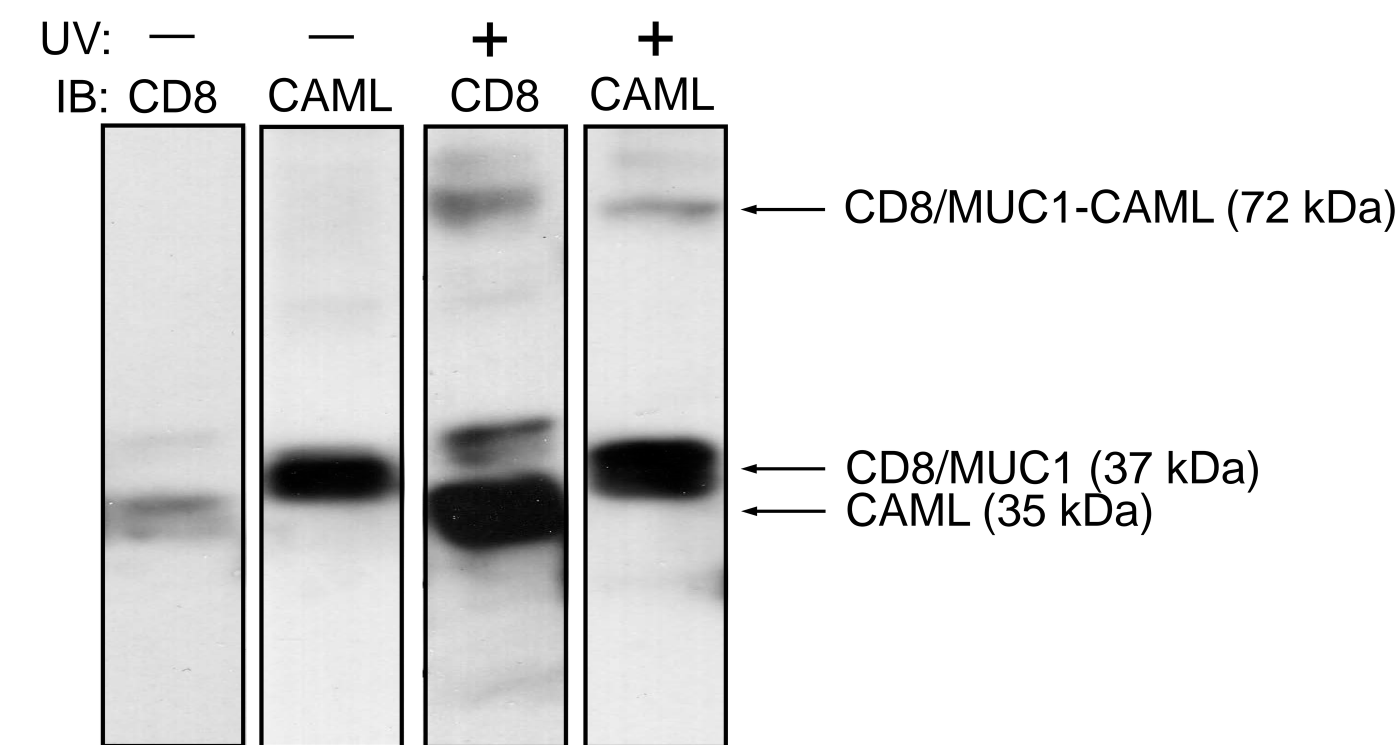
Methods: MUC1-CAML interaction was analyzed by intracellular protein cross-linking, *in vitro* coupled transcription/translation, and confocal immuno-fluorescence microscopy. Intracellular Ca^{2+} levels were measured using the Fluo-4 NW kit (Molecular Probes).

Results: MUC1 and CAML were covalently cross-linked in living cells using a novel intracellular protein cross-linking procedure. The observed size of the cross-linked proteins suggested a direct interaction without the involvement of intermediary components. This was confirmed by coupled *in vitro* transcription/translation using recombinant poly-peptides in the absence of additional intracellular proteins. By immunofluorescence microscopy, colocalization of both proteins on the plasma membrane was evident when cells expressed the MUC1 CT and CAML amino-terminal regions. Finally, coexpression of MUC1 and CAML led to increased intracellular Ca^{2+} levels compared with cells expressing either protein alone.

Conclusion: Our results have identified CAML as a novel binding partner of the MUC1 CT and suggest that MUC1 plays a role in calcium signaling in the airways.

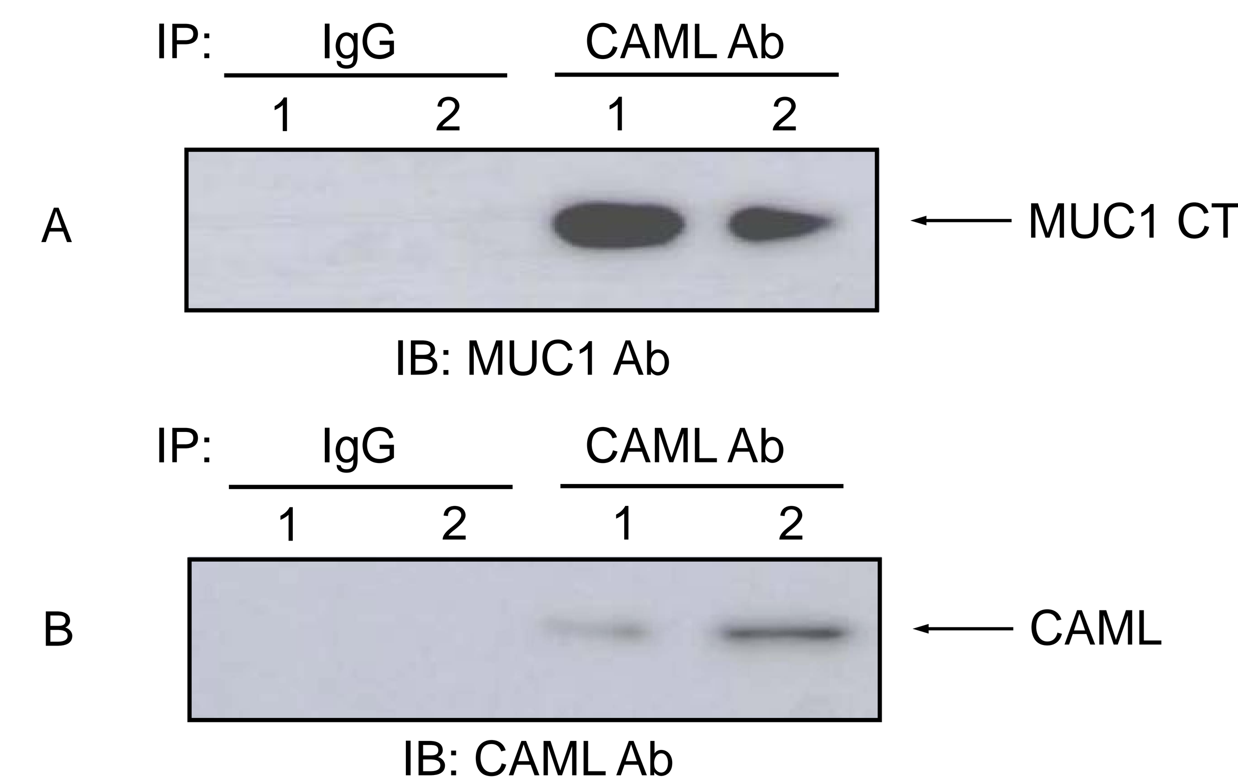
Results

Figure 1. MUC1-CAML Interaction by Intracellular Cross-linking



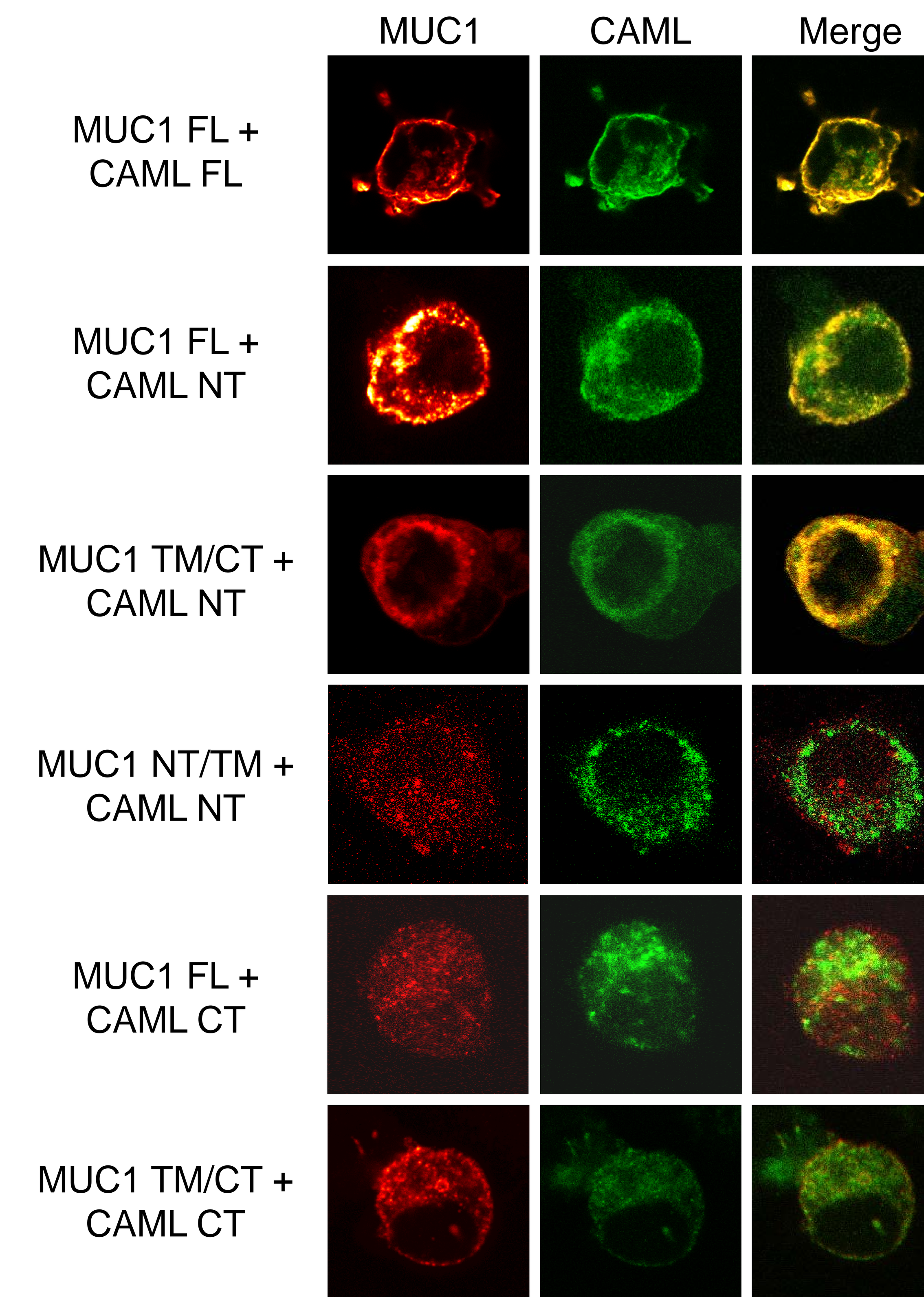
HEK293T cells were cotransfected with plasmids encoding CD8/MUC1 or CAML, the cells were metabolically labeled with photomethionine and photoleucine (Pierce), exposed or nonexposed to UV (365 nm) for 14 min, and cell lysates were analyzed by immunoblotting with CD8 or CAML antibodies. Note the appearance of the 72 kDa cross-linked CD8/MUC1-CAML complex after UV exposure.

Figure 2. MUC1-CAML Interaction by Coupled *In vitro* Transcription/Translation



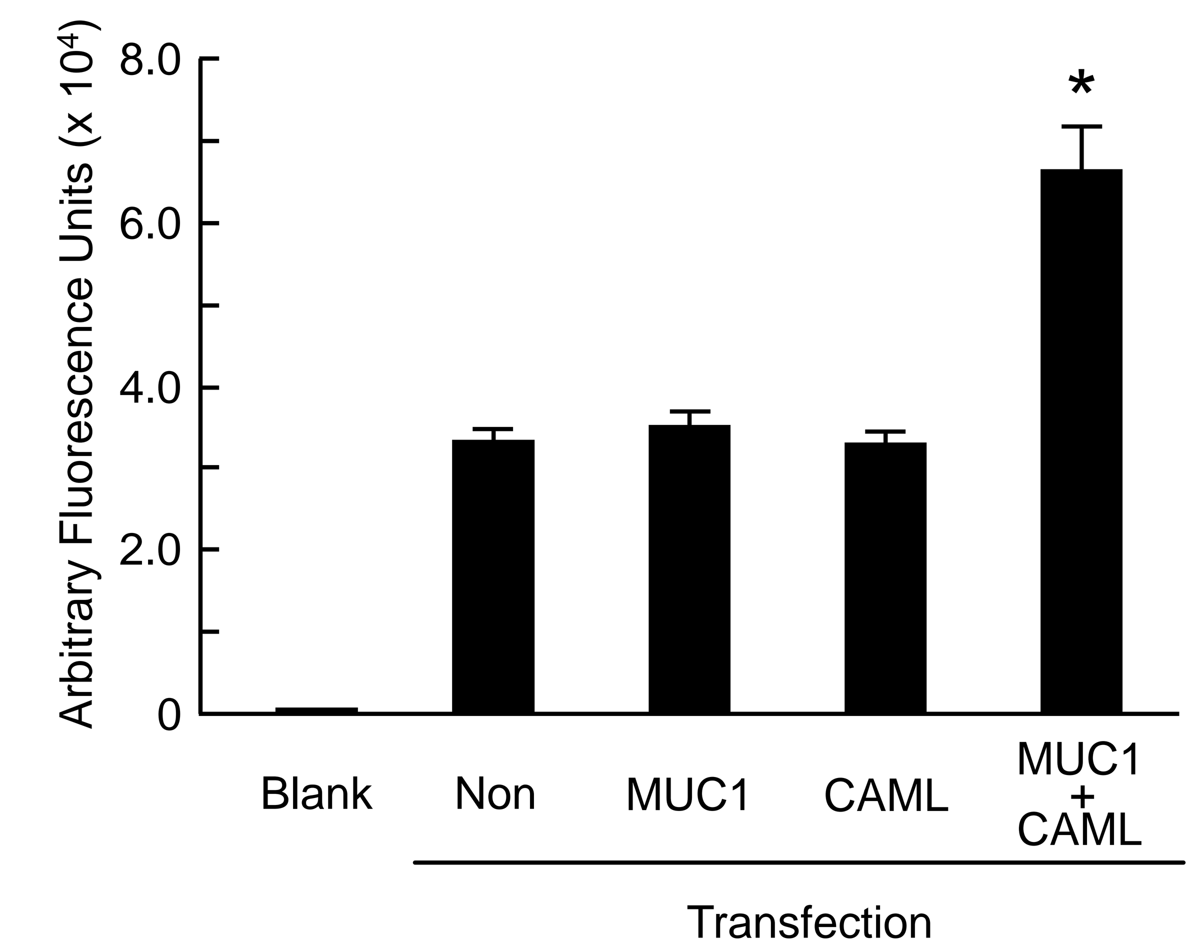
(A) Lane 1, MUC1 CT and CAML cDNAs were separately subjected to *in vitro* transcription/translation, the proteins were mixed, and analyzed by coIP as indicated. Lane 2, MUC1 CT and CAML cDNAs were mixed prior to transcription/translation and analyzed by coIP. (B) The blot in (A) was stripped and reprobed with the IP Ab as a loading control.

Figure 3. MUC1-CAML Coloclization by Confocal Microscopy



HEK293T cells were cotransfected with plasmids encoding the indicated MUC1 and CAML constructs, the cells were fixed on glass slides, stained with MUC1 (red) and CAML (green) Abs, and examined using a Zeiss LSM410 confocal microscope. Note colocalization of MUC1 FL or MUC1 CT with CAML FL or CAML NT after merge (yellow). FL, full-length; NT, NH_2-terminal; CT, COOH-terminal; TM, transmembrane.

Figure 4. Coexpression of MUC1 and CAML Increases Intracellular Ca^{2+} Levels



HEK293T cells were non-transfected (Non) or transiently transfected with plasmids encoding MUC1, CAML, or MUC1 + CAML cDNA constructs and intracellular Ca^{2+} levels were measured using the Fluo-4 fluorescence indicator. Blank refers to fluorescence generated in the absence of added cells (i.e. assay reagents only). Values are means ± SEM (N = 3). *, $p < 0.05$ comparing MUC1 + CAML cotransfection with MUC1 or CAML single transfections.

References

1. Kim, K.C., and Lillehoj, E.P. Am. J. Respir. Cell Mol. Biol. 39:644, 2008.
2. Guang, W. Kim, K.C., and Lillehoj, E.P. Am. J. Respir. Crit. Care Med. 177:A993, 2008.

Acknowledgments

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