Human MAdCAM-1 Antibodies as Targeted Immune Modulators

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Supplemental Material

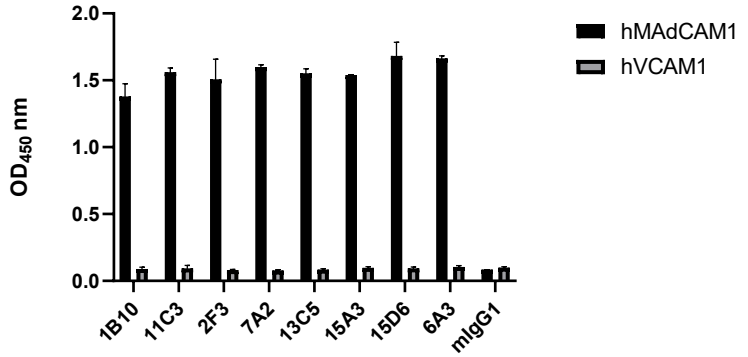


Figure S1: Cross Reactivity of mouse anti-human MAdCAM-1 mAbs to hVCAM-1. An ELISA was performed to determine whether the mAbs are cross reactive for VCAM-1 whereby wells of an ELISA plate were coated overnight at 4°C with either 1µg of either hMAdCAM-1 (see materials and methods section) or hVCAM-1 (Thermo Fisher Scientific). The plate was blocked with 1% BSA for 1h at room temperature prior to adding the antibodies at a fixed concentration of 3µg/ml and incubated at room temperature for 1h. Binding was detected using a secondary antibody (anti-mouse IgG h+l Ab conjugated to HRP)(Bethyl Laboratories)) and developed using TMB substrate (Thermo Fisher Scientific). Absorbance readings were recorded at 450 nm. All of the mAbs bound hMAdCAM-1 with the exception of IgG1 however none of the mAbs bound to hVCAM-1.

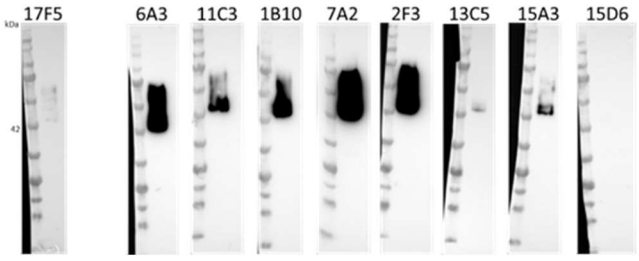


Figure S2: Binding Characterization of mouse anti-human MAdCAM-1 mAbs. The mAbs were validated for use in Western Blot whereby 1µg of full length hMAdCAM-1 was loaded onto NuPage 4-12%, 15-well Bis-Tris gels (Invitrogen), and electrophoresed at 200V for 35 minutes. The band corresponding to hMAdCAM-

1 was transferred to a nitrocellulose blotting membrane (Cytiva). After blocking non-specific binding sites on the membrane overnight using 5% milk at 4°C, each membrane was incubated at room temperature for 1hr with one of the mAbs (1µg). A commercially available (Thermo Fisher Scientific) mouse anti-human MAdCAM-1 mAb 17F5, served as a positive control. The membranes were washed with TBS + 0.1% Tween 20 before the addition of the secondary antibody (anti-mouse IgG h+l Ab conjugated to HRP)(Bethyl Laboratories)). Membranes were washed before staining with Luminata™ Crescendo (Millipore Sigma) and imaged on a Fusion FX (Vilber) chemiluminescent imager with a 30s exposure. All of the antibodies except 15D6 were able to detect hMAdCAM-1.

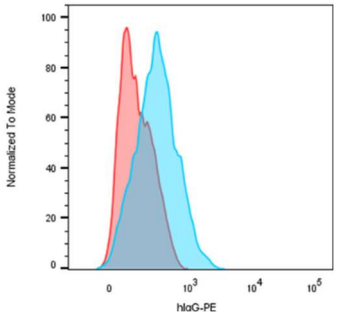
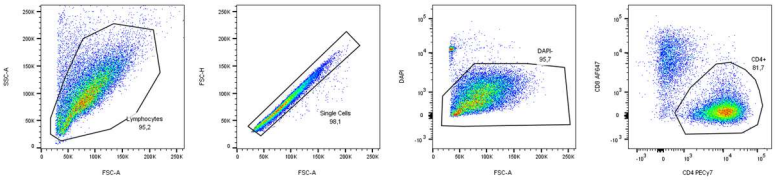


Figure S3: Integrin α4β7 expression. The expression of Integrin α4β7 occurs on human CD4⁺ T cells stimulated with α-CD3 and MAdCAM-1.Fc (in blue) in comparison to unstimulated cells (in red) in the presence of retinoic acid. Total T cells were incubated with anti-Integrin α4β7 (Hu117; R&D), followed by α-hIgG-PE (BioLegend), and analyzed using Flow Cytometry. The flow cytometry profile for one donor is shown here and is representative of all donors.

A.



B.

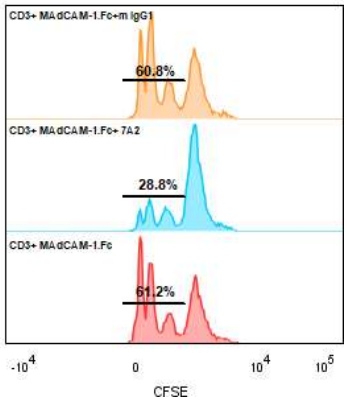


Figure S4: CFSE Trace and Gating Strategy. (A) Representative gating strategy and (B) CFSE trace of human CD4$^+$ T cells stimulated with α-CD3 and MAdCAM-1.Fc (in red) in comparison to cells treated with either mAb7A2 (in blue) or mIgG2a (in orange) in the presence of retinoic acid. mAb7A2 reduced the proliferation of CD4+ T cells.

Table S1: Epitope binning of representative mAb clones. Epitope binning analysis was performed on a representative mAb from each group defined in Figure 1. SPR was performed (BIAcore T200;Cytiva) and HBS running buffer (20mM of HEPES pH 7.4, 150mM NaCl, 0.005% Tween-20, 3.4mM EDTA) by immobilizing biotinylated human MadCAM-1 on a Streptavidin sensor chip (Cytiva). mAb "A" was injected at a flow rate of 30µL/min for 300s at a concentration 200× its K_d value. Next, a mixture of mAbs "A" and "B" were injected, both at a concentration 200x their respective K_d values. The RU was monitored for an additional 300s followed by regeneration. mAbs that bound to the same epitope are indicated in grey, clones that bound to different epitopes are indicated in blue, and orientation-dependent competitive clones are indicated in green. Concerning the latter, the clones are likely binding partially overlapping or proximally close epitopes where steric hindrance is preventing binding in one direction but not the other. Overall, each mAb assayed bound a unique epitope.

Competitor / Bound	6A3	1B10	11C3	15A3	13C5
6A3					
1B10					
11C3					
15A3					
13C5					