AK006 Drives Mast Cell Anergy as a Result of Antibody Binding Location and Distinct Protein Interaction Network

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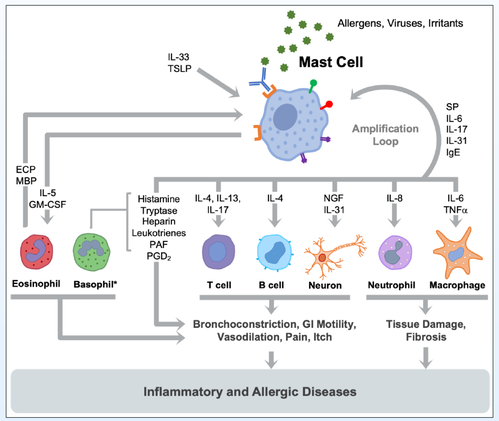
BACKGROUND

- Mast cells (MCs) regulate chronic inflammation through a myriad of activating cell surface receptors
- Dysregulation of MC activation through IgE-dependent and -independent mechanisms (ie cytokines, neuropeptides) contributes to allergic and non-allergic diseases
- MC-targeting strategies have focused on neutralizing individual mediators or activating receptors which may be insufficient to broadly reduce MC activity
- Molecules that dampen multiple pathways of MC activation, such as sialic acid-binding Ig-like lectins (Siglecs), represent novel therapeutic options for inflammatory diseases
- Siglec-6 and Siglec-8 are ITIM-bearing receptors highly expressed on MC
- Siglec-6 is an inhibitory receptor selectively expressed on human Mast Cells, and represents a novel target for the treatment of debilitating allergic, inflammatory, and proliferative diseases
- Likewise, Siglec-8 is a related ITIM-bearing receptor also expressed on human Mast Cells, as well as Eosinophils.
- Two therapeutic humanized antibodies towards Siglec-6 and Siglec-8, AK006 and AK002, respectively, have been developed which have been shown to broadly inhibit MC responses. However, their mechanisms of action have not been directly compared.

Figure 1. Mast Cells are Key Drivers of Acute and Chronic Inflammation

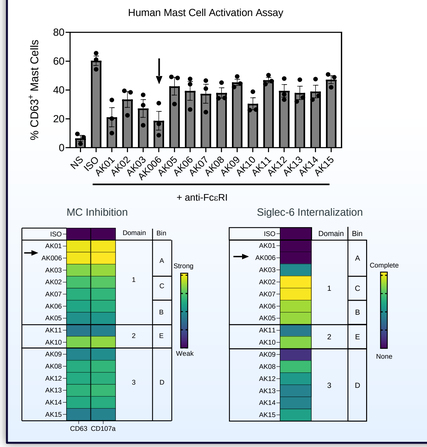


METHOD

- The activity of AK002 and AK006 was evaluated through biochemical characterization, ex vivo inhibition assays, in vivo studies, and transcriptome analysis using primary MCs.
- Additionally, proteomic profiling of MCs using quantitative mass spectrometry was performed to identify proteins associated with Siglec-6 and Siglec-8.

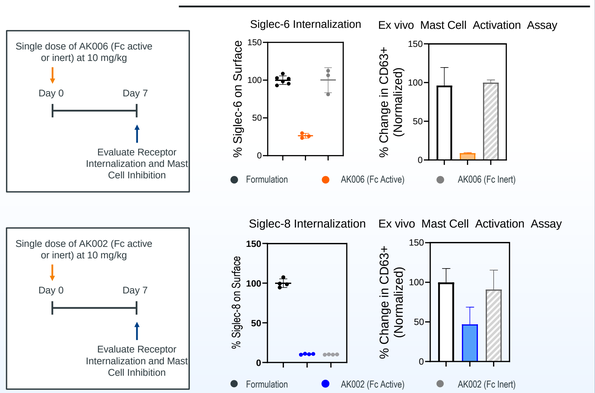
RESULTS

Figure 2. Siglec-6 mAb-mediated MC Inhibition and Internalization is Epitope Dependent



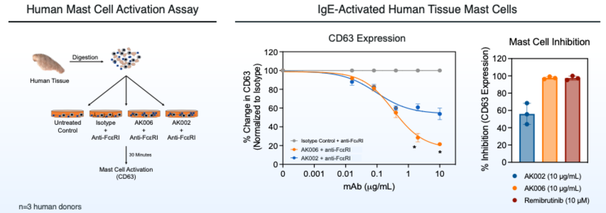
Siglec-6 mAbs show epitope-dependent receptor internalization and inhibition properties.
(Top Left) Diagram of Siglec-6 domains. (Top Center) Antibody binning of anti-Siglec-6 clones with relative levels of internalization measured via an internalization assay.
(Top Right) Percentage of CD63+ MCs non-stimulated or activated with anti-FcεRI antibody (CRA-1, 250 ng/mL) in the presence of Siglec-6 mAb clones (5 µg/mL) as determined by flow cytometry. (Bottom Right) Heatmap of MC inhibition induced by Siglec-6 mAb clones (5µg/mL) and their respective binding domains and bins.

Figure 3. AK006 and AK002 Requires Fc-engagement for Inhibition, but Only Siglec-6 Internalization is Dependent on Fc Receptors



Differential effect of Fc receptors on inhibition and internalization properties of AK002 and AK006.
(Top Left) Experimental set up of dosing animals with AK006 for 7 days, i.v. (Top Center) Internalization of Siglec-6 on Mast cells as measured by a non-competing Siglec-6 antibody clone 7 days after dosing. (Top Right) Whole PLMCs were obtained and then challenged with 500 ng/mL biotinylated anti-IgER antibody followed by neutravidin induced cross-linking. CD63 was measured to assess MC activation status.
(Bottom Left) Experimental set up of dosing animals with AK002 for 7 days, i.v. (Bottom Center) Internalization of Siglec-8 on Mast cells as measured by a non-competing Siglec-8 antibody clone 7 days after dosing. (Bottom Right) Whole PLMCs were obtained and then challenged with 500 ng/mL biotinylated anti-IgER antibody followed by neutravidin induced cross-linking for 20 minutes. CD63 was then measured by flow cytometry to assess MC activation status.

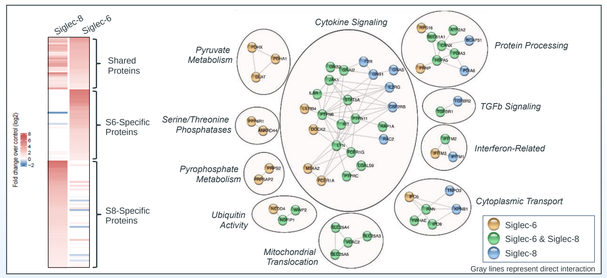
Figure 4. AK006 Delivers Deeper Inhibition of IgE-mediated MC Activation than AK002



n=3 human donors

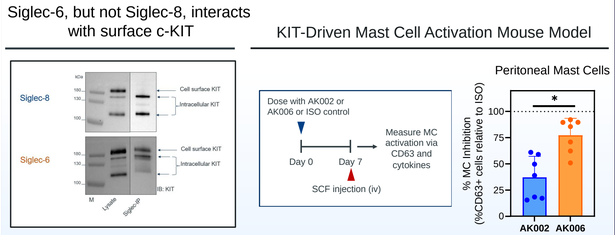
AK006 is more potent in inhibiting IgE-mediated signaling than AK002. (Left) Experimental design for activation of human lung mast cells with anti-IgER (CRA-1) in the presence of AK002 or AK006. (Right) Percentage of CD63 positive lung tissue MCs upon titration of AK006 (orange) or AK002 (blue).

Figure 5. Siglec-6 and Siglec-8 Protein Interactome are Unique



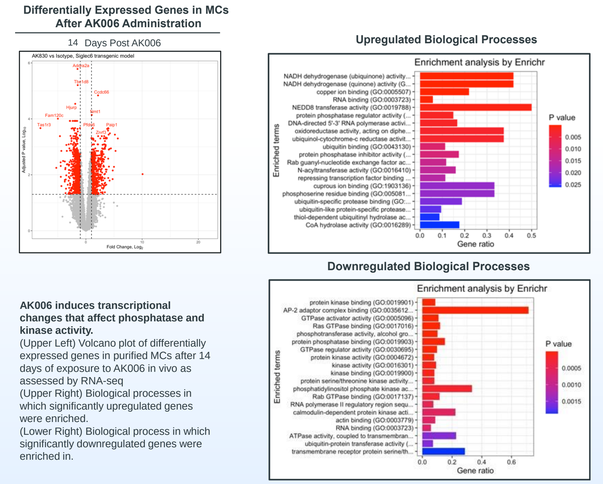
Siglec-6 and Siglec-8 interactome are unique.
(Upper Left) Overview heatmap of all proteins significantly co-immunoprecipitated with either Siglec-8 or Siglec-6.
(Upper Right) STRING analysis of the Siglec-6 and Siglec-8 interactomes with visual representation of the 10 largest clusters of protein interactions.

Figure 6. AK006 Delivers Deeper Inhibition of Non-IgE-mediated MC Activation than AK002



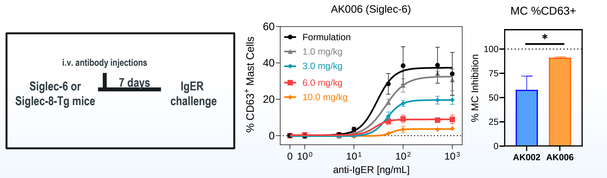
AK006 interacts with mature KIT and is more potent in inhibiting KIT signaling than AK002.
(Left) Western blots of lysates and IPs from S6- or S8-transgenic BMMCs stained with anti-KIT antibodies. (Right) Inhibition of CD63 expression when challenged with SCF and treated with either AK006 or AK002 normalized to Isotype.

Figure 7. AK006 Induces Time-Dependent Transcriptional Changes in Mast Cells



AK006 induces transcriptional changes that affect phosphatase and kinase activity.
(Upper Left) Volcano plot of differentially expressed genes in purified MCs after 14 days of exposure to AK006 in vivo as assessed by RNA-seq
(Upper Right) Biological processes in which significantly upregulated genes were enriched.
(Lower Right) Biological process in which significantly downregulated genes were enriched in.

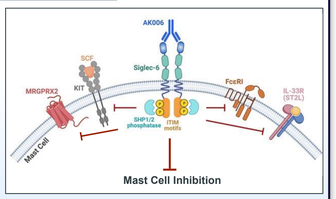
Figure 8. AK006 Induces Mast Cell Anergy *in vivo*



AK006 delivers doses dependent inhibition in vivo leading to attenuated MC responses.
(Left) Mice were dosed for 7 days with various antibody doses. PL was obtained and then challenged with biotinylated anti-IgER antibody at various doses followed by neutravidin induced cross-linking.
(Center) %CD63 was measured using flow cytometry.
(Right) AK006 induced significantly stronger inhibition than AK002 at 10 mg/kg following 500 ng/mL anti-IgER (MAR1) induced activation.

CONCLUSIONS

- AK006 displays differential MC inhibition as compared to AK002
- Targeting Siglec-6 with an agonist mAb, AK006, significantly suppressed IgE and non-IgE mediated MC activation
- These findings support AK006 as a potential therapeutic across multiple MC-driven diseases



References: XXXX

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