Siglec15 induces monocyte apoptosis and an antibody ES012 reverses siglec15-driven immunosuppression

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BACKGROUND

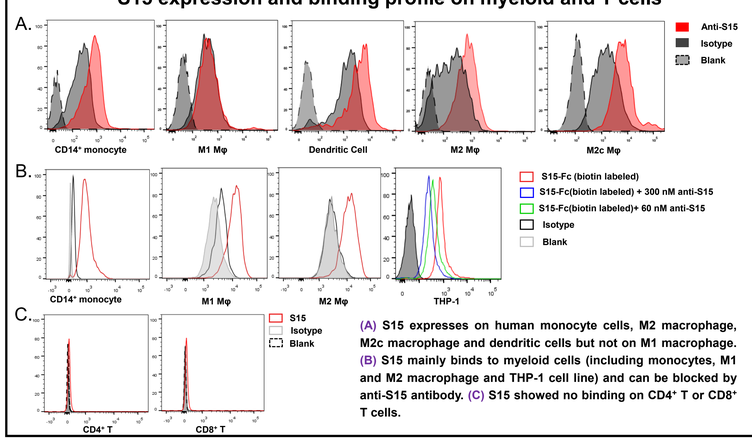
Siglec15 (S15) is a glycan-recognition proteins belongs to the Siglec (Sialic acid immunoglobulin superfamily lectins) family, preferentially recognizing the Neu5Aca2–6GalNAca– structure[1]. S15 is induced by M-CSF (macrophage colony-stimulating factor) and is highly expressed on TAM (tumor associated macrophage) and many tumor cells. S15 inhibits T cell activity via its binding to an unknown receptor on T cells[2]. While *in vitro* and *in vivo* evidence clearly demonstrated that S15 engagement suppressed antigen-specific T cell response and S15 antibody inhibited tumor growth in animal tumor models, the mechanism of S15 function on T cells and myeloid cells remains unclear. In this study, we seek to investigate S15 biology and generate anti-S15 antibodies for therapeutic use.

METHODS

Monocytes and T cells were purified from PBMC of healthy donors. M2, M1 macrophage and DC cells were induced in vitro from monocytes by different cytokines. The binding and function of S15 on different cell subsets were evaluated by in vitro assays. S15 antibodies generated, hybridoma fusion after mice immunization were screened and characterized by antigen binding, ligand blocking assay, rescue of monocyte apoptosis, reversion of monocyte inhibition and PBMC T cell activation assay. Selected leads underwent epitope binning by Bio-layer interferometry (BLI) and mapping by hydrogen deuterium exchange mass spectrometry (HDX-MS), they were then evaluated in MC38/hS15 bearing human S15 knock-in C57 mice for PK profile and in vivo efficacy. Finally, ES012 undergone preclinical assessment, including potential acute toxicity study in cynomolgus monkeys and pharmacokinetics (PK) study.

RESULTS

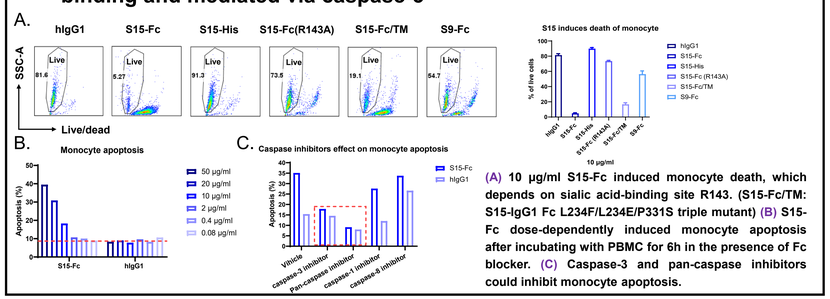
S15 expression and binding profile on myeloid and T cells



(A) S15 expresses on human monocyte cells, M2 macrophage, M2c macrophage and dendritic cells but not on M1 macrophage. (B) S15 mainly binds to myeloid cells (including monocytes, M1 and M2 macrophage and THP-1 cell line) and can be blocked by anti-S15 antibody. (C) S15 showed no binding on CD4⁺ T or CD8⁺ T cells.

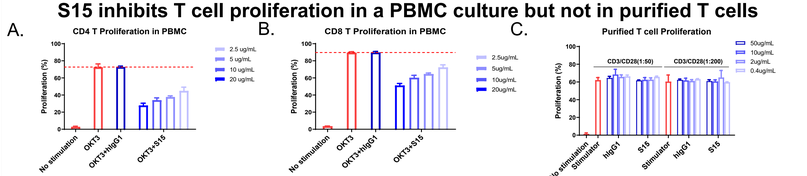
RESULTS

S15 induces monocyte apoptosis, which is dependent on sialic acid binding and mediated via caspase-3



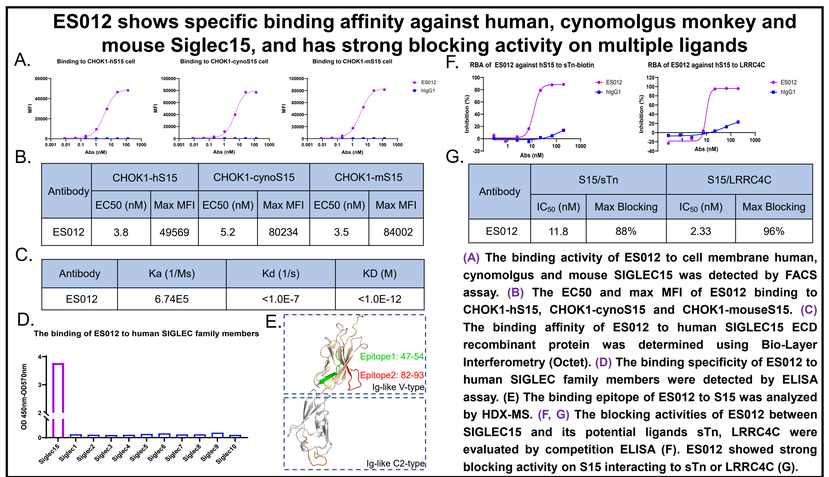
(A) 10 μg/ml S15-Fc induced monocyte death, which depends on sialic acid-binding site R143. (S15-Fc/TM: S15-IgG1 Fc L234F/L234E/P331S triple mutant) (B) S15-Fc dose-dependently induced monocyte apoptosis after incubating with PBMC for 6h in the presence of Fc blocker. (C) Caspase-3 and pan-caspase inhibitors could inhibit monocyte apoptosis.

S15 inhibits T cell proliferation in a PMC culture but not in purified T cells



(A, B) Siglec15-Fc could dose-dependently inhibit CD4⁺ (A) and CD8⁺T (B) cell proliferation by OKT3 stimulation in a PBMC culture. (C) Siglec15 doesn't directly inhibit T cells activation upon CD3/CD28 beads stimulation, or other stimulations, including PMA, ionomycin and PHA (data not show).

ES012 shows specific binding affinity against human, cynomolgus monkey and mouse Siglec15, and has strong blocking activity on multiple ligands



B.

Antibody	CHOK1-hS15		CHOK1-cynoS15		CHOK1-mS15	
	EC50 (nM)	Max MFI	EC50 (nM)	Max MFI	EC50 (nM)	Max MFI
ES012	3.8	49569	5.2	80234	3.5	84002

C.

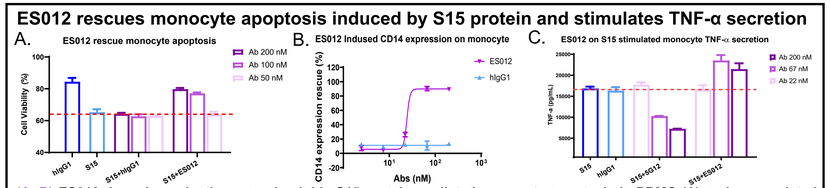
Antibody	Ka (1/Ms)	Kd (1/s)	KD (M)
ES012	6.74E5	<1.0E-7	<1.0E-12

G.

Antibody	S15/sTn		S15/LRRC4C	
	IC_{50} (nM)	Max Blocking	IC_{50} (nM)	Max Blocking
ES012	11.8	88%	2.33	96%

(A) The binding activity of ES012 to cell membrane human, cynomolgus and mouse SIGLEC15 was detected by FACS assay. (B) The EC50 and max MFI of ES012 binding to CHOK1-hS15, CHOK1-cynoS15 and CHOK1-mouseS15. (C) The binding affinity of ES012 to human SIGLEC15 ECD recombinant protein was determined using Bio-Layer Interferometry (Octet). (D) The binding specificity of ES012 to human SIGLEC family members were detected by ELISA assay. (E) The binding epitope of ES012 to S15 was analyzed by HDX-MS. (F, G) The blocking activities of ES012 between SIGLEC15 and its potential ligands sTn, LRRC4C were evaluated by competition ELISA (F). ES012 showed strong blocking activity on S15 interacting to sTn or LRRC4C (G).

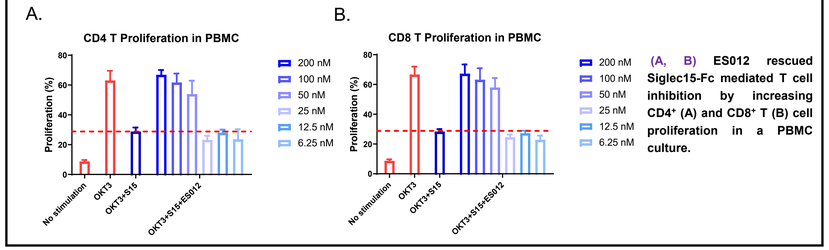
ES012 rescues monocyte apoptosis induced by S15 protein and stimulates TNF-α secretion



(A, B) ES012 dose-dependently rescued soluble S15 protein mediated monocyte apoptosis in PBMC (A) and up-regulated CD14 expression on live cells in a dose-dependent manner (B). (C) The effect of ES012 and 5G12 on monocyte TNFα secretion upon LPS stimulation incubated with S15 protein. ES012 increased TNF-α release while 5G12 blocked the release.

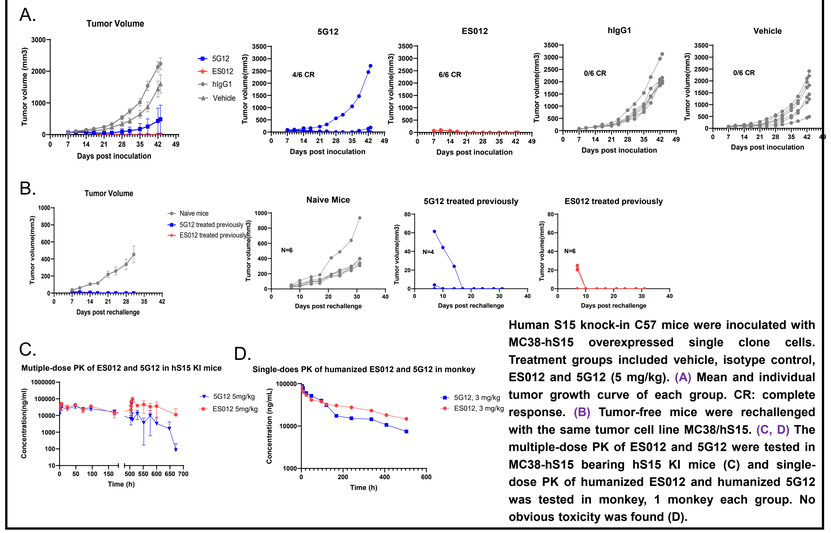
RESULTS

ES012 reverses the inhibition of S15 on OKT3-driven T cell proliferation in a PBMC culture



(A, B) ES012 rescued Siglec15-Fc mediated T cell inhibition by increasing CD4⁺ (A) and CD8⁺ T (B) cell proliferation in a PBMC culture.

In vivo efficacy study in MC38-hS15 bearing hS15 KI mice and PK profile of ES012



Human S15 knock-in C57 mice were inoculated with MC38-hS15 overexpressed single clone cells. Treatment groups included vehicle, isotype control, ES012 and 5G12 (5 mg/kg). (A) Mean and individual tumor growth curve of each group. CR: complete response. (B) Tumor-free mice were rechallenged with the same tumor cell line MC38/hS15. (C, D) The multiple-dose PK of ES012 and 5G12 were tested in MC38-hS15 bearing hS15 KI mice (C) and single-dose PK of humanized ES012 and humanized 5G12 was tested in monkey, 1 monkey each group. No obvious toxicity was found (D).

Summary

In summary, we have identified a novel function of S15 which is that S15 can induce monocyte apoptosis and its inhibitory effect on T cell function is indirect. Based on this newly discovered S15 biology, we have developed a potent, functional anti-Siglec15 mAb ES012 that has great potential to reverse immune suppression in the TME to promote anti-tumor immunity.

Reference

[1] Angata, T., et al., Siglec15: an immune system Siglec conserved throughout vertebrate evolution. Glycobiology, 2007. 17(8): p. 838-846.
[2] Wang, J., et al., Siglec15 as an immune suppressor and potential target for normalization cancer immunotherapy. Nat Med, 2019. 25(4): p. 656-666.