Sialic acid-binding immunoglobulin-like lectin (Sigelac)-15 is a rapidly internalised cell-surface antigen expressed by acute myeloid leukaemia cells

Huan Cao,1 Andreas Neerincx,2 Bernard de Bono,3 Ursula Lakner,4 Catherine Huntington,5 John Elvin,5 Emma Gudgin,6 Clare Pridans,6 Mark A. Vickers,1 Brian Huntly,6 John Trowsdale2 and Alexander D. Barrow7

1School of Medicine, Medical Sciences and Nutrition, University of Aberdeen, Aberdeen, 2Immunology Division, Pathology Department, University of Cambridge, Cambridge, UK, 3Auckland Bioengineering Institute, University of Auckland, Auckland, New Zealand, 4Medical Faculty, University of Tübingen, Tübingen, Germany, 5MedImmune, Cambridge, 6University of Edinburgh Centre for Inflammation Research, Edinburgh, UK, and 7Department of Microbiology and Immunology (DMI), The University of Melbourne, Melbourne, Australia

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Correspondence: Huan Cao, School of Medicine, Medical Sciences and Nutrition, University of Aberdeen, Aberdeen, UK. E-mail: h.cao@abdn.ac.uk

Summary

Sialic acid-binding immunoglobulin-like lectin (Siglec)-15 has recently been identified as a critical tumour checkpoint, augmenting the expression and function of programmed death-ligand 1. We raised a monoclonal antibody, A9E8, specific for Siglec-15 using phage display. A9E8 stained myeloid leukaemia cell lines and peripheral cluster of differentiation (CD)33+ blasts and CD34+ leukaemia stem cells from patients with acute myeloid leukaemia (AML). By contrast, there was minimal expression on healthy donor leucocytes or CD34+ stem cells from non-AML donors, suggesting targeting Siglec-15 may have significant therapeutic advantages over its fellow Siglec CD33. After binding, A9E8 was rapidly internalised (half-life of 180 s) into K562 cells. Antibodies to Siglec-15 therefore hold therapeutic potential for AML treatment.

Keywords: Siglec-15, acute myeloid leukaemia, antibody, phage display, endocytosis.

Introduction

Acute myeloid leukaemia (AML) is a significant health burden with an incidence of around four cases/100 000/year1 with ~3000 cases/year in the UK. Curative treatment is intensive, arduous for the patient, and requires long hospital admissions. In contrast to other malignancies, immunotherapeutic agents are yet to play a major role in treatment.

Identifying suitable tumour-specific antigens is a major barrier in developing immunotherapies.2 In AML, the most promising target has been the sialic acid-binding immunoglobulin-like lectin (Siglec), cluster of differentiation (CD)33, which is targeted by gemtuzumab ozogamicin (GO, Mylotarg) a toxin-(calicheamicin) conjugated antibody.3 Despite setbacks, a randomised trial of 237 patients aged ≥60 years and ineligible for intensive chemotherapy showed an improved overall survival (hazard ratio 0.69) in those assigned to GO induction and consolidation compared with best supportive care.4

Sialic acids are nine carbon-based sugars found at the termini of most mammalian glycan structures. The Siglecs are immunoglobulin superfamily receptors that bind to sialic acids and are commonly expressed on immune cells, particularly of the myeloid lineage.5 Most Siglecs are inhibitory, but some are activating, namely Siglecs-14, -15 and -16.6,7 A rapidly evolving sub-family of Siglecs, known as the CD33-related (CD33r) Siglecs, demonstrate potential as AML targets.8

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Siglec-15 is a new member of the Siglec family primarily expressed on a subset of myeloid cells. Siglec-15 has also been shown to bind the tumour antigen sialyl-Thomsen-nouvelle antigen (sTn). Siglec-15 is unusual in that it is equipped with both negative and positive signalling motifs. Siglec-15 contains a lysine residue in the transmembrane that potentially mediates association with signalling adaptor molecules, such as DNAX-activating protein 10 (DAP10), DAP12 and the Fc receptor common γ (FcRγ) chain, to promote cellular activation, yet also contains a cytoplasmic immunoreceptor tyrosine-based inhibitory motif (ITIM)-like motif known as immunoreceptor-tvrosine based switch motif (ITSM) that generally mediates inhibitory signals. In support of this, Siglec-15 has recently been identified as a novel check point inhibitor with mutually exclusive expression to the classical checkpoint inhibitor, programmed death-ligand 1 (PD-L1), suggesting antibodies to Siglec-15 may have a role as immune checkpoints that can complement PD-L1 inhibitors.

In the present study, we developed a monoclonal antibody (A9E8) to Siglec-15 using phage display. We show that A9E8 stains Siglec-15 on CD33+ peripheral blood mononuclear cells from patients with AML, but not from healthy donors. A9E8 also stains CD34+ leukaemic stem cells (LSCs) in AML, but not equivalent CD34+ cells from non-AML donors. Finally, A9E8 induces the rapid internalisation of Siglec-15 on myeloid leukaemia cells, such as K562, suggesting therapeutic potential for AML treatment.

Results

Generation of Siglec-15 specific antibody, A9E8
Phage display was employed to select a specific antibody, A9E8, against Siglec-15 (Figure S1), which binds to overexpressed cell surface Siglec-15 as well as endogenous Siglec-15 from leukaemia cell lines such as K562 (Figure S2; Figure S3). Clustered regularly interspersed short palindromic repeats (CRISPR) deletion of SIGLEC15 from the cell-line K562 resulted in loss of A9E8 staining (Figure S1D). Enhanced surface expression of full length Siglec-15 (N-terminal FLAG-tagged) was observed when paired with clones stably expressing DAP10, DAP12 and FcRγ (Figure S4).

Absence of Siglec-15 surface expression on peripheral blood leucocytes from healthy donors
Siglec-15 is absent on the surface of most mature leucocytes from healthy donors: T cells (0.2% CD3+), B cells (0.2% CD19+) and natural killer cells (0.3% CD3+CD56+), but a small (~0.8%) proportion of monocytes (CD14+) showed Siglec-15 surface expression (Figure S5A). The overall negative staining of mature circulating leucocytes was confirmed on nine healthy donors (Fig 1D). We report very weak Siglec-15 surface expression on 7% of cultured macrophages derived from peripheral blood monocytes and extremely low Siglec-15 surface expression on ~4% of dendritic cells before lipopolysaccharide (LPS) stimulation (Figure S5B), both of which were lost following 24 h of LPS stimulation (Figure S5B). These results show that in healthy donors, expression of Siglec-15 is low or absent on the cell surfaces of most mature circulating leucocytes as well as in vitro cultured macrophages and dendritic cells.

Siglec-15 is a prominent surface antigen on circulating myeloid blasts
In contrast, significantly higher Siglec-15 surface expression was found on peripheral blood cells from nine of the 12 patients with AML tested (Fig 1A–D), with an average of ~18% of the subpopulation being Siglec-15 positive as compared to only 0.6% for circulating peripheral blood mononuclear cells (PBMCs) from healthy donors (Fig 1D). CD33 and Siglec-15 show comparable level of percentage expression on circulating leucocytes (Fig 1E), giving a strong correlation (Pearson’s correlation r = 0.9823, n = 12) (Fig 1E). Three of the patients with AML were profiled in detail, where we see consistent co-expression of Siglec-15 with CD14 (Fig 1A–C). CD33 has been reported to be expressed on 90% of AML blasts. Surprisingly, we identified one AML patient amongst the 12, patient B (Fig 1B), who was CD33-negative, expressed a significant level of Siglec-15 on 20% of the peripheral cells. The strong correlation between Siglec-15 and CD33, as well as the existence of CD33-Siglec-15+ phenotypes is supported by public data found in Figure S6. The overall A9E8 binding specificity to circulating AML leucocytes over healthy cells is also reflected in the fluorescence intensities (Fig 1F). These data indicate that Siglec-15 is expressed on significantly higher percentage of circulating leucocytes in patients with AML than in healthy donors.

A9E8 induces a rapid internalisation of Siglec-15 from the cell surface
A fast rate of Siglec-15 internalisation would be advantageous for toxin-conjugated antibody targeting of Siglec-15-positive AML blasts. Rapid internalisation of Siglec-15 is expected from its cytoplasmic ITSM motif (sequence: SYNENL), conforming to the classical endocytosis YxxΦ motif (X, any amino acid; Φ, hydrophobic residue). Extremely rapid endocytosis of Siglec-15 was noted on K562 cell line through cross-linking of A9E8 with a half-life of only 174 s (Fig 2A). A9E8 does not induce endocytosis if Siglec-15 lacking its cytoplasmic tail (Fig 2B), indicating the observed phenomenon cannot be explained by dissociation of the A9E8 antibody from the cell surface. Rapid endocytosis is also observed in AML peripheral blood samples (Fig 2C), consistent with K562 cell line and an AML cell line, U937 (Figure S7). Z-stack confocal microscopy showed the presence of A9E8 antibody in permeabilised K562 cells (Fig 2D, E–v).
Circulating peripheral cells from non-AML patients but who have had granulocyte-colony stimulating factor (G-CSF) mobilisation showed no A9E8 staining (Figure S8A,B). These peripheral cells offer a higher representation of progenitor cells, including CD34+ stem cells (Figure S8A). These data provide support for the specificity of the A9E8 antibody in AML bone marrow, as healthy CD34+ stem cells would not be targeted. Furthermore, CD34+CD38- subpopulations of circulating peripheral cells from four AML and three non-AML G-CSF-treated patients were compared (Figure S8C–E). This so-called leukaemic stem cells (LSCs) compartment appears to have high levels of Siglec-15 expression in AML, but not in non-AML equivalents (Figure S8C–E).

Discussion

We used phage display to develop an antibody, A9E8, specific for Siglec-15 and observed expression on circulating cells in patients with AML with contrasting low/negligible expression on healthy cells. The antibody also induced rapid endocytosis, with a half-life of 3 min compared to >100 min for Siglec-5 and -9. These properties support A9E8 as a potential novel therapeutic agent for the treatment of AML.

CD33 is expressed on monocytes and macrophages of healthy individuals. Due to the relatively low expression of Siglec-15 on mature myeloid cells, A9E8 has the potential to be associated with less myelosuppression than GO.3,13 We
found that in one case of CD33\(^-\) AML, there was significant expression of Siglec-15, suggesting that Siglec-15 may be a viable target on blasts that lack CD33.\(^5,13,14\) While a potential toxin-conjugated A9E8 may target some osteoclasts that express Siglec-15, depletion of Siglec-15\(^+\) osteoclasts may provide the benefit of preventing bone-loss in AML disease and AML metastasis to bone.\(^15\)

Collectively, our present data demonstrate A9E8 is specific for AML blasts and LSCs, but not healthy cells, and induces rapid Siglec-15 internalisation on the K562 myeloid leukaemia cell line. A9E8 is therefore a promising antibody for targeting conjugated toxins to AML blasts and the LSC compartment. Further characterisation of the expression pattern of Siglec-15 on haematopoietic cells from patients with AML and healthy donors should be undertaken.

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Ethical approval

Ethical approval was obtained for the study ‘The causes of clonal blood cell disorders’ Research Ethics Committee reference number is 07/MRE05/44. All patients used for the study were consented.

Author contributions

Huan Cao performed the research, helped to design the research and drafted the manuscript. Andreas Neerincx helped to design the CRISPR work. Bernard de Bono, Ursula Lakner helped performed the research. Catherine Huntington and John Elvin helped to design the research and draft the manuscript. Emma Gudgin and Claire Pridans contributed essential reagents and samples. Mark A. Vickers helped to write the manuscript. Alexander D. Barrow, John Trowsdale and Brian Huntly all helped to design the research and write the manuscript.

Conflict of interest

MedImmune has filed the following patent: ‘Anti-Siglec-15 antibodies and uses thereof’. United States Patent 9447192. This patent has been reviewed in 2015.16

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Fig S1. Assessment of monoclonal antibody A9E8 specific for Siglec-15.
Fig S2. Siglec-15 transcript expression.
Fig S3. Siglec-15 surface expression on myeloid leukaemic cell lines.
Fig S4. Adaptor association of Siglec-15.
Fig S5. Siglec-15 is not expressed by most healthy peripheral blood leucocytes.
Fig S6. Expression of CD33 and SIGLEC15 are positively correlated in AML.
Fig S7. Endocytosis of A9E8 antibody on AML cell line U937 and AML peripheral blood cells.

Fig S8. Granulocyte-colony stimulating factor (G-CSF) mobilised peripheral blood staining with A9E8.
Fig S9. Amino acid alignment of splice variants of SIGLEC15.

Data S1. Materials and methods.

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