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INTRODUCTION

Systemic anaphylaxis is considered to be a typical immediate hypersensitivity response, determined by the activation of immune cells, via antigen-induced aggregation of IgE-sensitized FcεRI cells. Perhaps most the important cells, in the immediate hypersensitivity responses, are mast cells. We have previously shown that SPHK1 plays a key role in the intracellular signaling pathways triggered by FcεRI aggregation on human mast cells. More recently, we performed a genome-wide gene expression profiling of human mast cells, sensitized with IgE alone, or stimulated by FcεRI aggregation. We found that sphingosine kinase 1 (SPHK1) was one of genes activated at the earlier stages of mast cell activation, including during sensitization. Moreover, SPHK1 has been shown, by us and others, to be a key player in the intracellular signaling pathways triggered by several immune-receptors, including fMLP, C5a, and Fcγ and Fcε-receptors.

Here we have investigated the *in vivo* role of SPHK1 in allergy, using a specific siRNA to knockdown SPHK1 *in vivo*. Our results support a role for SPHK1 in the inflammatory responses that share clinical, immunological, and histological features of type I hypersensitivity. Thus, mice pretreated with the siRNA for SPHK1 were protected from the IgE mediated allergic reactions including: temperature changes, histamine release, cytokine production, cell-adhesion molecule expression, and immune cell infiltration into the lungs.

MATERIALS AND METHODS

Animals

All experiments were performed on male BALB/c mice, aged 6-10 weeks obtained from the Laboratory Animal Holding Unit, National University of Singapore, Singapore.

SPHK 1- siRNA

The specific siRNA for SPHK 1 sequence, 5'-GGGCAAGGCUCGACGUCdTdT-3' (sense) and 5'-GAGCUCGAGAGCCUUGCCdTT-3'(antisense). The annealed double-stranded lyophilized SPHK 1 siRNA (Qiagen Inc., CA, USA), was dissolved in the siRNA suspension buffer to obtain 20 siM solutions. The tubes were heated to 90°C for 1 min and incubated at 37°C for 60 min. Then the siRNAs were diluted accordingly to obtain the required concentration for the experiments.

Induction of Passive Systemic Anaphylaxis

BALB/c mice were lightly anesthetized and administered intravenously through the tail vein with 20µg of monoclonal mouse anti-DNP-IgE diluted in 200 µl of PBS. The positive control group was administered i.v. injection of 1mg of DNP-BSA in 100 µl of PBS after 24h of anti-DNP-IgE administration. Control mice received IgE alone or DNP alone in PBS. The treatment groups received (i.v.) with 4µg of siRNA for SPHK1 in 200ml PBS at 0h, 24h and 48h prior to IgE administration.

Monitoring of Rectal Temperature

Changes in core body temperature associated with systemic anaphylaxis were monitored by measuring changes in rectal temperature using a rectal probe coupled to a digital thermometer.

Histological study

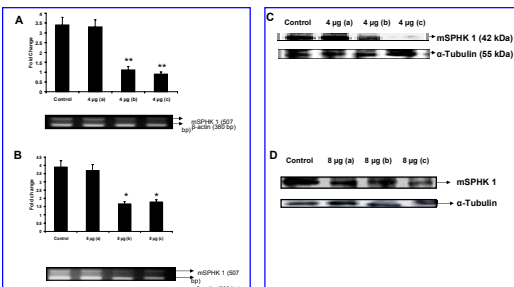
Samples of lung were obtained, fixed in 10% neutral buffered formalin, paraffin-embedded, cut into 5 mm sections and stained with hematoxylin-eosin according to standard procedures.

Immunohistochemistry

Immunohistochemistry was used to examine the expression of P-selectin, VCAM-1 and ICAM-1.

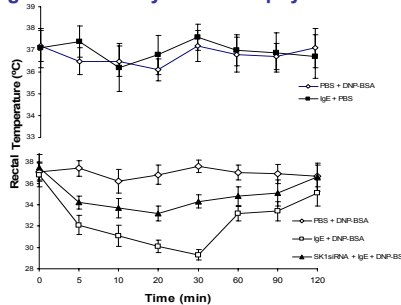
RESULTS

Fig1. RT-PCR and Western blot for the expression of mouse SPHK 1 in PBMCs.



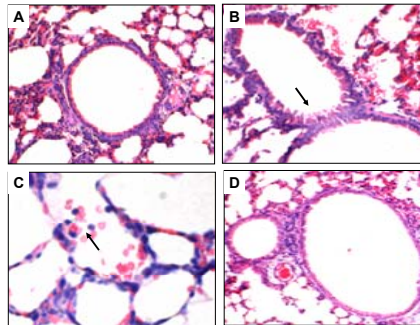
RT-PCR (A & B) and Western blot (C & D) showing the knockdown of murine SPHK 1 in PBMCs by repetitive administration of SPHK 1 specific siRNA (4 µg and 8 µg) i.v. (0.2 ml/min) at 0 hr, 24 hr, and 48 hr respectively within 1 minute in male BALB/c mice (n=5).

Fig 2. Assessment of rectal temperature during IgE-mediated systemic anaphylaxis



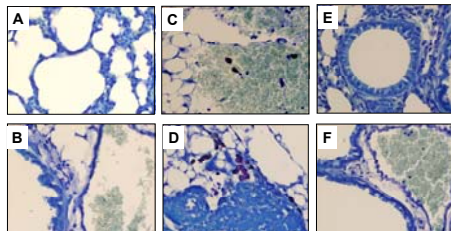
The monitoring of rectal temperature was started at the time of antigen injection. siRNA injected mice were administered with 4mg i.v at 0hr, 24hr and 48hr prior to antigen challenge. Data are shown as mean ± SD. * P<0.05. Five animals were used in each experimental condition.

Histologic Assessment of Lung Tissue Fig 3. Hematoxylin and Eosin staining



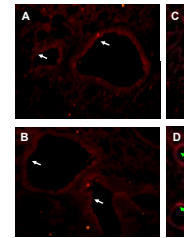
Sections of formalin-fixed lung tissue from a WT control mouse (A) and IgE+DNP-BSA triggered (B, C) siRNA (SPHK1)+ IgE+DNP-BSA (D) were stained with hematoxylin and eosin before examination by light microscopy. Lung sections from IgE+DNP-BSA triggered mice revealed the presence of inflammatory cells in the subepithelium of conducting airways (B), around blood vessels (B) and parenchyma (C) which was not seen in sections from WT mice (A) and siRNA treated (D).

Characterization of immune cells Fig 4. Toluidine blue staining



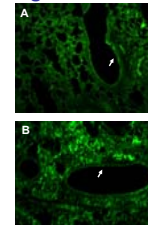
Sections of formalin-fixed lung tissue from a WT control mouse (A, B) and IgE+DNP-BSA triggered (C, D) siRNA (SPHK1)+ IgE+DNP-BSA (E, F) were stained with toluidine blue before examination by light microscopy. Lung sections from IgE+DNP-BSA triggered mice revealed the presence dark blue-stained mast cell granules (arrows) in the blood vessels (C), parenchyma (D). Mast cells were not seen section from WT (A, B) and siRNA treated (E, F).

Immunohistochemistry of P-selectin Fig 5. P-Selectin



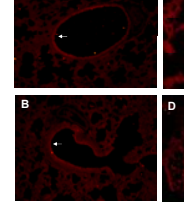
Immunofluorescence of lung P-selectin in the IgE+DNP-BSA airway epithelium, which (white arrows) treated mice (Fig 6. VCAM1

Fig 6. VCAM1



Immunofluorescence of lung VCAM1 in the IgE+DNP-BSA airway epithelium (red arrows) and siRNA treated mice (C, D) around the airway (violet arrows), which was not observed in WT (A, B) and siRNA treated mice (E, F).

Fig 7. ICAM1



Immunofluorescence of lung ICAM1 in the IgE+DNP-BSA airway epithelium (white arrows) and siRNA treated mice (C, D) around the airway (green arrows), which was not observed in WT (A, B) and siRNA treated mice (E, F).

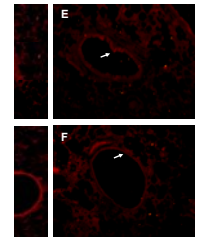
CONCLUSION

- Repetitive administration of siRNA significantly causes the reduction of SPHK 1 mRNA levels.
- IgE mediated systemic anaphylaxis is significantly enhanced in mice as assessed by changes in temperature.
- Histological studies revealed that immune cell infiltration and mast cell characterization.
- The overall inflammatory response of immune cells were significantly reduced by the knock down of Sphk1.
- Promising therapeutic potential to treat type-I allergy and other allergic conditions.

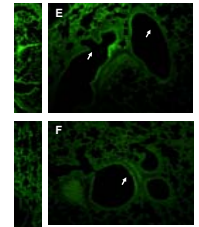
ACKNOWLEDGMENTS

This work was supported by...

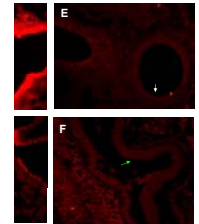
tion of Cell Adhesion



showing the expression of P-selectin in the IgE+DNP-BSA airway epithelium, which (white arrows) treated mice (A, B) and siRNA treated mice (C, D) around the airway (violet arrows), which was not observed in WT (A, B) and siRNA treated mice (E, F).



showing the expression of VCAM1 in the IgE+DNP-BSA airway epithelium (red arrows), which was not observed in WT (A, B) and siRNA treated mice (E, F).



showing the expression of ICAM1 in the IgE+DNP-BSA airway epithelium (white arrows) and siRNA treated mice (C, D) around the airway (green arrows), which was not observed in WT (A, B) and siRNA treated mice (E, F).