Episodic angioedema with eosinophilia (Gleich syndrome) is a multilineage cell cycling disorder

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Supplemental Methods:

Serologic Assessments

Serum IL-5, IL-8, IL-9, IL-13, sIL-2Rα, eotaxin-1, GM-CSF, G-CSF, IFN-γ, MIP-1-β, TNFα and IL-1-β levels were assessed by suspension array technology in multiplex (Millipore). The limits of detection were 0.1, 0.3, 1.1, 0.3, 7.5, 2.1, 2.3, 3.9, 0.4, 3.2, 0.2, and 0.7 pg/mL, respectively.

Immunohistochemistry methods

Immunohistochemical staining for lymphocytes was performed on a DAKO autostainer (Agilent Technologies, Carpinteria, CA) using a CD3 monoclonal antibody (1:200 dilation) and CD4 monoclonal antibody (1:80 dilation) and detected using diaminobenzine as the chromogen.

Immunohistochemical staining for eosinophil peroxidase was performed with a mouse anti-mouse monoclonal antibody (EPX; 1:500, provided by Dr. J.J. Lee). Heat-induced epitope retrieval was accomplished with Leica retrieval solution (Citric buffer) for 25 min and antibody was detected using the BondMax biotin-avidin free polymer-based detection system with diaminobenzine as the chromogen. Samples were analyzed on a Leica BondMax autostainer (Leica Microsystems, Bannockburn, IL).

Mast cells were identified in tissue by tryptase immunostaining performed on an automated immunostainer (Benchmark XT, Ventana Medical Systems, Inc., Tucson, AZ), according to the manufacturer's protocol. Antigen retrieval was accomplished using

a Protease 1 solution and detected with a Ventana UltraView DAB Detection Kit. Mast Cell Tryptase (clone AA1, 1:400; Dako Corp, CA) was used as the primary antibody. Mast cells were quantified in the bone marrow and skin by averaging counts over 10 high-powered fields.

Intracellular flow cytometry methods

Peripheral blood mononuclear cells (PBMCs) were isolated using density gradient separation (Ficoll-Paque Plus, GE Healthcare) and cryopreserved in liquid nitrogen. PBMCs were thawed, washed twice, resuspended in AIM V medium (Invitrogen) with 20 ng/ml phorbol myristate acetate, 1 μM ionomycin calcium salt and 10 μg/ml brefeldin A (all EMD Biosciences, San Diego, CA) and cultured at $4x10^6$ cells/well in 24 well plates in a 5% CO_2 incubator. After 6 h, 60 mg DNase (EMD Biosciences) were added to each well. Cells were harvested after 5 minutes, washed twice in cold PBS, labeled with LIVE/DEAD Fixable Violet Dead Cell Stain (Invitrogen), washed twice in cold PBS, and fixed in 4% paraformaldehyde (Sigma, St Louis, Mo). For staining, cells were blocked in PBS with 0.1% saponin (Sigma) and 5% nonfat dried milk for 30 minutes and then stained with the following panel: CD3-Pacific Blue (UCHT1-BD Pharmingen), CD4-Qdot 605 (S3.5 Invitrogen), CD8- PE-Cy5.5 (3B5 Invitrogen), CD154-APC-Cy7 (24-31 Biolegend), IL-4-PE (MP4-25D2, BD Pharmingen), IL-5-APC (TRFK5 BD Pharmingen), IL-13-PE (JES105A2 BD Pharmingen), IFN-γ-AF700 (B27 BD Pharmingen). Samples were acquired on an LSRII cytometer (BD Biosciences) and analyzed using FlowJo (v9.4.8 Stanford California).

Human Androgen Receptor (HUMARA) assay methods

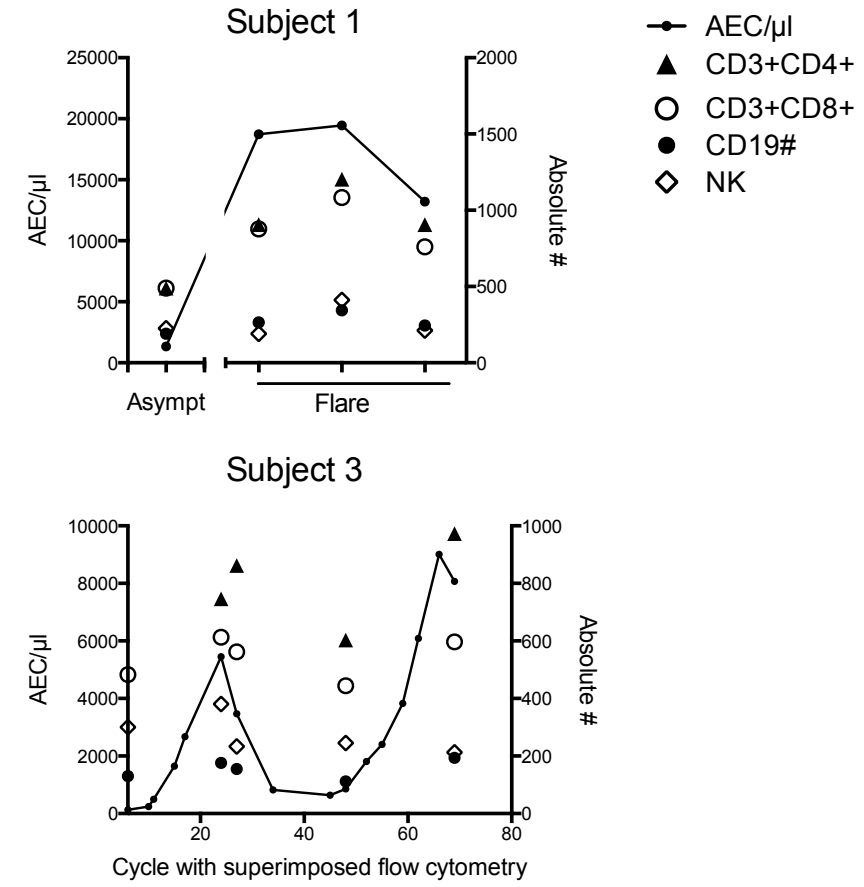
DNA was extracted using Purgene Tissue and Blood kit (Qiagen). DNA concentration and purity was calculated and 1.25 µg of DNA was incubated with 1:10 proteinase K at 37°C for 75 minutes. The total volume was split in half and was digested with or without 0.5µL of 10U *Hpa*II for 10 minutes at 65°C. The HUMARA locus was amplified using the primers (1pmol/ µL): FW 5'- /56-FAM/TCC AGA ATC TGT TCC AGA GCG TGC – 3' ; RV 5' – GCT GTG AAG GTT GCT GTT CCT CAT – 3', and the PCR protocol: initial denaturation at 95°C for 5 minutes, 40 cycles of denaturation at 95°C for 45 seconds, annealing at 61°C for 45 seconds, and elongation at 72°C for 1 minute. Products were then separated by capillary electrophoresis and the relative quantity of gene product peaks was assessed to determine skewing.

Supplemental Data Figure Legend:

Supplement 1. Cycling of T cell subset (CD3+CD4+, CD3+CD8+, B cells (CD19+) and NK cell (CD16+CD56+) numbers parallel the eosinophil count in episodic angioedema with eosinophilia in Subject 1 during a flare and subject 3 during one full cycle.

Supplement 2. HUMARA analysis showing polyclonal populations of cells in a female subject with episodic angioedema with eosinophilia (subject 4). E-eosinophils, CD4+-CD4+ lymphocytes, N-Neutrophils, B-B lymphocytes, PC-Positive control HES subject with a lymphocyte clone.

Supplement 1



Supplement 2