Supplemental Methods

Animals

All animal studies were approved by the Institutional Animal Care and Use Committee of the University of California, Santa Barbara and by the Regierung of Oberbayern, Munich, Germany. The *St3gal6 Δ* alleles were bred into the C57BL/6 background at least 5 generations prior to experiments.

Flow Cytometry

Single cell suspensions from the lymph nodes and Peyer's patches were prepared and red blood cells (RBCs) were lysed with RBC lysis buffer (eBioscience). Cells were incubated with antibodies in FACS buffer (2% FCS in PBS) for 20 min at 4˚C. For the experiment of E- and P-selectin ligand expression on neutrophil, peripheral blood leukocytes (PBLs) were isolated as previously described.[1] The isolated PBLs were incubated with Gr-1 antibody and either the E- or P-selectin-Fc chimera (R&D Systems) bound to fluorescein isothiocyanate (FITC)-conjugated anti-human IgG antibody (Sigma) in binding medium of Dulbecco modified Eagle medium (Gibco/Invitrogen) plus 2% IgG-free bovine serum albumin (BSA, Jackson Immunoresearch), with addition of 2 mM CaCl2 or 5 mM EDTA, for 30 min at 4˚C.[2] Lectin binding analysis on neutrophil was performed as previously described.[1] For lectin binding analysis on platelets and RBCs, whole blood cells were incubated with antibodies in FACS buffer for 20 min at 4˚C. Antibodies against CD3e (145-2C11), CD11a (2D7), CD11b (M1/70), CD18 (C71/16), CD24 (M1/69), CD41 (MWReg30), CD45R/B220 (RA3-6B2), CD62L (MEL-14), CD162 (PSGL-1, 2PH1), Gr-1 (RB6-8C5) and TER-119 (TER-119) were purchased from BD Pharmingen. *Erythrina crystagalli* lectin (ECA), *Ricinus communis agglutinin*-I (RCA-I), *Maackia amurensis* (MAL), Peanut agglutinin (PNA) and Elderberry lectin (SNA) were purchased from Vector Laboratories. Data were acquired using a FACSCalibur Flow Cytometer and analyzed by CellQuest Software (Becton Dickinson). For sialidase treatment, PBLs or whole blood cells were incubated with 0.3 units per peripheral blood 1 ml of *Arthrobacter ureafaciens* sialidase (Sigma) for 1 hr at 37˚C.

Ex vivo and *in vitro* flow chamber assays

Rectangular glass capillaries (VitroCom, cross section 200 µm x 2000 µm) were used as *ex vivo* flow chambers as described.[3] Recombinant murine (rm) P-selectin (20 µg/ml) and rmE-selectin (20 µg/ml) were immobilized on the flow chamber surface over night at 4 °C followed by incubation of the flow chamber with casein 10% (Sigma) for 2 hr at room temperature. Thereafter, flow chambers were washed with normal saline and connected to PE tubing which served at the same time as carotid artery catheter. The flow chamber was fixed under an upright fluorescence microscope (BX51, Olympus) with a saline immersion objective (×20/0.95 NA, Olympus) and perfused with whole blood from mice which is driven through the flow chamber by the arterial blood pressure of the mouse. Leukocyte rolling in the flow chamber was assessed as number of rolling leukocytes per field of view (FOV), observed over 10 min and recorded via a CCD camera system (CF8/HS; Kappa) on a Panasonic S-VHS recorder. For *in vitro* flow chamber experiments, neutrophils were isolated from wild type mice ($5x10^6$ cells/ml) as described[4] and treated with 0.1 units/ml *Arthrobacter ureafaciens* sialidase or *Streptococcus pneumoniae* sialidase (QA Bio) for 1 hr at room temperature. Flow chambers were perfused at a wall shear stress of 1 dyne/cm^2 using a high precision perfusion pump (Harvard Apparatus).

Peritoneal Inflammation

Mice were administered 1 ml of 3% thioglycollate (Sigma) in PBS by intraperitoneal injection as previously described.[5] At the indicated times, mice were sacrificed and their peritoneal cavities lavaged with 10 ml of ice-cold PBS containing 1% BSA and 0.5 mM EDTA. Red blood cells were removed by RBC lysis buffer (eBioscience). Peritoneal cell exudates were stained with anti-Gr-1 antibody (BD Pharmingen) and the pan-macrophage marker antibody F4/80 (Caltag/Invitrogen) and analyzed by flow cytometry.

Histology

For analysis of L-selectin ligand expression, 5 µm frozen sections of lymph nodes or Peyer's patches were incubated with the L-selectin-Fc chimera (R&D Systems) and anti-aTubulin antibody (Santa Cruz Biotechnology) followed by goat anti-human IgM FITC-conjugated secondary antibody (Sigma) and goat anti-rabbit IgG Texas Red-conjugated secondary

antibody (Santa Cruz Biotechnology). Serial sections were stained with hematoxylin and eosin (H & E).[5] Images were analyzed by TissueFAXS and TissueQuest microscope software systems (TissueGnostics USA Ltd.) as described previously.[6,7]

Supplemental References

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