Supplemental Materials

METHODS

Human peripheral blood mononuclear cells purification, flow-cytometric analysis and immunofluorescence studies

PBMC were isolated by Ficoll-Hypaque density-gradient centrifugation. The following antibodies were used for analysis of Tfh cells in human blood: Allophycocyanin/Cyanine7-conjugated CD4, Phycoerythrin (PE) conjugated Foxp3 (all from Biolegend, San Diego, CA), Brilliant Violet 421™ conjugated CXCR5 (from BD Biosciences, San Diego, CA) and PE-conjugated IL-1β (from R&D system, Minneapolis, MN). The FLICA probes are non-cytotoxic fluorescent labeled inhibitors of caspases and can covalently bind to active caspase enzymes. To assess the activity of caspase-1, the FAM-FLICA caspase-1 probe (ImmunoChemistry Technologies, Bloomington, MN) was used according to the manufacturer's protocol. For intracellular and intranuclear staining, the Foxp3 Staining Set (eBioscience) was used according to the manufacturers' instruction. For intracellular cytokine staining, the cells were pretreated with Cell Activation Cocktail (with Brefeldin A) (Biolegend) for 6 h. Then, cells were washed twice with PBS containing 1% BSA and stained for surface molecules. After fixation and permeabilization, the cells were stained with anti-IL-1β Ab. For Foxp3 staining, surface-stained PBMC were fixed and permeabilized without stimulation. Appropriate isotype controls were used. Stained cells were analyzed by multiparameter flow cytometry and analyzed with FlowJo software**.**

Isolation of mouse spleen and lymph nodes lymphocytes, flow cytometric analysis and germinal center studies

B6 and its congenic strains, MRL/lpr and MZM2328 mice were sacrificed under anesthesia by cervical dislocation. The spleen and lymph nodes were removed and dissociated in RPMI 1640 medium containing 10% fetal bovine serum (FBS). Splenic lymphocytes were isolated using Mouse 1× Lymphocyte Separation Medium (Dakewe Biotech, Shenzhen, China), following manufacturer's instructions. For lymph node cells from mice, the rounded side of a plunger from a 5 ml syringe was used to crush the tissues through the 70 μm cell strainer to generate single cell suspensions. The cell suspensions were transferred into a 15 ml centrifuge tube and centrifuged at 400 × g for 10 min. Cell pellets were washed twice and suspended in staining buffer (PBS containing 2% FBS). Single-cell suspensions were stained with the following antibodies and subjected to cytometric analysis as described above. Fluorochrome-conjugated mAb against mouse CD3 (145-2C11), CD4 (RM4-5), CD21/35 (7E9), CD19 (6D5), CXCR5 (L138D7), IgD (11-26c.2a), and Ki67 (11F6) were from Biolegend (San Diego, CA). In some experiments, affinity purified rabbit anti-mouse CD3e Ab (Sinobiological, China) conjugated with Alexa Fluor labeling kits (Invitrogen, Grand Island, NY) were used. A FAM-FLICA caspase-1 probe (ImmunoChemistry Technologies) was used to identify act. caspase-1. For germinal center staining, frozen sections of spleen were used.

Spleen PNA staining

Paraffin-embedded sections of spleen tissue were stained with biotinylated peanut agglutinin (PNA) (Vector Laboratories, Burlingame, USA) followed by incubation with the ABC kit (Vector Laboratories, Burlingame, USA). Then, the sections were stained with diaminobenzidine (DAB) and counterstained with hematoxylin.

***In vitro* induction of B-cell activation by Tfh cells and IgG production by Tfh cells**

Autologous B cells ($CD19^+$), act. caspase-1^+ Tfh cells ($CD4+CXCR5+GITR^-CD19^-$ act. caspase-1^+) and act. caspase-1^- Tfh cells ($CD4+CXCR5+GITR^-CD19^-$ act. caspase-1^-) were sorted from lymph nodes of MRL/*lpr* mice at age of 16 weeks using the BD FACSAria II cell sorter (BD Biosciences, CA). B cells (4×10^4/well) were plated alone or with 2×10^4 act. caspase-1^+ Tfh cells or 2×10^4 act. caspase-1^- Tfh cells in the presence of 2 μg/ml soluble anti-CD3 (Biolegend) and 5 μg/ml anti-IgM (Jackson Immunoresearch Laboratories) for 6 days. At day 6, cells were collected and stained with fluorophore-conjugated Ab for flow cytometry. Activated B cells (CD19+GL-7+) were detected by flow cytometry. Stained cells were analyzed by multiparameter flow cytometry (CytoFLEX S, Beckman Coulter) and analyzed with FlowJo software. For assaying *in vitro* IgG production, 10^4 Tfh cells were incubated with $5x10^5$ splenic B cells for 5 days. Supernatants were harvested for quantifying mouse IgG by ELISA.