Mice

Nfil3$^{-/-}$ mice were generated as previously described.[1] *Nfil3*$^{-/-}$ mice backcrossed with C57BL/6 for at least 10 generations were used for all experiments. The act-mOVA/K$^{b-/-}$ mice were a generous gift from Dr. Stephen Schoenberger (La Jolla Institute for Allergy & Immunology).[2] All mice were bred and maintained under specific pathogen-free conditions. All experimental mouse protocols were adhered to Institutional Animal Care and Use Committee guidelines and were approved by the IACUC of University of Iowa.

Real-time RT-PCR

RNA was isolated from purified cells or stimulated cells as indicated and cDNA was synthesized using the SuperScript® First-Strand Synthesis System (Invitrogen). Quantification of mRNA expression was performed using SYBR® GREEN PCR Master Mix on the 7900HT Fast Real-Time PCR System (Applied Biosystems). The expression levels of each gene were normalized to the expression of *Hprt1*. The following primers were used: *Nfil3*, 5′-GAACTCTGCCTTAGCTGAGGT-3′ and 5′-ATTCCCGTTTTCTCCGACACG-3′; *Hprt1*, 5′-GTTGGATACAGGCCAGACTTTGTTG-3′ and 5′-GATTCAACTTGCGCTCATCTTAGGC-3′; *Batf3*, 5′- CAGAGCCCCAAGGACGATG-3′ and 5′-GCACAAAGTTCATAGGACACAGC-3′; *Irf8*, 5′-CGGGGCTGATCTGGGAAAAT-3′ and 5′-CACAGCGTAACCTCGTCTTC-3′; *Id2*, 5′-ATGAAAGCCTTCAGTCCGGTG-3′ and 5′-AGCAGACTCATCGGGTCGT-3′; *Irf4*, 5′-TCCGACAGTGGTTGATCGAC-3′ and 5′-CCTCACGATTGTAGTCCTGCTT-3′.

Flow cytometry

Cell suspensions from spleen, thymus, blood and BM culture were stained for using following antibodies: CD11c (N418, HL3), CD4 (RM4-5), CD8a (53-6.7), DEC-205 (DEC-205), CD24 (M1/69), PDCA-1 (927) SIRP-α (p84), CD45RA (14.8), Thy1.2 (30-H12), c-kit (2B8), Sca-1 (D7), Flt3 (A2F10), CD127 (A7R34), CD115 (AFS98), CD3 (145-2C11), B220 (RA3-6B2), CD19 (eBio1D3), CD11b (M1/70), Gr-1 (RB6-8C5), Ter119 (Ter-119), pan-NK (DX5), IL-12 (C15.6), if necessary, streptavidin (APC-Cy7). These antibodies were purchased from BD Biosciences, eBiosciences, and Biolegend. For IL-12 detection, stimulated DCs were stained for CD11c and CD45RA, fixed, permeabilized, and then stained with anti-IL-12 antibody. MHC class I tetramers (K^b) specific for OVA$_{257-264}$ were prepared using published protocols.[3,4] OVA$_{257-264}$ peptide (SIINFEKL) was custom ordered from Bio-synthesis. The tetramer staining protocol was previous described.[5] Cells were analyzed with LSR II (BD Biosciences), and data were interpreted with CellQuest (BD Biosciences) or FlowJo (Tree Star).

Isolation of dendritic cells

Spleen cDCs were isolated as described previously.[6] Briefly, minced spleens were treated with collagenase D (Roche) for 30 min at 37^{o}C and red blood cells (RBCs) were lysed. To deplete T cells, B cells, and pDCs, cells were incubated with anti-Thy1.2 and anti-B220 microbeads. Unbound cells were further incubated with anti-CD11c microbeads to purify cDCs.

***In vivo* DC induction**

Mice were subcutaneously injected with 5×10^6 B16 melanoma cells expressing FL as described previously.[7] Eight days after 8 injection, splenocytes were examined for DC population by flow cytometry.

Bone marrow culture
RBCs-depleted BM cells were cultured at 1.5×10^6 cells/ml in the presence of 50 ng/ml mouse FL (Peprotech) for 9 days. To identify each DC subset cells were stained for CD11c, CD45RA, CD24, and SIRP-α.

Viral transduction
Retroviral constructs for BATF3 and NFIL3 were generated by cloning BATF3 cDNA or NFIL3 cDNA into pMIG. Whole BM cells were cultured in the presence of 50 ng/ml FL for 2 days, then spin-infected with viruses produced by transient transfection of viral constructs into 293T cells. After infection, cells were grown in the presence of 50 ng/ml FL for another 7 days before being subjected to flow cytometry. Infected cells were identified as GFP^+ cells.

In vivo cross-priming assay
Irradiated splenocytes (5×10^6 cells) from act-mOVA/$K^{b-/-}$ mice were injected into *Nfil3*$^{+/+}$ or *Nfil3*$^{-/-}$ mice intravenously. Five days after injection, the magnitude of the $OVA_{257-264}$-specific $CD8^+$ T-cell response in PBL and spleen was determined by MHC class I-peptide tetramer staining.[5]

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