Slc15a4, AP-3, and Hermansky-Pudlak syndrome proteins are required for Toll-like receptor signaling in plasmacytoid dendritic cells

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Despite their low frequency, plasmacytoid dendritic cells (pDCs) produce most of the type I IFN that is detectable in the blood following viral infection. The endosomal Toll-like receptors (TLRs) TLR7 and TLR9 are required for pDCs, as well as other cell types, to sense viral nucleic acids, but the mechanism by which signaling through these shared receptors results in the prodigious production of type I IFN by pDCs is not understood. We designed a genetic screen to identify proteins required for the development and specialized function of pDCs. One phenovariant, which we named *feeble*, showed abrogation of both TLR-induced type I IFN and proinflammatory cytokine production by pDCs, while leaving TLR responses intact in other cells. The *feeble* phenotype was mapped to a mutation in *Slc15a4*, which encodes the peptide/histidine transporter 1 (PHT1) and has not previously been implicated in pDC function. The identification of the *feeble* mutation led to our subsequent observations that AP-3, as well as the BLOC-1 and BLOC-2 Hermansky-Pudlak syndrome proteins are essential for pDC signaling through TLR7 and TLR9. These proteins are not necessary for TLR7 or TLR9 signaling in conventional DCs and thus comprise a membrane trafficking pathway uniquely required for endosomal TLR signaling in pDCs.

adapter protein 3 | lysosome-related organelle | solute carrier | type I interferon | vesicular trafficking

Plasmacytoid dendritic cells (pDCs) are a rare subset of DCs found in the blood and peripheral lymphoid organs. They differ from conventional DCs in that they are derived from lymphoid rather than myeloid precursors and by differences in cell-surface markers. Despite their rarity, pDCs are the source of most of the type I IFN produced in vivo during most viral infections (1–3). pDCs also secrete proinflammatory cytokines and chemokines that initiate the innate immune response and help orchestrate the subsequent adaptive immune response. However, their role in host defense remains uncertain because depletion studies have not yet demonstrated a clear susceptibility phenotype in any viral infection (3, 4).

pDCs are activated upon engagement of Toll-like receptors (TLRs), which recognize molecular signatures of microbes including viruses (reviewed in ref. 5). In particular, ssRNA and ssDNA engage TLR7 and TLR9, respectively, within acidified endosomal compartments. Signaling through both of these receptors is MyD88- and IRAK-4–dependent, and in pDCs, additionally dependent on inhibitor κ-B kinase-α (IKKα), osteopontin, and IFN regulator factor 7 (IRF7) for type I IFN production (6, 7). At least three chaperone molecules (PRAT4A, GP96, and UNC93B) are necessary for trafficking TLR7 and TLR9 to their correct subcellular location in most or all cell types (8–10); a specialized trafficking system for TLRs in pDCs, which may be required for TLR-induced type I IFN production, has been proposed but not described (11).

Here, we describe a series of mutations, detected in an in vivo screen for proteins required for pDC function, which selectively abrogate TLR7 and TLR9 signaling in pDCs. We show that lysosome-related organelle (LRO) trafficking and biogenesis proteins, such as adapter-related protein complex-3 (AP-3) and Hermansky-Pudlack syndrome (HPS) proteins of the biogenesis of lysosome-related organelle complex (BLOC)-1 and BLOC-2 groups, are specifically required for type I IFN and cytokine production in pDCs. Moreover, Slc15a4, an obscure solute channel protein, is essential for TLR-mediated signaling in pDCs. Our data reveal a specialized membrane trafficking mechanism necessary for TLR signaling in pDCs, which could explain their unique responses to viral infection.

Results

Forward Genetic Screen to Identify Proteins with Nonredundant Roles in Type I IFN Production by pDCs. To detect defects in pDC development or function, we injected randomly mutagenized mice with the TLR9 agonist CpG-A mixed with the cationic lipid DOTAP, and measured type I IFN in serum six hours later. As expected, mice with defects in TLR9 signaling or type I IFN production, *Tlr9*$^{-/-}$, *Myd88*$^{poc/poc}$, *Irak4*$^{otiose/otiose}$, *Unc93b1*$^{3d/3d}$, and *Ifnar1*$^{-/-}$, failed to produce detectable type I IFN following CpG challenge (Fig. 1*A*). Among more than 4,500 third generation (G3) C57BL/6J mice homozygous for random ENU-induced germline mutations produced as previously described (12), mice derived from 5 different pedigrees failed to produce serum type I IFN after CpG-A challenge (Fig. 1*B*). The first mutation, denoted *CpG6*, also impaired TNF production by peritoneal macrophages in response to CpG-B oligodeoxynucleotide stimulation. *Tlr9* was therefore sequenced, which revealed a G→A transition in exon 2 of *Tlr9* (Fig. S1*A*). A second mutation, named *inept*, was found to affect not only pDC responses to CpG-A, but also to resiquimod. The *inept* mutation, an A→G transition in exon 4 of *Irf7*, was identified by whole genome sequencing using the Applied Biosystems SOLiD 3+ sequencing platform and verified by Sanger dideoxynucleotide sequencing (Fig. S1*B*). The *gemini* mutation, which was initially identified in a screen for proteins required for the innate immune response to MCMV infection, was subsequently found to also abrogate the type I IFN response to in vivo CpG-A challenge. In addition to these phenotypes, *gemini* mice were characterized by marked neutrophilia and a paucity of

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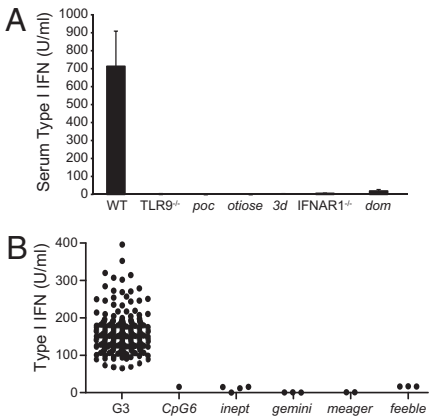

Fig. 1. Identification of mutants with in vivo defects in pDC type I IFN production. Mice were injected with the TLR9 ligand CpG-A along with DOTAP and serum type I IFN levels measured six hours later. The screen was validated by testing mice with mutations in genes known to be essential for the type I IFN response to CpG-A. *Tlr9*$^{-/-}$, *MyD88*$^{poc/poc}$, *Irak4*$^{otiose/otiose}$, *Unc93b1*$^{3d/3d}$, *Ifnar1*$^{-/-}$, and *Stat1*$^{dom/dom}$ mice all displayed little to no serum type I IFN (*A*). Screening ENU-mutagenized G3 mice revealed five unique pedigrees with defects in the CpG-induced type I IFN response. These mutations included alleles in *Tlr9* (*CpG6*), *Irf7* (*inept*), and *Irf8* (*gemini*). *Gemini* and *meager* mice were assayed on a different day (*B*). All error bars represent SEM.

monocytes and mature macrophages (Fig. S1*C*). The MCMV susceptibility and cellular phenotypes were mapped to chromosome 8 by bulk segregation analysis (BSA) (13) (Fig. S1*D*), and candidate gene sequencing revealed a nonsense mutation in the DNA binding domain of IRF8. Mice with a targeted mutation in *Irf8* lack pDCs (14, 15), and this likely accounts for the inability of *gemini* mice to respond to CpG-A in our in vivo screen. A fourth pedigree, named *meager*, is currently under study to identify the causative mutation. Finally, a fifth mutation, denoted *feeble,* was expanded as a homozygous stock for further analysis once it was clear that their inability to produce type I IFN in response to CpG-A was not caused by a mutation in any of the genes known to function in TLR signaling, pDC development and function, or IFN production and signaling.

***Feeble* Mice Display a Specific Defect in pDC Function.** Homozygous *feeble* mice fail to produce type I IFN following in vivo challenge with TLR7 or TLR9 ligands (Fig. 2*A*) despite having normal frequencies of splenic pDCs (Fig. 2*C*). pDCs also develop in vitro in Flt3L-treated bone marrow cultures from *feeble* mice (Fig. 2*C*); however, these cells do not secrete type I IFN or TNF-α, IL-12, or IL-6 in response to TLR7 and TLR9 ligands (Fig. 2 *A* and *B*). pDCs were enriched to high purity from the Flt3L cultures to prevent detection of cytokines generated by other cell populations. The defect in type I IFN production by *feeble* pDCs is not due to an inability to secrete type I IFN-because *Ifna* transcripts and intracellular type I IFN protein could not be detected in in vitro-generated *feeble* pDCs, even when exogenous type I IFN was added to circumvent the requirement for the IFN feed-forward loop (Fig. 3 *A* and *B*). Uptake of CpG by *feeble* pDCs is normal, suggesting that the *feeble* defect occurs after TLR ligands are internalized (Fig. 3*C*).

In contrast to our observation that pDCs from *feeble* mice fail to respond to TLR ligands, we found that conventional dendritic cells (cDCs) from these mutants (generated in vitro from GM-CSF-treated bone marrow cells) produce near normal amounts of TNF-α following stimulation with all TLR stimuli, including TLR7 and TLR9 ligands (Fig. S2*A*). Furthermore, TRIF-dependent signaling through TLR4 is intact in cDCs from *feeble* mice (Fig. S2*B*). Finally, *feeble* cDCs transfected with double-stranded DNA produce IFN, demonstrating that DNA sensing through DNA cytosolic sensors is intact in these mice (Fig. S2*C*). Collectively, these observations show that the *feeble* mutation selectively impairs the ability of pDCs, but not cDCs, to respond to TLR ligands and produce IFN.

Mapping and Identification of the *feeble* Mutation. We mapped the *feeble* phenotype by crossing homozygous mutant mice to C3H/HeN mice and backcrossing the F_1 progeny to the mutant stock: based on 46 meioses, the mutation mapped to chromosome 5 with a peak LOD score of 4.58 (Fig. 4*A*), and on 112 meioses, localized to a 7.2 Mbp critical region between markers located 125.310 and 132.581 Mbp from the centromere. This region contained 55 genes, of which all coding bases were sequenced at the genomic DNA level, using a combination of semiautomated capillary sequencing and whole genome sequencing. We identified a T→A transversion in the donor splice site in intron 2 of *Slc15a4*, which results in at least two aberrant transcripts (Fig. 4*B* and Fig. S3*A*). The first aberrant transcript lacks 260 bp derived from exons 2 and 3 leading to a frame shift with 73 aberrant amino acids before chain termination. The second transcript lacks all nucleotides

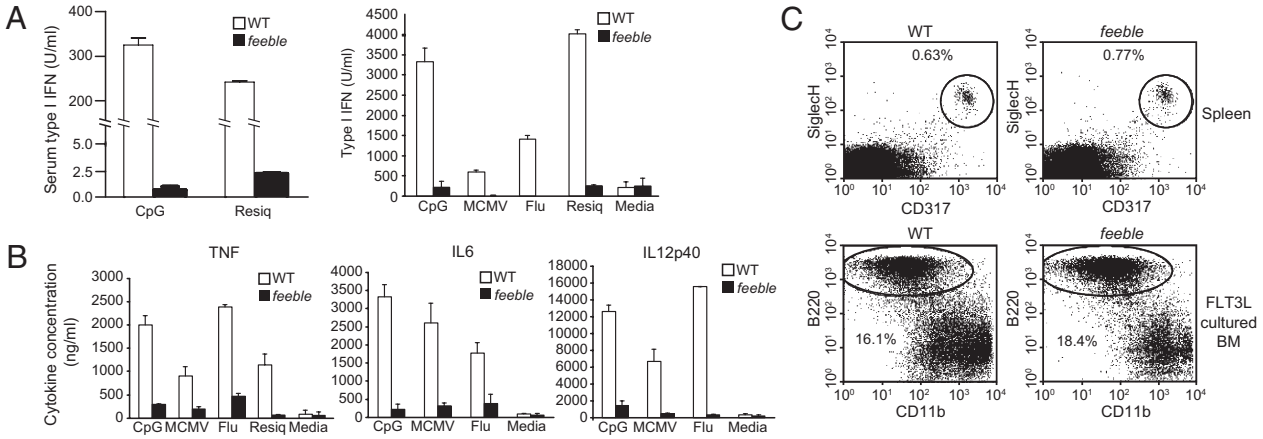

Fig. 2. The *feeble* mutation causes defective pDC function. Injection of *feeble* homozygous mice with CpG-A (TLR9 stimulation) or resiquimod (TLR7 stimulation) does not induce serum type I IFN (*A*, *Left*), and highly enriched pDC cultures generated from homozygous *feeble* bone marrow lack type I IFN responses to the indicated TLR stimuli. Data are representative of three mice (*A*, *Right*). In vitro-derived *feeble* pDCs generate reduced levels of secreted proinflammatory cytokines in response to the indicated stimuli. Data are representative of three mice (*B*). Normal pDC numbers are present in the *feeble* spleen, and normal percentages of pDCs develop from *feeble* bone marrow in vitro (*C*). All error bars represent SEM.

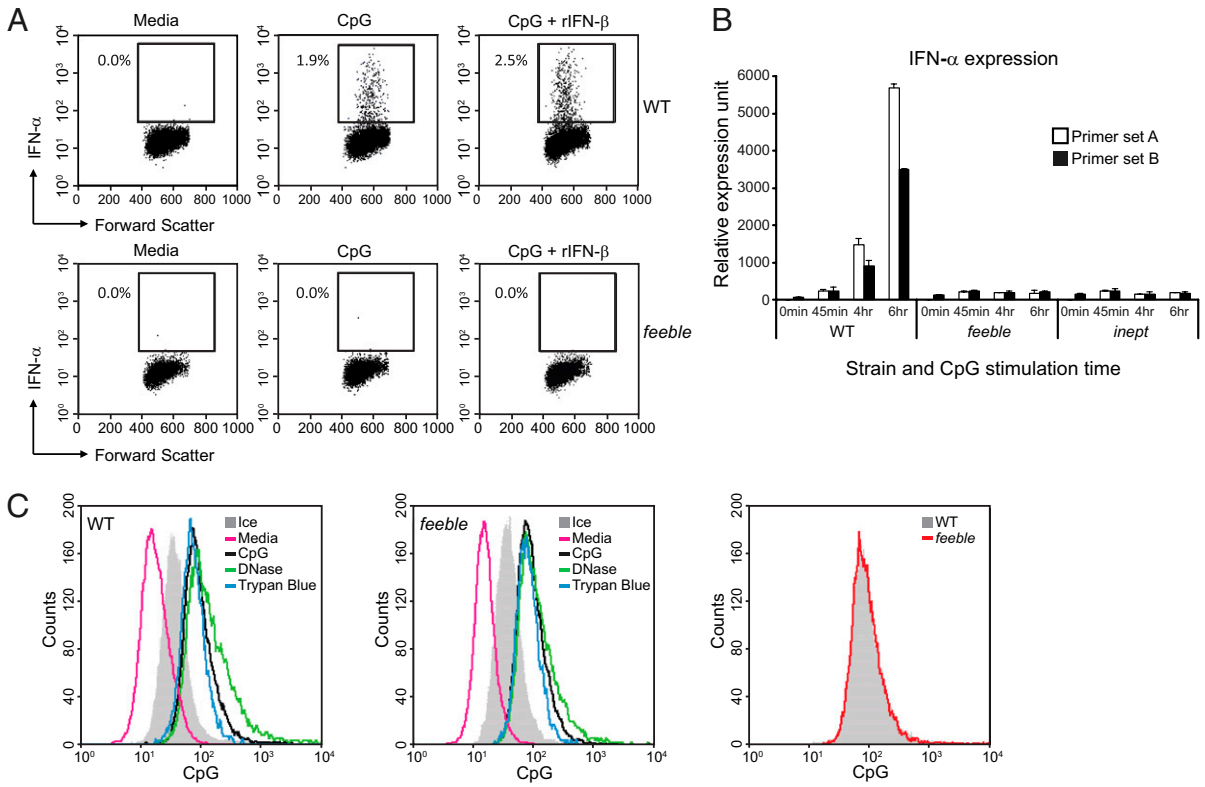

Fig. 3. The *feeble* mutation affects type I IFN mRNA expression and protein production. Lack of intracellular type I IFN in response to CpG stimulation in *feeble* pDCs even in the presence of exogenous recombinant IFN (rIFN-β). Populations were first gated on live cells expressing B220. FSC, forward scatter (*A*). Lack of IFN-α gene induction in CpG-stimulated *feeble* pDCs using two degenerate primer sets. *Inept* pDCs with a mutation in *Irf7* were used as controls (*B*). Uptake of Alexa488 conjugated CpG is normal in *feeble* pDCs (*C*). Except for "Media," all conditions included addition of CpG. Incubations were performed at 37 °C except for "Ice". CpG on ice reflects background extracellular fluorescence occurring without active uptake. Treatment with DNase was to remove cell surface-bound DNA. Trypan blue was to quench extracellular fluorescence. Right hand graph depicts a merge of wild type and *feeble* data. All error bars represent SEM.

specified by exon 2 and the same nucleotides specified by exon 3 that are missing from the first aberrant transcript, resulting in the loss of 348 bp and 116 amino acids.

Slc15a4 is a 574 amino acid, twelve-membrane spanning protein that is a member of the solute carrier superfamily of intrinsic membrane transporters (Fig. S3*B*). *Slc15a4* transcripts are highly expressed in pDCs, with only low-level expression in cDCs (Fig. 4*C*). The aberrant splicing transcripts caused by the *feeble* mutation would result in a protein truncated within transmembrane domain 5 (TMD5) or a protein lacking both TMD5 and TMD6.

Identification of a Novel Membrane Trafficking Pathway Necessary for pDC Function. Slc15a4 possesses a dileucine motif [(D/E)xxxL(L/I)] in its N-terminal cytoplasmic domain. AP-3 associates with acidic dileucine-based motifs to shuttle target proteins from the trans-Golgi to lysosomes and lysosome-related organelles (LRO) (16). We therefore hypothesized that AP-3 is also required for pDCs to produce IFN in response to nucleic acids. To directly test this, we determined whether mice with mutations in AP-3b1, one of several protein subunits of the AP-3 complex, have defects in pDC function. Indeed, $Ap3b1^{pearl/pearl}$ and $Ap3b1^{bullet\ gray/bullet\ gray}$ mice (17, 18) failed to produce type I IFN after in vivo challenge with CpG DNA (Fig. 5*A*). Further analysis of $Ap3b1^{pearl/pearl}$ mice revealed that although splenic pDCs are present (Fig. 5*B*) and can be generated in vitro from bone marrow, the cells cannot produce type I IFN and TNF-α in response to TLR9 stimulation (Fig. 5*C*). cDCs from $Ap3b1^{pearl/pearl}$ mice are functionally normal (Fig. 5*D*). Moreover, treatment of pDCs at the time of CpG stimulation with an inhibitor of the AP-3 activating GTP binding protein ARF1 results in inhibition of type I IFN gene induction by pDCs (Fig. 5*E*). In summary, like *feeble* mice, *pearl* mice have a selective defect in the ability of pDCs, but not cDCs, to signal through TLR9.

Mutations in AP-3 subunits cause Hermansky-Pudlak syndrome (HPS; OMIM #203300) in humans and HPS-like disease in mice (17, 19–21), which is characterized by oculocutaneous albinism, impaired platelet aggregation, and in some cases, pulmonary fibrosis, neutropenia, and mild immunodeficiency. In addition to AP-3, biogenesis of lysosome-related organelle complexes (BLOC)-1, BLOC-2, and BLOC-3 are required to generate LRO (22). Using our in vivo CpG screen, we tested mice with mutations in genes encoding HPS proteins, and observed that these molecules are also necessary for pDC function (Fig. 5*A*). The mutations examined included the *salt and pepper* allele of *Dtnbp1*, which encodes dysbindin, a component of the BLOC-1 complex (23), and the *toffee* allele of *Hps5*, which encodes a component of the BLOC-2 complex (24). Mutations in the lysosomal trafficking regulator gene *Lyst* and the GTPase-encoding *Rab27a* also cause albinism, platelet dysfunction, and immunological defects (25, 26). However, mice with homozygous mutations in these genes responded normally to in vivo CpG challenge as did mice homozygous for a mutation in the pigmentation-specific gene *Slc45a2* (Fig. S4).

Discussion

Using an unbiased forward genetic screen designed to identify proteins with nonredundant roles in pDC development or function, we have shown that the solute carrier protein superfamily member Slc15a4 is required for the ability of pDCs to respond to nucleic acids through TLR7 and TLR9. Slc15a4 contains a dileucine motif characteristic of proteins that undergo AP-3 dependent trafficking to LRO. This property of Slc15a4 led us to

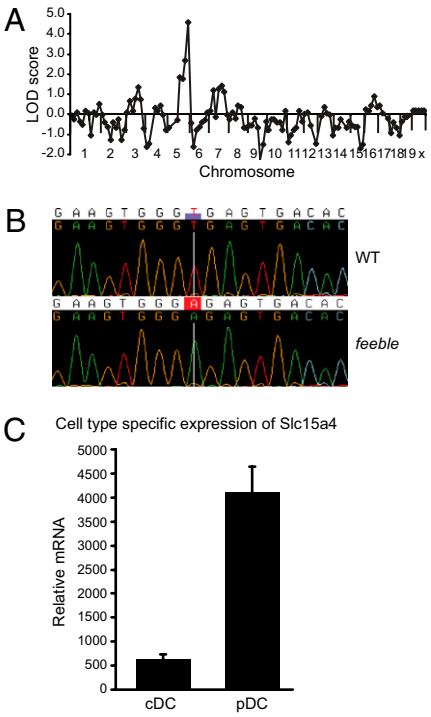

Fig. 4. Mapping and identification of the *feeble* mutation. LOD score mapping the *feeble* mutation to chromosome 5 (*A*) and sequence showing the T-to-A transversion in the *Slc15a4* gene (*B*). *Slc15a4* mRNA is expressed in pDCs but not cDCs (*C*).

the subsequent observation that the LRO trafficking system is essential for TLR signaling in pDCs; hence, mutations that affect the AP-3 complex, dysbindin, and HPS5 also ablate TLR signaling in pDCs. Mutations that impair the function of these proteins result in Hermansky-Pudlak syndrome, which is caused by LRO defects in melanosomes, platelet dense granules, lamellar bodies of type II alveolar epithelial cells, and lytic granules of CTL and NK cells. These cell-type specific organelles share various characteristics with lysosomes and endolysosomes, such as an acidic intralumenal pH (27). A similar organelle within pDCs may be required for TLR-dependent induction of both type I IFN and proinflammatory cytokines, but is not required for cDC function.

We propose that LRO trafficking capacitates the endosome by directing the incorporation of proteins that allow TLR signaling within the specialized environment of the pDC, and that one of these proteins is the Slc15a4 transporter. The importance of channel proteins in LRO physiology is demonstrated by the nonredundant roles of two twelve-membrane spanning proteins, OCA2 and Slc45a2, in melanosomal biogenesis and trafficking (28–30). Both of these proteins are necessary for the processing and trafficking of tyrosinase and other proteins necessary for melanin synthesis in the melanosomes (31), and may also be important to maintain melanosomal pH (32). Similar to Slc15a4, the OCA2 polypeptide sequence contains dileucine motifs in its N-terminal region that are recognized by AP-3, which directs OCA2 to the mature melanosome (33).

Slc15a4 has been characterized as a proton-coupled oligopeptide transporter in vitro (34). When expressed in cell culture, Slc15a4 can localize to lysosomes (35). In addition, Slc15a4 is expressed in the early endosomal compartment in the human epithelial cell line HEK293T, and based on siRNA knockdown experiments (36) has been shown to be necessary for Nod1 signaling in response to intracellular bacterial peptidoglycan-derived muramyl peptides. Similar to the related protein Slc15a1 (37, 38), Slc15a4 may be required to transport processed bacterial peptides into the cytosol. We hypothesize that Slc15a4 is necessary for endosomal TLR signaling perhaps by transporting a critical component into or out of the endosome. In view of its proposed peptide/proton symporter function, Slc15a4 may also have a role in maintaining the appropriate pH of the endosomal compartment as endosomal acidification is required for optimal TLR7 and TLR9 activation.

Our results suggest the intriguing possibility that patients with HPS may be unable to mount robust type I IFN responses during viral infections owing to defects of pDC function. We further suggest that the *feeble* mutation of *Slc15a4*, which does not cause most of the manifestations of HPS in mice, may present a model system with which to study the isolated consequences of pDC dysfunction. As *Slc15a4* was named as a candidate susceptibility locus for systemic lupus erythematosus (SLE) (39), we consider that the encoded protein may be an attractive target for pharmacotherapy aimed at preventing aberrant type I IFN production as occurs in diseases such as SLE.

Materials and Methods

Animals. All mouse strains used in this study were housed in The Scripps Research Institute Animal Facility. All studies were performed in accordance with the guidelines established by the Institutional Animal Care and Use Committee of The Scripps Research Institute. ENU mutagenesis was performed on C57BL/6J mice ordered from The Jackson Laboratory as described previously (12). Each mutagenized (G0) male was bred to a C57BL/6J female, and the resulting G1 males were crossed to C57BL/6J females to yield G2 mice. G2 females were backcrossed to their G1 sires to yield G3 mice. *Otiose, pococurante, 3d, Lps2, CpG6, inept, gemini, bullet gray, toffee, salt and pepper*, and *june gloom* mice are described in more detail at http://mutagenetix.scripps.edu. *Tlr9*$^{-/-}$, *Ifnar1*$^{-/-}$, *Ap3b1*$^{pearl/pearl}$, and *Rab27a*$^{ash/ash}$ were obtained from The Jackson Laboratory.

In Vivo CpG Challenge. Mice were injected i.v. with 2μg CpG-A plus DOTAP (Roche). Six hours later, blood was collected and serum type IFN was measured by bioassay. For *bullet gray*, *pearl*, *toffee*, and *salt and pepper* mice, G3 mice were run in parallel on the same day. L929 mouse fibroblasts stably transfected with an IFN-sensitive luciferase element (L929-ISRE) were incubated with supernatant for five hours, washed once with PBS, lysed with reporter lysis buffer (Promega), and incubated overnight at −80 °C. After thawing, 35 μL luciferase reagent (Promega) was added to each well, and luminescence read immediately on an Lmax plate reader (Molecular Devices).

DC Cell Cultures and Flow Cytometry. Bone marrow cells were extracted from femurs and tibias and cultured with GM-CSF or Flt3L as previously described (40). Splenocytes from C57BL/6J, *feeble*, and *Ap3b1*$^{pearl/pearl}$ mice were stained with mAbs 440c (anti-Siglec-H) (41) and CD317 (anti-BST2) (42).

To measure CpG uptake, BM-pDCs were cultured with CpG2216 conjugated to Alexa488 for 2 h at 37 °C. Cells were washed and stained with B220-APC. Cells were gated on B220$^+$ and CpG uptake assessed. Controls included BM-pDCs cultured with media alone, CpG on ice, added DNase to remove cell surface DNA or Trypan Blue.

TLR Stimulation. Lipopolysaccharide [*Salmonella* minnesota R959 (Re)] was obtained from Alexis Biochemicals. ODN 2216 (CpG-A) was obtained from MWG/Operon. Resiquimod was obtained from 3M. Peptidoglycan (PGN) was purchased from Fluka. Double-stranded DNA was obtained from Amersham Pharmacia Biotech. Lipofectamine was obtained from Invitrogen. The concentrations of TLR agonists used in experiments follow: LPS (500pg/mL), resiquimod (100ng/mL), PGN (2μg/mL), CpG-A (20 μg/mL). MCMV (Smith strain) was prepared as described previously (43). Influenza virus (PR8 strain) was grown in chicken eggs. Cells were stimulated with 1 MOI of MCMV or PR8 strain influenza. Cells were incubated at 37 °C for 16 h, and culture media was collected for IFN bioassay or cytokine ELISAs (eBioscience). For in vitro stimulation of BM-pDCs, cells were enriched to >90% purity using Miltenyi MACS to deplete CD19$^+$ cells and enrich B220$^+$ cells (40). Brefeldin A was obtained from Sigma and used at 5 μg/mL.

RT-PCR. Degenerate primers were used to detect multiple isoforms of *Ifna*. Primer set A consists of panIFN-α forward 5′ATGGCTAG(A/G)CTCTGTGCTTTCCT and reverse 5′ AGGGCTCTCCAGA(T/C)TTCTGCTCTG. Primer set B consists of panIFN-α forward 5′CCACAGGATCACTGTGTACCTGAGA and reverse 5′CTGATCACCTCCCAGGCACAG.

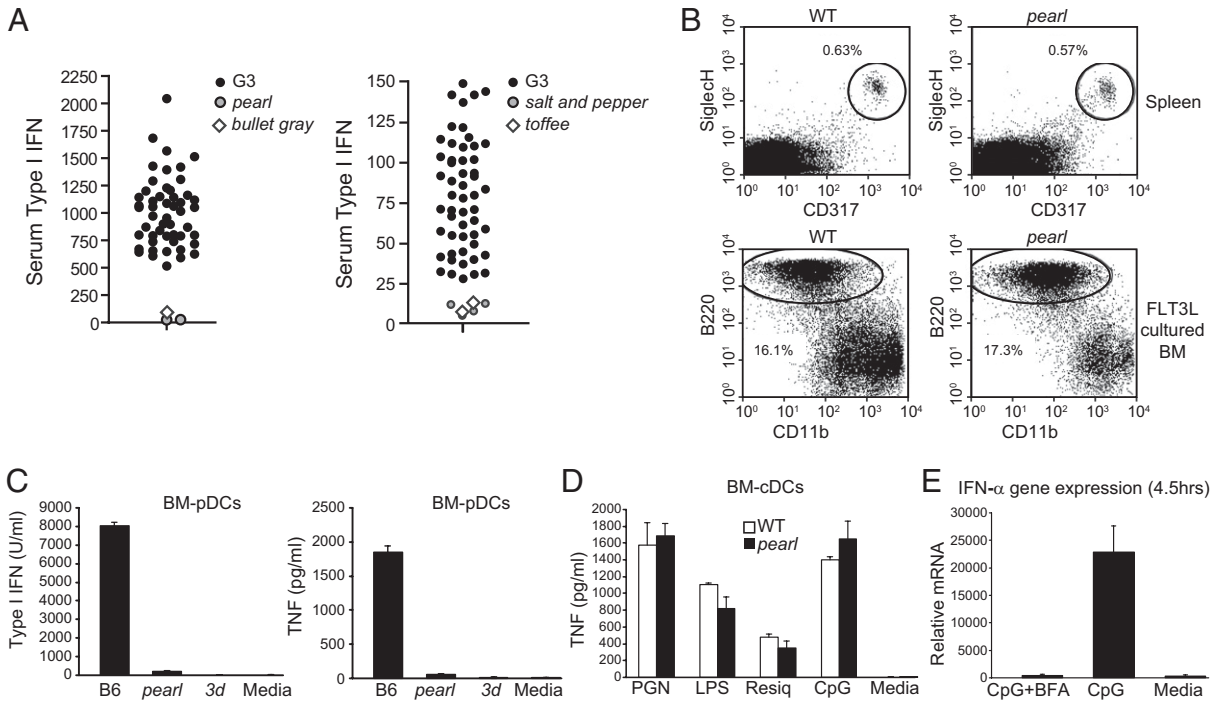

Fig. 5. A membrane trafficking pathway is important for pDC function. Mice with defects in the Ap3b1 subunit of the AP-3 complex (*pearl* and *bullet gray*) and mutations in genes encoding BLOC-1 (*salt and pepper*) and BLOC-2 (*toffee*) components lack serum type I IFN in response to CpG injection compared with ENU-mutagenized G3 mice (*A*). *Ap3b1*$^{pearl/pearl}$ mice display normal numbers of splenic pDCs and generate normal percentages of bone marrow-derived pDCs. C57BL/6J controls are the same as in Fig. 2 (*B*). Highly enriched in vitro-derived *Ap3b1*$^{pearl/pearl}$ pDCs display defective type I IFN responses (*C*), but bone marrow-derived cDCs appear to be normal (*D*). *Unc93b1*$^{3d/3d}$ pDCs were used as controls in *C*. The ARF1 inhibitor, Brefeldin A, prevents type I IFN gene production in wild type pDCs (*E*). All error bars represent SEM.

Slc15a4 expression in BM-DCs or enriched BM-pDCs was determined using four primer sets. First set forward 5′CTTGGCTCTGATTCCTTACTGGACAG and reverse 5′TGCTCATCCCACTGAAGGACAAGC; second set forward 5′TGTACCATGCTGCTGACTTGCC and reverse 5′CATTGGGTGGATGAGCAGTCATACAG; third set forward 5′GATGAGCACCGCATCAAACATGG and reverse 5′TACCAGGTCCAGCCTTTTACTCTCCAGG; fourth set forward 5′TGCCAGAGAAGAAGAAGAAGAGTCCC and reverse 5′CGTACTTCACAGACACAATGAGGAAGAG.

Note Added in Proof. While this paper was in review, Sasai et al. (44) independently reported that PDCs from *Pearl* mice fail to make type I interferon in response to DNA.

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1. Siegal FP, et al. (1999) The nature of the principal type 1 interferon-producing cells in human blood. *Science* 284:1835–1837.
2. Liu YJ (2005) IPC: Professional type 1 interferon-producing cells and plasmacytoid dendritic cell precursors. *Annu Rev Immunol* 23:275–306.
3. Swiecki M, Colonna M (2010) Unraveling the functions of plasmacytoid dendritic cells during viral infections, autoimmunity, and tolerance. *Immunol Rev* 234: 142–162.
4. Krug A, et al. (2004) TLR9-dependent recognition of MCMV by IPC and DC generates coordinated cytokine responses that activate antiviral NK cell function. *Immunity* 21: 107–119.
5. Kawai T, Akira S (2007) TLR signaling. *Semin Immunol* 19:24–32.
6. Kawai T, et al. (2004) Interferon-alpha induction through Toll-like receptors involves a direct interaction of IRF7 with MyD88 and TRAF6. *Nat Immunol* 5: 1061–1068.
7. Shinohara ML, et al. (2006) Osteopontin expression is essential for interferon-alpha production by plasmacytoid dendritic cells. *Nat Immunol* 7:498–506.
8. Takahashi K, et al. (2007) A protein associated with Toll-like receptor (TLR) 4 (PRAT4A) is required for TLR-dependent immune responses. *J Exp Med* 204:2963–2976.
9. Yang Y, et al. (2007) Heat shock protein gp96 is a master chaperone for toll-like receptors and is important in the innate function of macrophages. *Immunity* 26: 215–226.
10. Kim YM, Brinkmann MM, Paquet ME, Ploegh HL (2008) UNC93B1 delivers nucleotide-sensing toll-like receptors to endolysosomes. *Nature* 452:234–238.
11. Honda K, et al. (2005) Spatiotemporal regulation of MyD88-IRF-7 signalling for robust type-I interferon induction. *Nature* 434:1035–1040.
12. Hoebe K, Du X, Goode J, Mann N, Beutler B (2003) *Lps2*: A new locus required for responses to lipopolysaccharide, revealed by germline mutagenesis and phenotypic screening. *J Endotoxin Res* 9:250–255.
13. Xia Y, et al. (2010) Bulk segregation mapping of mutations in closely related strains of mice. *Genetics*, in press.
14. Schiavoni G, et al. (2002) ICSBP is essential for the development of mouse type I interferon-producing cells and for the generation and activation of CD8alpha(+) dendritic cells. *J Exp Med* 196:1415–1425.
15. Tsujimura H, Tamura T, Ozato K (2003) Cutting edge: IFN consensus sequence binding protein/IFN regulatory factor 8 drives the development of type I IFN-producing plasmacytoid dendritic cells. *J Immunol* 170:1131–1135.
16. Höning S, Sandoval IV, von Figura K (1998) A di-leucine-based motif in the cytoplasmic tail of LIMP-II and tyrosinase mediates selective binding of AP-3. *EMBO J* 17:1304–1314.
17. Feng L, et al. (1999) The beta3A subunit gene (Ap3b1) of the AP-3 adaptor complex is altered in the mouse hypopigmentation mutant pearl, a model for Hermansky-Pudlak syndrome and night blindness. *Hum Mol Genet* 8:323–330.
18. Rutschmann S, Moresco EM, Mack A, Beutler B (2010) Record for bullet gray. *MUTAGENETIX (TM)* Available at http://mutagenetix.scripps.edu/. Accessed September 28, 2007.
19. Dell'Angelica EC, Shotelersuk V, Aguilar RC, Gahl WA, Bonifacino JS (1999) Altered trafficking of lysosomal proteins in Hermansky-Pudlak syndrome due to mutations in the beta 3A subunit of the AP-3 adaptor. *Mol Cell* 3:11–21.
20. Jung J, et al. (2006) Identification of a homozygous deletion in the AP3B1 gene causing Hermansky-Pudlak syndrome, type 2. *Blood* 108:362–369.
21. Huizing M, et al. (2002) Nonsense mutations in ADTB3A cause complete deficiency of the beta3A subunit of adaptor complex-3 and severe Hermansky-Pudlak syndrome type 2. *Pediatr Res* 51:150–158.
22. Di Pietro SM, Dell'Angelica EC (2005) The cell biology of Hermansky-Pudlak syndrome: Recent advances. *Traffic* 6:525–533.
23. Li W, et al. (2003) Hermansky-Pudlak syndrome type 7 (HPS-7) results from mutant dysbindin, a member of the biogenesis of lysosome-related organelles complex 1 (BLOC-1). *Nat Genet* 35:84–89.

24. Di Pietro SM, Falcón-Pérez JM, Dell'Angelica EC (2004) Characterization of BLOC-2, a complex containing the Hermansky-Pudlak syndrome proteins HPS3, HPS5 and HPS6. *Traffic* 5:276–283.
25. Barbosa MD, et al. (1996) Identification of the homologous beige and Chediak-Higashi syndrome genes. *Nature* 382:262–265.
26. Wilson SM, et al. (2000) A mutation in Rab27a causes the vesicle transport defects observed in ashen mice. *Proc Natl Acad Sci USA* 97:7933–7938.
27. Cutler DF (2002) Introduction: Lysosome-related organelles. *Semin Cell Dev Biol* 13: 261–262.
28. Gardner JM, et al. (1992) The mouse pink-eyed dilution gene: Association with human Prader-Willi and Angelman syndromes. *Science* 257:1121–1124.
29. Rinchik EM, et al. (1993) A gene for the mouse pink-eyed dilution locus and for human type II oculocutaneous albinism. *Nature* 361:72–76.
30. Newton JM, et al. (2001) Mutations in the human orthologue of the mouse underwhite gene (uw) underlie a new form of oculocutaneous albinism, OCA4. *Am J Hum Genet* 69:981–988.
31. Costin GE, Valencia JC, Vieira WD, Lamoreux ML, Hearing VJ (2003) Tyrosinase processing and intracellular trafficking is disrupted in mouse primary melanocytes carrying the underwhite (uw) mutation. A model for oculocutaneous albinism (OCA) type 4. *J Cell Sci* 116:3203–3212.
32. Puri N, Gardner JM, Brilliant MH (2000) Aberrant pH of melanosomes in pink-eyed dilution (p) mutant melanocytes. *J Invest Dermatol* 115:607–613.
33. Sitaram A, et al. (2009) Localization to mature melanosomes by virtue of cytoplasmic dileucine motifs is required for human OCA2 function. *Mol Biol Cell* 20:1464–1477.
34. Yamashita T, et al. (1997) Cloning and functional expression of a brain peptide/histidine transporter. *J Biol Chem* 272:10205–10211.
35. Sakata K, et al. (2001) Cloning of a lymphatic peptide/histidine transporter. *Biochem J* 356:53–60.
36. Lee J, et al. (2009) pH-dependent internalization of muramyl peptides from early endosomes enables Nod1 and Nod2 signaling. *J Biol Chem* 284:23818–23829.
37. Buyse M, Tsocas A, Walker F, Merlin D, Bado A (2002) PepT1-mediated fMLP transport induces intestinal inflammation in vivo. *Am J Physiol Cell Physiol* 283:C1795–C1800.
38. Vavricka SR, et al. (2004) hPepT1 transports muramyl dipeptide, activating NF-kappaB and stimulating IL-8 secretion in human colonic Caco2/bbe cells. *Gastroenterology* 127:1401–1409.
39. Han JW, et al. (2009) Genome-wide association study in a Chinese Han population identifies nine new susceptibility loci for systemic lupus erythematosus. *Nat Genet* 41: 1234–1237.
40. Diacovo TG, Blasius AL, Mak TW, Cella M, Colonna M (2005) Adhesive mechanisms governing interferon-producing cell recruitment into lymph nodes. *J Exp Med* 202: 687–696.
41. Blasius A, et al. (2004) A cell-surface molecule selectively expressed on murine natural interferon-producing cells that blocks secretion of interferon-alpha. *Blood* 103: 4201–4206.
42. Blasius AL, et al. (2006) Bone marrow stromal cell antigen 2 is a specific marker of type I IFN-producing cells in the naive mouse, but a promiscuous cell surface antigen following IFN stimulation. *J Immunol* 177:3260–3265.
43. Tabeta K, et al. (2004) Toll-like receptors 9 and 3 as essential components of innate immune defense against mouse cytomegalovirus infection. *Proc Natl Acad Sci USA* 101:3516–3521.
44. Sasai M, Linehan MM, Iwasaki A (2010) Bifurcation of Toll-like receptor 9 signaling by adaptor protein 3. *Science* 329:1530–1534.