IAI Journals.ASM.org

G Proteins $G\alpha_{i1/3}$ Are Critical Targets for *Bordetella pertussis* Toxin-Induced Vasoactive Amine Sensitization

Sean A. Diehl,[a,b] Benjamin McElvany,[a] Rajkumar Noubade,[b] Nathan Seeberger,[b] Brock Harding,[b] Karen Spach,[b] Cory Teuscher[b,c]

Departments of Medicine-Infectious Diseases,[a] Medicine-Immunobiology,[b] and Pathology,[c] University of Vermont College of Medicine, Burlington, Vermont, USA

Pertussis toxin (PTX) is an AB5-type exotoxin produced by the bacterium *Bordetella pertussis*, the causative agent of whooping cough. *In vivo* intoxication with PTX elicits a variety of immunologic and inflammatory responses, including vasoactive amine sensitization (VAAS) to histamine (HA), serotonin (5-HT), and bradykinin (BDK). Previously, by using a forward genetic approach, we identified the HA H_1 receptor (*Hrh1*/H_1R) as the gene in mice that controls differential susceptibility to *B. pertussis* PTX-induced HA sensitization (Bphs). Here we show, by using inbred strains of mice, F_1 hybrids, and segregating populations, that, unlike Bphs, PTX-induced 5-HT sensitivity (Bpss) and BDK sensitivity (Bpbs) are recessive traits and are separately controlled by multiple loci unlinked to 5-HT and BDK receptors, respectively. Furthermore, we found that PTX sensitizes mice to HA independently of Toll-like receptor 4, a purported receptor for PTX, and that the VAAS properties of PTX are not dependent upon endothelial caveolae or endothelial nitric oxide synthase. Finally, by using mice deficient in individual $G\alpha_{i/o}$ G-protein subunits, we demonstrate that $G\alpha_{i1}$ and $G\alpha_{i3}$ are the critical *in vivo* targets of ADP-ribosylation underlying VAAS elicited by PTX exposure.

Infection with the bacterium *Bordetella pertussis* can lead to whooping cough, usually typified by paroxysomal coughing and, in severe cases, oropharyngeal tissue swelling, hypertension, pneumothorax, and long-lasting aftereffects (1). The principal active agent in *B. pertussis*, pertussis toxin (PTX), is an AB5-type secreted exotoxin (2–4). The A (active) subunit catalyzes the ADP-ribosylation and thereby impairment of α-subunit signaling in $G\alpha_{i/o}$-linked heterotrimeric guanine nucleotide regulatory protein (G-protein) complexes (5). This covalent modification prevents the $G\alpha\beta\gamma$ complex from associating with G protein-coupled receptors (GPCRs) on the cell membrane (6). The B (binding) pentamer is thought to bind cell surface receptors on a variety of mammalian cells and facilitate the cellular entry of the A subunit (7). PTX elicits diverse physiological responses *in vivo*, including leukocytosis and altered glucose regulation (8), increased blood-brain barrier (BBB) permeability, and systemic vasoactive sensitization (VAAS) to biogenic amines (9). PTX is also used as an ancillary adjuvant to approximate an infectious environmental influence in animal models of tissue-specific autoimmune disease (10). Genetically, one of PTX's physiological effects, VAAS to histamine (HA), is controlled by a single autosomal dominant locus (*Bphs*, for *B. pertussis*-induced HA sensitization) and has been used as an intermediate phenotype for genetic studies of organ-specific autoimmune diseases (11, 12).

Although several cell types have the capacity to produce HA, mast cells are thought to be a main source during tissue inflammation (13). Mast cells also produce large amounts of serotonin (5-HT) and bradykinin (BDK), which, together with HA and other lipid and glycosylated mediators, are stored in granules. During inflammation, soluble and cell surface molecules can act as secretagogues to stimulate degranulation and release of factors from mast cells, which can affect vascular permeability (14), leading to antigen leakage from or inappropriate leukocyte access to tissues (13).

We previously studied VAAS in response to HA by using a forward genetic approach to identify the HA H_1 receptor (*Hrh1*/H_1R) as the gene underlying Bphs (11, 12), a subphenotype for tissue-specific autoimmune diseases elicited with the aid of PTX. A T cell-specific role for H_1R in disease susceptibility was found (15), but a bone marrow chimeric approach revealed that expression of H_1R in the nonhematopoietic compartment was responsible for Bphs (16), prompting the closer look at the mechanism of Bphs that we took in this study.

During the positional cloning of *Bphs*, we observed that SJL/J mice were also susceptible to PTX-induced 5-HT sensitization (Bpss, for *B. pertussis*-induced 5-HT sensitization) while C3H.$Bphs^{SJL/J}$ mice were not, thereby excluding *Hrh1* as a candidate for Bpss (17). These results indicate that the phenotypic variation in responsiveness to Bphs and Bpss reflects genetic control of distinct intermediate phenotypes rather than allelic variation in genes controlling overall susceptibility to VAAS. To address this possibility and to gain insight into the genetic control of VAAS to other vasoactive amines, we challenged a panel of PTX-sensitized inbred, F_1, and N2 backcross mice with 5-HT and BDK and studied them for Bpss and Bpbs (*B. pertussis*-induced BDK sensitization), respectively. Unlike *Bphs*/*Hrh1*, which is dominant, we show that *Bpss* and *Bpbs* are recessive traits and are controlled by separate complex genetic mechanisms unlinked to 5-HT or BDK receptors.

It is known that PTX-induced VAAS to HA (VAASH) requires intoxication with active holotoxin (18) in a mechanism that presumably involves ADP-ribosylation of the $G\alpha_{i/o}$ class of G pro-

Received 2 August 2013 **Returned for modification** 2 October 2013
Accepted 19 November 2013
Published ahead of print 9 December 2013
Editor: S. R. Blanke
Address correspondence to Sean A. Diehl, sean.diehl@med.uvm.edu.
Supplemental material for this article may be found at http://dx.doi.org/10.1128/IAI.00971-13.
Copyright © 2014, American Society for Microbiology. All Rights Reserved.
doi:10.1128/IAI.00971-13

TABLE 1 Bphs, Bpss, and Bpbs of inbred mice[a]

Strain	Bphs		Bpss							Bpbs					
				PTX + 5-HT at:							PTX + Bdk at:				
	Carrier + HA (25 mg/kg)	PTX + HA (25 mg/kg)	Carrier + 5-HT (50 mg/kg)	50 mg/kg	12.5 mg/kg	3.1 mg/kg	0.78 mg/kg	0.39 mg/kg	LD_{50}	Carrier + Bdk (80 mg/kg)	80 mg/kg	40 mg/kg	20 mg/kg	10 mg/kg	LD_{50}
SM/J	0/4	4/4[b]	0/4	4/4[b]	5/5	5/6	3/6	0/4	1.0	0/4	4/4[b]	2/4	0/4	0/4	40.0
SJL/J	0/4	4/4[b]	0/4	12/12[c]	12/12	6/11	0/8		2.6	0/4	4/4[b]	4/4	1/4	0/4	22.5
AKR/J	0/4	0/4	0/4	12/12[c]	10/10	8/17	0/8		3.2	0/4	4/4[b]	3/4	0/4	0/4	35.6
MRL/MpJ	0/4	0/4	0/4	4/4[b]	6/6	4/8	0/4		2.9	0/4	2/4	1/4	0/4	0/4	
LG/J	0/4	4/4[b]	0/4	4/4[b]	7/8	0/4			5.4	0/4	2/4	0/4	0/4	0/4	
RFM/UnNCr	0/4	4/4[b]	0/4	6/6[d]	0/6	0/4			26.2						
PL/J	0/4	4/4[b]	0/4	6/6[d]	0/8	0/4			29.8						
DBA/1J	0/4	4/4[b]	0/4	5/5[d]	0/6	0/4			28.6						
SWR/J	0/4	4/4[b]	0/4	6/6[d]	0/4	0/4			22.0						
C57BL/6J	0/4	4/4[b]	0/4	9/15	0/14	0/8				0/4	4/4[b]	4/4	0/4	0/4	28.3
DBA/2J	0/4	4/4[b]	0/4	4/15	0/14	0/4				0/4	4/4[b]	2/4	0/4	0/4	40.0
A/J	0/4	4/4[b]	0/4	2/15	0/12	0/4				0/4	4/4[b]	2/4	0/4	0/4	40.0
A/HeJ	0/4	4/4[b]	0/4	3/6	0/6	0/4				0/4	4/4[b]	4/4	2/4	0/4	20.0
NOD/LtJ	0/4	4/4[b]	0/4	2/6	0/8	0/4									
FVB/NCr	0/4	4/4[b]	0/4	2/6	0/8	0/4									
C57BL/10J	0/4	4/4[b]	0/4	1/6	0/4	0/4				0/4	2/4	0/4	0/4	0/4	
NZB/BlnJ	0/4	4/4[b]	0/4	3/8	0/6	0/4				0/4	4/4[b]	3/4	2/4	0/4	22.1
NZW/LacJ	0/4	4/4[b]	0/4	2/9	0/6	0/4				0/4	4/4[b]	4/4	2/4	0/4	20.0
129X1/SvJ	0/4	4/4[b]	0/4	6/10	5/14	1/5	0/4			0/4	4/4[b]	3/4	2/4	0/4	22.1
BALB/cJ	0/4	4/4[b]	1/4	3/10	6/17	0/5	0/4			0/4	4/4[b]	4/4	2/4	0/4	20.0
BALB/cByJ	0/4	4/4[b]	0/4	0/9	0/6	0/4				0/4	4/4[b]	2/4	0/4	0/4	40.0
CBA/JCr	0/4	0/4	0/4	0/8	0/8	0/4									
B10.D2n	0/4	4/4[b]	0/4	0/9	0/6	0/4				0/4	2/4	0/4	0/4	0/4	
B10.S/SgSnJ	0/4	4/4[b]	0/4	1/10	0/6	0/4				0/4	1/4	0/5	0/5	0/5	
C3H/HeJ	0/4	0/4	0/4	4/14	4/15	0/4				0/4	4/4[b]	4/4	1/4	0/4	22.5
C3H/HeN	0/4	0/4	0/4	0/4	0/4	0/4				0/4	4/4[b]	4/4	2/4	0/4	20.0
C3H.*Bphs*^{SJL}	0/4	4/4[b]	0/4	0/4	0/4	0/4				0/4	4/4[b]	4/4	2/4	0/4	20.0

[a] Animals were sensitized with 200 ng PTX by i.v. injection and challenged 3 days later by i.v. injection of HA (25 mg/kg), 5-HT (0.39 to 50 mg/kg), or BDK (10 to 80 mg/kg). Three or four mice were examined at a given dose. Dosages are based on mg/kg (dry weight) free base or peptide. Deaths were recorded at 30 min, and the results are expressed as the number dead/number studied. LD_{50}s of 5-HT and HA were calculated with GraphPad Prism. Contrasts for combined phenotypes: SM = SJL = AKR = MRL; SM < LG = SJL = AKR = MRL; LG < RFM = PL = DBA/1 = SWR. The significance of differences between carrier- and PTX-treated groups was determined by Fisher's exact test. The significance of differences in LD_{50}s among and between the strains was determined by the extra sum of squares *F* test. Bpss overall $F = 15.9$, $P < 0.0001$; Bpbs overall $F = 1.3$, $P = 0.2$.

[b] $P = 0.05$.

[c] $P < 0.0001$.

[d] $P < 0.01$.

teins (19). However, there are several $G\alpha_{i/o}$ proteins and splice variants but the specific *in vivo* target(s) associated with PTX-induced VAAS has remained unclear for nearly 30 years. We show here by using knockout (KO) mice that $G\alpha_{i1}$ and $G\alpha_{i3}$ are the specific targets of ADP-ribosylation required for PTX-induced VAASH.

MATERIALS AND METHODS

Ethics statement. All experimental procedures were approved by the University of Vermont Institutional Animal Care and Use Committee (approval number 10-020) in accordance with relevant institutional guidelines and the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health.

Mice. Most of the inbred strains in Table 1 were purchased from The Jackson Laboratory, except for RFM/UnNCr, FVB/NCr, CBA/JCr, and C3H/HeN mice, which were purchased from the National Cancer Institute/Charles River Laboratories. F_1 hybrid strains and N2 backcross mice, as in Tables 2 and 3, respectively, were generated in-house by using parental strains purchased from Jackson. *Hrh1*-deficient mice (B6.129P-*Hrh1*^{tm1Wat}) (20), C3H.SJL-*Bphs*^{s} congenic mice (11), were maintained in-house at the University of Vermont. *Bdkrb2*-deficient mice (B6.129S7-*Bdkrb2*$^{tm1Jfh/J}$) (21), *Nos3*-deficient mice (B6.129P2-*Nos3*$^{tmiUnc/J}$) (22), caveolin-1-deficient mice (B6.Cg-*Cav1*^{tm1Mls}/J) (23), *Tlr4* mutant C57BL/10ScNJ mice (24), and control C57BL/10J mice were purchased from Jackson. PKCα-deficient (B6.129-*Prkca*^{tm1Jmk}) (25), PKCβ-deficient (129/Sv-*Prkcb*^{tm1Tara}) (26), PKCγ-deficient (B6.129P2-*Prkcg*^{tm1Stl}) (27), PKCδ-deficient (129/SV×Ola.*Prkcd*^{tm1Qxu}) (5), PKCε-deficient (129/Sv × C57BL/6.*Prkce*^{tm1Ltg}) (28), and PKCζ-deficient (129/Sv.*Prkcz*^{tm1Jmos}) mice (29) were a kind gift from Michael Leitges, Max Planck Institute for Experimental Endocrinology, Hannover, Germany.

$G\alpha_o$-deficient mice (129SvEv.129SvEv-*Gnao1*^{tm1LBi}) (30), $G\alpha_{o1}$-deficient mice (129SvOla.129SvEv-*Gnao1.1*^{tm1LBi}) (31), $G\alpha_{o2}$-deficient mice (129SvOla.129SvEv-*Gnao1.2*^{tm1LBi}) (31), $G\alpha_{i1}$-deficient mice (129Sv.129SvEv-*Gnai1*^{tm1Drs}) (32), $G\alpha_{i2}$-deficient mice (B6.129SvEv-*Gnai2*^{tm1LBi}) (33), $G\alpha_{i3}$-deficient mice (129Sv.129SvEv-*Gnai3*^{tm1LBi}) (31), and $G\alpha_{i1/3}$ doubly deficient mice (129SvEv-*Gnao1*^{tm1LBi} × 129SvEv-*Gnai3*^{tm1LBi}) (31) were obtained from Lutz Birnbaumer (National Institute of Environmental Health Sciences, Research Triangle Park, NC) and maintained as wild-type (WT, +/+) mice, heterozygotes (+/−), or homozygotes (−/−) on 129SvEv ($G\alpha_o$, $G\alpha_{i1}$, $G\alpha_{i2}$, $G\alpha_{i3}$, $G\alpha_{i1} \times G\alpha_{i3}$), CD1 outbred ($G\alpha_o$), FVB ($G\alpha_{i2}$, 10th backcross), C57BL/6 ($G\alpha_{i2}$, 8th backcross), and crossbred C57BL/6:129SvEv 50:50 ($G\alpha_{o1}$, $G\alpha_{o2}$, $G\alpha_{i2}$) backgrounds. All animals were provided normal mouse chow and water *ad libitum*.

Vasoactive amine sensitization and challenge. Bphs was carried out as described previously (11, 16). Bpss and Bpbs followed a similar protocol. Briefly, 200 ng of PTX (List Biological Laboratories, Campbell, CA) in phosphate-buffered saline was administered intravenously (i.v.) by tail

TABLE 2 Inheritance of Bphs, Bpss, and Bpbs in F_1 hybrid strains[a]

Phenotype and strain	Classification	No. of mice with phenotype/total (*P* value)			Contrasts
		Group 1	Group 2	Group 3	
Bphs					
SJL/J	$Bphs^+$ $Bpss^+$ $Bpbs^+$	6/6[b]	6/6[c]	5/6[d]	
C3H/HeJ	$Bphs^-$	0/6[b]	0/6[c]	0/6[d]	
(C3H × SJL)F_1		6/6[b] (0.0001)	6/6[c] (0.0001)	4/6[d] (0.0094)	SJL/J = (C3H × SJL)F_1 > C3H/HeJ
C57BL/6J	$Bphs^+$ $Bpbs^+$	6/6[b]	6/6[c]	1/6[d]	
CBA/J	$Bphs^-$	0/6[b]	0/6[c]	0/6[d]	
(B6 × CBA)F_1		6/6[b] (0.0001)	6/6[c] (0.0001)	2/6[d] (0.3)	C57BL/6J = (B6 × CBA)F_1 > CBA/J
Bpss					
SJL/J	$Bphs^+$ $Bpss^+$ $Bpbs^+$	12/12[e]	12/12[f]	6/11[g]	
PL/J (control)	$Bpss^+$	6/6[e]	0/8[f]	0/4[g]	
(PL × SJL)F_1		8/9[e] (0.35)	1/9[f] (<0.0001)	0/5[g] (0.03)	SJL/J > PL/J = (PL × SJL)F_1
SJL/J		12/12[e]	12/12[f]	6/11[g]	
BALB/cJ	$Bpss^-$	3/10[e]	6/17[f]	0/5[g]	
(BALB × SJL)F_1		6/9[e] (0.001)	4/8[f] (0.002)	0/5[g] (0.02)	SJL/J > BALB/cJ = (BALB × SJL)F_1
AKR/J	$Bphs^+$ $Bpss^+$ $Bpbs^+$	12/12[e]	10/10[f]	8/17[g]	
DBA/2J	$Bpss^-$	4/15[e]	0/14[f]	0/4[g]	
(AK × DBA)F_1		4/8[e] (0.0003)	0/8[f] (<0.0001)	0/5[g] (0.05)	AKR/J > DBA/2J = (AK × DBA)F_1
SJL		10/10[e]	10/10[f]	6/10[g]	
B10.S	$Bpss^-$	1/10[e]	1/10[f]	0/10[g]	
(B10 × SJL)F_1		1/10[e] (<0.0001)	0/10[f] (<0.0001)	0/10[g] (0.0006)	SJL/J > B10.S = (B10 × SJL)F_1
Bpbs					
SJL/J	$Bphs^+$ $Bpss^+$ $Bpbs^+$	6/6[h]	6/6[i]	4/6[j]	
B10.S	$Bpbs^-$	1/6[h]	0/6[i]	0/6[j]	
(B10 × SJL)F_1		2/6[h] (0.009)	1/6[i] (0.0007)	0/5[j] (0.008)	SJL/J > B10.S = (B10 × SJL)F_1

[a] F_1 hybrids were generated by using the indicated parental strains. Mice were then challenged for Bphs, Bpss, or Bpbs at the indicated doses. Significance of difference in susceptibility between parental strains and F_1 hybrids was determined using Fisher's exact test.
[b] HA challenge dose, 25 mg/kg free base.
[c] HA challenge dose, 12.5 mg/kg free base.
[d] HA challenge dose, 3.1 mg/kg free base.
[e] 5-HT challenge dose, 50 mg/kg free base.
[f] 5-HT challenge dose, 12.5 mg/kg free base.
[g] 5-HT challenge dose, 3.1 mg/kg free base.
[h] BDK challenge dose, 80 mg/kg peptide.
[i] BDK challenge dose, 40 mg/kg peptide.
[j] BDK challenge dose, 20 mg/kg peptide.

vein injection in a volume of 0.1 ml on day 0. On day 3, mice were challenged i.v. with HA dihydrochloride, 5-HT hydrochloride, or BDK acetate (all from Sigma, dosed at milligrams per kilogram of body weight by using the free amine molecular weight). Deaths were recorded at 30 min post-challenge and are shown as the number of animals affected over the total.

TABLE 3 Bphs, Bpss, and Bpbs in N2 backcross mice[a]

Phenotype and strain	No. of mice		*P* value	
	Affected	Unaffected	1 locus	2 loci
Bpss				
SJL	10	0		
(B10.S × SJL)F_1	0	10		
(B10.S × SJL) × B10.S N2	20	80	0.50	0.005
Bpbs				
SJL	10	0		
(B10.S × SJL)F_1	0	10		
(B10.S × SJL) × B10.S N2	28	72	0.75	<0.0001

[a] SJL, (B10.S × SJL)F_1, and (B10.S × SJL) × B10.S N2 mice were challenged for Bpss (25 mg/kg 5-HT) and Bpbs (40 mg/kg BDK) as in Table 1. Results were tested by chi-square goodness of fit for both one- and two-locus recessive models of inheritance.

Microsatellite genotyping. We performed microsatellite typing as previously described, with liver DNA using informative microsatellite markers that distinguished B10.S and SJL/J mice and are linked to all known 5-HT receptors and the *Bdkrb1/2* locus (34, 35).

RESULTS

Distinct genetic control of Bphs, Bpss, and Bpbs. To further extend our hypothesis that distinct genetic mechanisms control susceptibility to PTX-induced sensitization to individual biogenic amines, we examined 27 different inbred mouse strains for Bphs, Bpss, or Bpbs. We identified two strains that were sensitive to a challenge with all three amines (SJL/J and SM/J), which is consistent with previous reports showing the SJL/J strain is sensitive to both HA and 5-HT challenges (17). Several strains were sensitive to only two amines, but we also observed strains that were monosensitive. For example, C57BL10/J mice were susceptible only to Bphs, C3H/HeJ or C3H/HeN mice were susceptible only to Bpbs, and MRL/MpJ mice were susceptible only to Bpss (see Table S1 in the supplemental material). Furthermore, even within a given phenotype, we observed variability in the 50% lethal doses (LD_{50}s). The LD_{50} of Bpss for SM/J mice was 1 mg/kg 5-HT, whereas that for PL/J mice was 30 mg/kg (Table 1). Bpbs exhibited

LD_{50}s of 20 to 40 mg/kg. The dose of HA used as a control for all strains (25 mg/kg) is approximately 2 LD_{50}s for susceptible strains and readily discriminates Bphs from Bphs-resistant strains (17). The observance of all possible amine sensitivity combinations across an inexhaustive survey of inbred mouse strains indicates that the phenotypic variation in susceptibility to VAAS reflects the genetic control of distinct intermediate phenotypes rather than allelic variation in genes controlling overall susceptibility to intoxication or VAAS.

Susceptibility to Bphs is controlled by *Hrh1*, and this phenotype is inherited in an autosomal dominant fashion (11). We expanded these studies to investigate the inheritance of Bpbs and Bpss by phenotyping (susceptible × resistant)F_1 hybrid mice. (C3H/HeJ × SJL/J)F_1 mice were Bphs, consistent with previous studies (12). Sudweeks et al. observed that (SJL/J × CBA/J)F_1 mice were also Bphs (12). Here we extended this finding by phenotyping (C57BL/6J × CBA/J)F_1 mice for Bphs and observed the same fully penetrant dominant inheritance (Table 2). These data are also consistent with genotype analysis of *Hrh1*, since both SJL/J and C57BL/6J mice harbor the Bphs (*Hrh1*^{s}) allele while C3H/HeJ and CBA/J mice harbor the Bphs resistance *Hrh1*^{r} allele (11).

Bpbs and Bpss are two-gene recessive traits. To assess *Bpbs* and *Bpss* inheritance, we generated (susceptible × resistant)F_1 hybrid mice on the basis of the phenotype data in Table 1. For both *Bpss* and *Bpbs*, we used SJL/J mice as the prototypical susceptible strain (SJL/J mice were susceptible to all three vasoactive amines, Bphs$^+$ Bpss$^+$ Bpbs$^+$; Table 1). To rule out strain-specific contributions to the Bpss phenotype, we also employed an F_1 hybrid generated by using AKR/J mice as the susceptible strain (like SJL/J mice, AKR/J mice are Bpss$^+$ Bpbs$^+$). As a positive control for interstrain crosses in Bpss, we assessed (SJL/J × PL/J)F_1 mice (PL/J is Bpss$^+$), and these mice indeed remained Bpss$^+$ and were statistically indistinguishable from either parental strain (Table 2). By analyzing the Bpss phenotype of (SJL/J × BALB/cJ), (SJL/J × B10.S), and (AKR/J × DBA/2J)F_1 hybrids, we found that Bpss was recessive since the F_1 mice displayed the phenotype of the Bpss-resistant parental strain (Table 2). We observed a similar recessive inheritance pattern for Bpbs, where (SJL/J × B10.S)F_1 mice were Bpbs resistant, like the B10.S parental strain (Table 2). Collectively, these data again confirm that the phenotypic variation in susceptibility to VAAS reflects the genetic control of responsiveness to individual VAAs rather than susceptibility and resistance to intoxication in general.

The fact that Bphs was reported to be controlled by a single autosomal dominant gene (36) was confirmed when we identified *Hrh1* as the gene controlling Bphs (11). In order to determine the number of loci controlling Bpss and Bpbs, we generated (B10.S × SJL/J) × B10.S N2 backcross mice in sufficient numbers to be able to statistically evaluate by chi-square test both one- and two-locus models of genetic control. The data for both Bpss and Bpbs are consistent with a two-gene model of recessive inheritance (Table 3).

Lack of linkage of Bpss and Bpbs to 5-HT or BDK receptors, respectively. Given that Bphs was identified as the product of *Hrh1* (11), we wondered whether Bpss and Bpbs could be similarly controlled by 5-HT and BDK receptor polymorphism. To test this hypothesis, we determined whether or not Bpss or Bpbs was linked to any of the known 5-HT or BDK receptors, respectively. To this end, (B10.S × SJL/J) × B10.S N2 backcross mice were phenotyped for Bpss or Bpbs and genotyped by using informative microsatellite markers linked to the 14 5-HT genes encoding functional receptors on chromosomes 1, 4, 5, 9, 13, 14, 16, 18, 19, and X and to *Bdkrb1/2* on chromosome 12. Four additional putative 5-HT receptor sequences (*Htr1da*, *Htr1db*, *Htr1ea*, and *Htr1eb*) have been deposited in PubMed Gene. *Htr1da* and *Htr1db* were based on partial *Htr1d* sequence data by Weydert et al. suggesting the possible existence of two *Htr1d* genes on mouse chromosome 4 (37). However, *Htr1da* was shown not to encode a unique functional receptor, and the record for *Htr1db* has been replaced with that for *Htr1d*, which we analyzed. With respect to *Htr1ea* and *Htr1eb*, the situation is similar; *Htr1ea* is actually *Htr1e* and *Htr1eb* is *Htr1f*, both of which we analyzed. Therefore, our analysis covered all known functional 5-HT receptor genes. By chi-square analysis, we did not observe significant linkage of *Bpss* to any of the known 5-HT receptors, nor did we observe linkage of *Bpbs* to *Bdkrb1/2* (Table 4). However, we did observe that *Bdkrb2*-deficient mice (21) were completely protected from Bpbs (Table 5), indicating that while not genetically linked to *Bpbs*, Bdkrb2 signaling is required to elicit PTX-induced sensitization to BDK.

eNOS and caveolin-1 in Bphs. To further investigate the molecular mechanism by which PTX intoxication elicits HA sensitization, we employed genetically modified mice lacking key individual components thought to be involved in PTX activity or the signal propagation involved in HA-mediated vasoregulation. Vascular endothelium expresses the enzyme nitric oxide synthase (eNOS, the protein encoded by *Nos3*), which catalyzes the formation of nitric oxide from L-arginine. This pathway plays a major role in the regulation of vascular tone and blood pressure through the generation of cyclic GMP (cGMP) (38–40). eNOS activation occurs in response to elevation of Ca^{2+} concentrations in the cell (17, 41). Activation of H_1R increases intracellular Ca^{2+} levels as one of the proximal signaling events (42, 43). In addition, H_1R activation has been shown to elevate cGMP levels *in vivo* (44). To address whether *Nos3* is required for Bphs, we sensitized WT C57BL/6J mice and *Nos3*-deficient mice (B6.129 background) (22) with PTX and challenged them with HA. *Nos3*-deficient mice were just as Bphs as WT mice (Table 6), suggesting that VAAS following PTX intoxication is not dependent on *Nos3*. In endothelial cells (ECs), eNOS is highly expressed in caveolae, which are specialized membrane-associated organelles important for the barrier and vasoregulatory functions of these cells. To test whether other features of caveolae distinct from eNOS are important in the regulation of VAASH, we tested the Bphs response of caveolin-1-deficient mice. Since cross regulation of caveolin-1 and eNOS function has been shown (45), we also investigated Bphs in caveolin-1 (*Cav1*) and *Nos3* doubly deficient mice. In contrast to control *Hrh1*-deficient mice, both *Cav1*$^{-/-}$ and *Cav1*$^{-/-}$ *Nos3*$^{-/-}$ mice were Bphs (Table 7). Together, these results indicate that neither the formation of caveolae nor activation of eNOS in ECs is a critical regulator of VAASH.

Individual PKC isoforms do not mediate Bphs. In most mammalian tissues, H_1R induces the activation of phospholipase C (PLC) via PTX-resistant $G\alpha_{q/11}$-proteins to generate inositol-1,4,5-triphosphate (IP_3) and diacylglycerol (DAG) from phosphatidylinositol-4,5-bisphosphate (43). IP_3 leads to increased intracellular Ca^{2+}, and DAG leads to activation of specific isoforms of protein kinase C (PKC). We therefore examined the requirements for activation of PKC downstream of H_1R in eliciting Bphs. There are at least 12 PKC isoforms (46), and we surveyed selected gene-deficient mice representing representative members of the different isozyme subclasses for their roles in susceptibility to Bphs. We

TABLE 4 Bpss and Bpbs are not linked to 5-HT and BDK receptors, respectively[a]

Receptor	Chr[b]	Distance (cM[c])	Size (Mbp)	Marker	Distance (cM)	Size (Mbp)	No. of mice: Affected S	Affected H	Affected B	Unaffected S	Unaffected H	Unaffected B	χ^2 value	*P* value
Bpss														
Htr2b	1	52	88	*D1Mit10*	56.6	93	3	12	2	22	14	39	4.8	0.4
Htr5b		63	123											
Htr6	4	65	139	*D4Mit203*	60	129	2	12	6	25	34	17	4.9	0.4
Htr1d		66	136											
Htr5a	5	15	28	*D5Mit148*	18	32	6	9	3	16	46	14	4.5	0.5
Htr3a	9	29	49	*D9Mit105*	35	63	9	6	3	21	34	19	6.6	0.3
Htr3b		29	49											
Htr1b		46	82											
Htr1a	13	58	106	*D13Mit75*	59	107	3	11	6	20	37	18	1.2	0.9
Htr2a	14	42	75	*D14Mit71*	44	97	6	11	3	17	36	23	2.3	0.8
Htr1f	16	39	65	*D16Mit14*	34	51	4	9	7	19	32	25	3.9	0.6
Htr4	18	37	62	*D18Mit81*	41	67	4	11	5	19	39	18	0.4	1
Htr7	19	33	36	*D19Mit88*	34	37	6	8	6	20	34	22	1.8	0.9
Htr2c	X	66	143	*DXMit36*	63	143	2/4[d]	5	3/6[d]	5/22[d]	16	12/27[d]	3.2/0.1[d]	0.7/0.8[d]
Bpbs, *Bdkrb1/2*	12	52	107	*D12Mit7*	50	104	7	11	10	14	36	15	3.4	0.6

[a] (B10.S × SJL/J) × B10.S N2 backcross mice were phenotyped for Bpss or Bpbs as described for Table 3 (25 mg/kg 5-HT and 40 mg/kg BDK, respectively). B, B10.S; H, heterozygous; S, SJL/J (at indicated position with chromosome location for the gene and closest marker indicated). Genotype frequency differences among affected and unaffected BC1 mice were evaluated by chi-square analysis in two-by-two contingency tables. Significance was tested by Fisher's exact test.
[b] Chr, chromosome.
[c] cM, centimorgans.
[d] Female/male values.

selected three "conventional" DAG/Ca^{2+}-dependent isozymes (PKC-α, -β, and -γ), two DAG-dependent and Ca^{2+}-independent "novel" class members (PKC-δ and -ε), and one DAG- and Ca^{2+}-independent "atypical" member (PKC-ζ) for PTX sensitization and subsequent HA challenge. All of these gene-deficient mice are on the B6 background, but all carry 129 strain DNA because of gene targeting in strain 129 embryonic stem cells. However, we found no difference in Bphs in the background control strains over a 16-fold HA dose range (Table 8). As expected, H_1RKO mice were Bphs resistance, again demonstrating the critical role of *Hrh1* in eliciting Bphs. Lastly, we observed that all B6.129 PKC KO mice were susceptible to Bphs (Table 8). Thus, irrespective of the mode of activation or tissue expression patterns, neither PKC-α, -β, -γ, -δ, -ε, nor -ζ individually appears to control *Bphs*.

Bphs is not controlled by Tlr4. Experiments with murine splenocytes using radiolabeled PTX and lipopolysaccharide (LPS) showed that both compounds bind to Toll-like receptor 4 (47). In 2004, Kerfoot et al. reported that induction of experimental autoimmune encephalomyelitis (EAE) with PTX as an ancillary adjuvant and PTX-mediated lymphocyte rolling and adhesion were diminished in the absence of *Tlr4* (48). In conjunction with a recent report showing that commercial PTX preparations can contain LPS/endotoxin (49), we found that our source of PTX contained approximately 40 endotoxin units (EU) per PTX sensitization inoculum. This concentration is equivalent to 0.001 EU/kg of body weight (data not shown), which is far below the 5-EU/kg cutoff in preclinical animal models (50). In light of these considerations, we tested the hypothesis that the VAAS activity of PTX in the Bphs model was dependent on *Tlr4*. For these experiments, we sensitized WT C57BL/10J mice and C57BL/10ScNJ mice with active holotoxin and challenged them with HA 3 days later. C57BL/10ScNJ mice have a deletion of the *Tlr4* gene that results in the absence of both mRNA and protein ($Tlr4^{lps\text{-}del}$) and thus is defective in response to LPS stimulation (24). $Tlr4^{lps\text{-}del}$ differs from the $Tlr4^{Lps\text{-}d}$ mutation of C3H/HeJ mice, a point mutation that causes an amino acid substitution, resulting in a hypomorphic *Tlr4* gene product (24). Studying the responsiveness of C57BL/10ScNJ mice therefore precludes the possibility that PTX can still mediate its effects through a non-LPS-responsive allele. We found that both C57BL/10J and C57BL/10ScNJ mice were

TABLE 5 Bpbs in WT and *Bdkrb2*-deficient mice[a]

Strain	No. of mice dead/total after challenge with BDK at: 80 mg/kg	40 mg/kg	20 mg/kg	10 mg/kg	χ^2 value, *df*	*P* value
C57BL/6J	3/3	3/3	1/3	0/3		
B6.129S7-*Bdkrb2*^{tm1Jfh}/J	0/4	0/4	0/4	0/4	26.0, 7	0.0005

[a] WT or *Bdkrb2*-deficient mice were sensitized with 200 ng PTX by i.v. injection and challenged 3 days later by i.v. injection of BDK. Significance was determined by Fisher's exact test.

TABLE 6 Bphs is not mediated by eNOS[a]

Strain	No. of mice dead/total after challenge with HA at: 50 mg/kg	12.5 mg/kg	3.13 mg/kg
C57BL/6J	4/4	4/4	2/4
B6.129.P2-*Nos3*$^{t,m,i/Unc}$/J	4/4	4/4	1/4

[a] WT and *Nos3*-deficient mice were analyzed for Bphs 3 days after the i.v. injection of 200 ng PTX. HA dosages are based on mg/kg (dry weight) free base. Deaths were recorded at 30 min, and the results expressed as the number dead/number studied.

TABLE 7 Disruption of endothelial caveolae does not affect Bphs[a]

Strain	No. of mice dead/total (% of total) after challenge with HA at:			
	25 mg/kg	12.5 mg/kg	6.25 mg/kg	3.125 mg/kg
WT	2/2 (100)	2/2 (100)	2/2 (100)	0/2 (0)
Cav1$^{-/-}$	2/2 (100)	2/2 (100)	3/3 (100)	2/5 (40)
Cav1$^{-/-}$ × *Nos3*$^{-/-}$	2/2 (100)	2/2 (100)	3/3 (100)	1/4 (25)
Hrh1$^{-/-}$	0/3	0/3	0/3	0/3

[a] WT, *Cav1*-deficient, *Cav1*/*Nos3* doubly deficient, and *Hrh1*-deficient mice were analyzed for Bphs. Deaths were recorded at 30 min, and the results expressed as the number dead/number studied.

Bphs (Table 9). These data are also consistent with our previous study showing that PTX-induced angiogenesis of brain microvascular ECs was not affected by the lack of *Tlr4* signaling (51). Thus, our data suggest that while certain defined effects of PTX, such as leukocyte recruitment, may require *Tlr4*, the HA-sensitizing activity of PTX does not.

$G\alpha_{i1/3}$ are critical PTX targets for elicitation of VAASH. The ADP-ribosylating activity of PTX is a well-established mechanism of action whereby PTX inhibits the function of $G\alpha_{i/o}$ proteins (52) and is required to elicit the myriad physiological responses following *in vivo* intoxication, including VAAS and the immune potentiating effects in EAE (18). Conjugation of ADP-ribose to a key cysteine residue in the C terminus of $G\alpha_{i/o}$ (but not $G\alpha_s$, $G\alpha_q$, or $G\alpha_{11}$) proteins prevents the association of the $G\alpha_{i/o}G\beta\gamma$ complex with the GPCR (5, 26). Therefore, PTX is a powerful tool to discriminate whether a given GPCR signals through $G\alpha_{i/o}$-dependent or $G\alpha_{i/o}$-independent mechanisms (19). However, there are several $G\alpha_i$ proteins ($G\alpha_{i1}$, $G\alpha_{i2}$, $G\alpha_{i3}$, and $G\alpha_z$) and $G\alpha_o$ proteins ($G\alpha_{o1}$ and $G\alpha_{o2}$, splice variant products of the *Gnao* gene) (28). To determine which $G\alpha_{i/o}$ proteins are targeted by PTX for HA sensitization, we directly assessed the responses of mice genetically deficient in specific $G\alpha_i$ or $G\alpha_o$ proteins to HA challenge. We did not test $G\alpha_z$, since this protein lacks the key cysteine for PTX-mediated ADP-ribosylation (29) and is therefore not affected by PTX. Our hypothesis was that genetic deficiency in one or more $G\alpha_{i/o}$ proteins would mimic HA hypersensitivity; i.e., such mice would be Bphs without prior PTX sensitization.

We first addressed the role of $G\alpha_o$ proteins as targets for ADP-ribosylation in VAASH. $G\alpha_{o1}$ and $G\alpha_{o2}$ are different gene products because of alternative exon usage in the *Gnao* gene (30). *Gnao*-deficient mice lack both $G\alpha_{o1}$ and $G\alpha_{o2}$ splice products because of disruption of *Gnao* exon 6, which is common to both gene products (30). Neither *Gnao*-deficient mice nor mice specifically lacking either $G\alpha_{o1}$ or $G\alpha_{o2}$ were spontaneously sensitive to HA (Fig. 1). These results demonstrated that the HA-sensitizing activity of PTX was not due to its ADP-ribosylation of $G\alpha_o$ proteins.

Next we addressed the putative role of $G\alpha_i$ proteins as targets for PTX. $G\alpha_{i1}$, $G\alpha_{i2}$, and $G\alpha_{i3}$ are encoded by distinct genes, *Gnai1*, *Gnai2*, and *Gnai3*, respectively (29), and are widely expressed (28). Compared to WT mice, $G\alpha_{i1}{}^{-/-}$ ($P = 0.0002$) and $G\alpha_{i3}{}^{-/-}$ ($P = 0.008$) mice were significantly more sensitive to HA, whereas $G\alpha_{i2}{}^{-/-}$ mice were not (see Table S2 in the supplemental material). We also analyzed the response of $G\alpha_{i1}{}^{-/-}$ $G\alpha_{i3}{}^{-/-}$ double-KO mice and found them to also be significantly more susceptible to spontaneous HA sensitivity than WT mice are ($P = 0.001$) (see Table S2). Additionally, if one directly compares the susceptibility to spontaneous sensitivity to HA among the various KO strains, there is a significant overall effect of genotype ($P < 0.0001$) with no significant difference among *Gnao*$^{-/-}$, $G\alpha_{o1}{}^{-/-}$, $G\alpha_{o2}{}^{-/-}$, and $G\alpha_{i2}{}^{-/-}$ mice ($P = 0.5$) or among $G\alpha_{i1}{}^{-/-}$, $G\alpha_{i3}{}^{-/-}$, and $G\alpha_{i1}{}^{-/-}$ $G\alpha_{i3}{}^{-/-}$ mice ($P = 0.3$); however, there is a highly significant difference in susceptibility to spontaneous HA sensitivity between the two groups ($P < 0.0001$). Taken together, these results demonstrate that $G\alpha_{i1}$ and $G\alpha_{i3}$ are clear targets of ADP-ribosylation associated with PTX-induced VAASH.

TABLE 8 Bphs in PKC KO mice[a]

Strain	No. of mice dead/total after challenge with HA at:		
	50 mg/kg	12.5 mg/kg	3.13 mg/kg
129XlISvJ	10/10	10/10	4/6
C57BLl6J	6/6	7/8	4/6
B6129PF$_2$/J	4/4	4/4	5/6
Hrh1$^{-/-}$	0/4	0/4	0/4
Prkca (PKCα)$^{-/-}$	3/3	3/3	3/3
Prkcb (PKCβ)$^{-/-}$	3/3	3/3	3/3
Prkcc (PKCγ)$^{-/-}$	6/6	8/8	9/9
Prkcd (PKCδ)$^{-/-}$	4/4	3/3	3/3
Prkce (PKCε)$^{-/-}$	11/11	3/3	3/3
Prkcz (PKCζ)$^{-/-}$	4/4	3/3	1/2

[a] WT controls and PKC-α, -β, -γ, -δ, -ε, and -ζ singly deficient mice were analyzed for Bphs. Deaths were recorded at 30 min, and the results expressed as the number dead/number studied.

TABLE 9 Bphs in *Tlr4* mutant mice[a]

Strain	No. of mice dead/total after challenge with HA at:		
	50 mg/kg	12.5 mg/kg	3.125 mg/kg
C57BL/10J	5/5	6/6	1/6
C57BL/10ScNJ	5/5	6/6	0/6

[a] C57BL/10J (*Tlr4*$^{+}$) and C57BL/10ScNJ (*Tlr4*-deficient) mice were analyzed for Bphs. Deaths were recorded at 30 min, and the results expressed as the number dead/number studied.

DISCUSSION

We have focused on understanding the genetic basis for VAAS elicited by PTX sensitization. Changes in vascular permeability elicited during inflammation may promote antigen leakage and contribute to pathogen dissemination. Dysregulated vascular per-

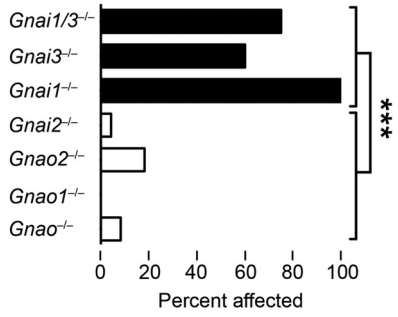

FIG 1 Loss of $G\alpha_{i1}$ or $G\alpha_{i3}$ mimics PTX to elicit HA sensitization. Mice deficient in $G\alpha_{i/o}$ subunits were analyzed for HA sensitivity in the absence of PTX exposure. Mice received 100 mg/kg HA i.v., and deaths were recorded within 30 min. Statistical significance was determined by one-way ANOVA using 8 to 23 mice per group. ***, $P < 0.001$.

meability may allow for inappropriate tissue access to immune cell surveillance, leading to tissue damage (13). In this regard, PTX has long been known to alter vascular permeability through its ability to promote VAAS (53). The vasoactive amines HA, 5-HT, and BDK have all been shown to influence vascular permeability, in particular, the BBB (16, 53, 54). Here we have shown that after PTX sensitization, (i) hypersensitivity to HA, 5-HT, or BDK (Bphs, Bpss, and Bpbs, respectively) is uniquely genetically controlled; (ii) unlike the genetic control of *Bphs*, that of *Bpss* and *Bpbs* and is not linked to any of the known 5-HT or BDK receptors; (iii) Bpbs is mediated by *Bdkrb2*; and (iv) $G\alpha_{i1/3}$ proteins are the physiological *in vivo* targets of ADP-ribosylation by PTX associated with VAASH.

Under inflammatory conditions, vascular permeability is modulated by activated leukocytes acting on the vascular unit either through secreted or cell surface factors (13, 54). Mast cells are classically known to produce and store HA, 5-HT, and other amine/lipid/glycosylated mediators (55). More recently, mast cells have been shown to also produce BDK (14). Mast cells perfuse tissues and rapidly respond to external local stimuli which may act as secretagogues to stimulate the release of stored granular contents, including vasoactive amines. In fact, the critical role of mast cell-derived products in the control of vascular permeability was recently shown (14). Concomitant with the relaxed vascular permeability promoted by mast cell degranulation, neutrophils may gain tissue access to promote damage and promote subsequent recruitment of adaptive immune cells (13). From these data, one may then posit that environmental exposure may affect mast cells or the target vasculature to influence vascular permeability.

Mast cell number, function (56, 57), and susceptibility to VAAS vary across different genetic backgrounds (58, 59). Although Bphs was mapped by using the SJL/J-derived *Hrh1* allele (11, 12), up to 75% of the available inbred mouse strains harbor this same allele (11; R. Noubade, unpublished data). We and others have indeed observed that SJL/J mice were also susceptible to Bpss (17, 53), though Bpss was not mediated by *Hrh1* (17). Like HA and 5-HT, a BDK challenge after PTX intoxication also leads to death due to acute hypovolemic and hypotensive shock. Given these phenotypic similarities and the extensive genetic variation across inbred laboratory mouse strains, we tested whether a conserved genetic mechanism controls general VAAS by PTX. To address this possibility, we examined responses to HA, 5-HT, and BDK in a panel of inbred mice. Among these strains, we observed the full spectrum of VAAS to different individual mediators, indicating that the phenotypic variation in responsiveness to PTX reflects the genetic control of distinct intermediate phenotypes rather than allelic variation in genes controlling overall susceptibility to PTX intoxication.

Furthermore, using F_1 hybrid crosses, we found that Bpss and Bpbs are autosomal recessive, while Bphs is autosomal dominant (12). The susceptibility pattern for Bpss and Bpbs in (SJL/J × B10.S) × SJL/J N2 crosses was consistent with a two-gene model of genetic control. Unlike *Bphs*, which showed genetic linkage to a key HA receptor, *Hrh1* (11), for both *Bpss* and *Bpbs*, susceptibility was not linked to genes encoding the cognate ligand receptors. Furthermore, although *Bpbs* was not linked to *Bdkrb1/2*, this phenotype was abrogated in *Bdkrb2*-deficient mice, indicating that *Bdkrb2* is nonetheless required for *Bpbs*. Taken together, our results confirm and extend the finding that the phenotypic variation in susceptibility to VAAS following PTX sensitization reflects the unique genetic control of specific intermediate phenotypes, i.e., Bphs, Bpss, and Bpbs, rather than susceptibility and resistance to intoxication in general.

Previously, we investigated the cell type requirements for H_1R expression in Bphs. Using bone marrow chimeras, we found that the nonhematopoietic compartment determined susceptibility to Bphs (16). We found that EC-specific expression of H_1R was not sufficient to mediate Bphs (16). Here we extended our analysis of ECs in VAASH. Inhibition of $G\alpha_i$ proteins by PTX has been shown to increase eNOS (*Nos3*) expression (60), yet *Nos3*-deficient mice were not protected from Bphs. Similarly, disruption of *caveolin-1* either alone or in conjunction with *Nos3* disruption did not affect Bphs. Another possibility that we have not analyzed here is that VAASH is due to PTX-mediated inhibition of sphingosine-1-phosphate (S1P) receptor signaling in EC. S1P regulates barrier function, and mice deficient in S1P in plasma were more susceptible to HA-induced vascular leakage (61). Nonetheless, administration of PTX to mice expressing EC-specific H_1R did not render them sensitive to VAASH (16), which argues against this notion. Taken together, these results did not formally rule out the possibility that *Hrh1* on EC is required but suggested that coexpression of H_1R in another component of the vascular unit may be required for Bphs. Given the roles for H_1R in vascular smooth muscle and neural function (62, 63), we hypothesize that H_1R signaling in non-ECs of the vascular unit may be an important component of Bphs (16). Indeed, in the central nervous system (CNS), astrocytic end feet projecting into the EC/smooth muscle layers that wrap cerebral arterioles are important in relaying neural signals that control arterial tone in the brain (64).

Our results illustrate a central role for Gα protein activation and GPCRs in vasoregulation. The mast cell mediators HA, 5-HT, and BDK all promote vascular leakage/edema (65), and all signal through GPCRs. Nonetheless, diversity in molecular responses is controlled by receptor specificity and the activation of different effector Gα proteins (28). Forty-five years ago, Bergman and Munoz introduced the concept of counterbalanced signals controlling VAAS, with a special emphasis on the interplay between HA and the adrenergic-receptor (AR) pathways (66). These authors also suggested that PTX might sensitize to HA by targeting AR pathways. More recent observations are also consistent with the existence of opposing Gα protein effects in VAAS. Many ARs are coupled to $G\alpha_i$ proteins (α_{2A}, α_{2B}, α_{2C}) (67), whereas key receptors for the vasoactive amines (H_1R, 5-HT2, and the B2 BDK receptor) are all linked to $G\alpha_{q/11}$ (28). It was recently shown that the anaphylactic shock response is controlled by endothelial $G\alpha_{q/11}$ signaling (68). Our data suggest that inactivation of $G\alpha_{i1/3}$ proteins by PTX is a key destabilizing effect that may allow unabated signaling through $G\alpha_{q/11}$ or $G\alpha_s$-linked GPCRs such as those for HA, 5-HT, and BDK.

PTX catalyzes the ADP-ribosylation of $G\alpha_{i/o}$ proteins associated in the Gα/β/γ heterotrimer and prevents the interaction of the trimeric signaling complex with GPCRs (6). In mice, there are eight $G\alpha_{i/o}$ genes (*Gnai1* to *-3*, *Gnao*, *Gnaz*, and *Gnat1* to *-3*) which, because of alternative splice forms of *Gnao*, encode at least 10 different protein products (28). PTX has been generally considered a broad-spectrum $G\alpha_{i/o}$ inhibitor with only limited insight into either $G\alpha_{i/o}$ specificity or specific function. Here we have identified $G\alpha_{i1}$ and $G\alpha_{i3}$ as the targets of ADP-ribosylation associated with Bphs. Mice lacking $G\alpha_{i1}$ and $G\alpha_{i3}$ were spontaneously

susceptible to Bphs, a response normally observed in intact mice only after intoxication with active PTX holotoxin. It is possible that PTX targets other than $G\alpha_{i1/3}$ contribute to Bphs. This would be detected only if we treated $G\alpha_{i1/3}$-deficient mice with PTX and observed 100% penetrance. We did not formally address this possibility because we are not aware of any WT or KO mouse strain besides $G\alpha_{i1}$- or $G\alpha_{i1/3}$-deficient mice that exhibit spontaneous HA sensitivity at 100% penetrance (17, 36). Therefore, we interpret our present results to be due to incomplete penetrance possibly due in part to HA dosing (17, 36). Furthermore, given that Bphs was seen in all other $G\alpha_{i/o}$-deficient mice, our results show the specificity of PTX for $G\alpha_{i1/3}$ with regard to HA sensitization.

We hypothesize that H_1R signaling (which uses $G\alpha_{q/11}$) in the vascular unit is counterbalanced by $G\alpha_{i1/3}$-dependent signals to maintain proper vascular tone and permeability. The mice used here were ubiquitously deficient in $G\alpha_{i1/3}$, precluding the assignment of $G\alpha_{i1/3}$ function to a specific cell type. The GPCR(s) that links to $G\alpha_{i1/3}$ in the control of Bphs is also not known, although ARs remain a possibility. Since the identification of *Hrh1* as *Bphs* in 2002 (11), more has been learned about the other three HA receptors, two of which—H_3R and H_4R—are linked to $G\alpha_{i/o}$ and are therefore sensitive to PTX (63). H_4R expression is restricted to the hematopoietic system, but our finding that Bphs is controlled by the nonhematopoietic system (16) rules out this receptor as a $G\alpha_{i/o}$-linked candidate for PTX inhibition in Bphs. Expression of H_3R, however, is found in the CNS and in the autonomic nervous system (69) and has been found to regulate BBB permeability and susceptibility to both CNS autoimmune disease (70) and CNS infection in the case of cerebral malaria (71). Accordingly, another potential explanation for the physiological Bphs response could involve PTX-mediated inhibition of H_3R to destabilize baseline vascular permeability and allow HA to signal unabated through another of its receptors (H_1R) to induce acute vasodysregulation.

Collectively, our data reveal that $G\alpha_{i1/3}$ proteins play an important role in the sensing of PTX and the regulation of vascular permeability in response to vasoactive amines. While H_1R signaling in the nonhematopoietic compartment appears to ultimately control Bphs, the hematopoietic compartment is likely to serve as a source of vasoactive amines, for example, with mast cells as major producers of HA. Thus, it is important to understand Gα function in specific cell types at the interface of vasculature and tissue. Under inflammatory conditions, $G\alpha_{i2}$ expression in ECs, but not in leukocytes, is important in the extravasation of leukocytes into inflamed lung tissue (72). PTX exposure strongly impairs immune cell migration due to inhibition of $G\alpha_{i/o}$-linked chemokine receptor signaling (73). Deletion of $G\alpha_{i2}$ affects T and B cell homing under steady-state conditions (33, 74, 75) and leads to T cell hyperreactivity (76). However, not all functions of $G\alpha_{i2}$ are unique to this molecule, since competition for CXCR3-mediated signaling between $G\alpha_{i2}$ and $G\alpha_{i3}$ occurs, where $G\alpha_{i3}$ may compete for and quench $G\alpha_{i2}$-mediated chemotaxis in *in vitro* migration assays (77). Extravasation of immune cells into tissues under inflammatory conditions involves a combination of chemokines (78, 79) and hence may involve different $G\alpha_i$ members. Our data linking PTX to $G\alpha_{i1/3}$ function and the ability of PTX to enhance the development of inflammatory disease (10) implicate $G\alpha_{i1}$- and $G\alpha_{i3}$-linked GPCRs in this process.

Our data may also give insight into the molecular mechanisms discriminating immunity to *B. pertussis* provided through exposure to whole-cell pertussis (wP) via infection or vaccination or via vaccination with the acellular pertussis (aP) vaccine (which replaced wP in 1997). aP refers to a vaccine in which PTX is inactivated by chemical fixation or by genetic means. This modification decreases the incidence of side effects, but data showing that aP may exhibit less durability and protection than wP are mounting (80). Taken with our data, it is possible that the $G\alpha_{i1/3}$-linked GPCRs inhibited by PTX are critical for durable immunity to *B. pertussis*. Indeed, production of inflammatory cytokines was increased in stimulated splenocytes from $G\alpha_{i1/3}$-deficient mice while that of regulatory cytokines was decreased (81), lending support to the notion that $G\alpha_{i1/3}$ damping may contribute to a robust systemic immune response. Identification of these critical GPCRs may lead to strategies for their blockade in order to maximize the benefits of aP while increasing vaccine durability. The hypersensitivity to vasoactive amines released during the course of inflammation, as we have modeled here with Bphs, may be an indicator of such an effective strategy.

ACKNOWLEDGMENTS

We thank Michael Leitges (Max Planck Institute, Hannover, Germany) and Lutz Birnbaumer (National Institute for Environmental Health Sciences, Research Triangle Park, NC) for kinds gifts of PKC- and $G\alpha_{i/o}$-deficient mice, respectively. We thank the staff of the University of Vermont Animal Facility for expert care.

This research was supported by grants from the NIH (NS061014, NS069628, and NS36526).

REFERENCES

1. **von König CH, Halperin S, Riffelmann M, Guiso N.** 2002. Pertussis of adults and infants. Lancet Infect. Dis. **2:**744–750. http://dx.doi.org/10.1016/S1473-3099(02)00452-8.
2. **Yajima M, Hosoda K, Kanbayashi Y, Nakamura T, Nogimori K, Mizushima Y, Nakase Y, Ui M.** 1978. Islets-activating protein (IAP) in Bordetella pertussis that potentiates insulin secretory responses of rats. Purification and characterization. J. Biochem. **83:**295–303.
3. **Yajima M, Hosoda K, Kanbayashi Y, Nakamura T, Takahashi I, Ui M.** 1978. Biological properties of islets-activating protein (IAP) purified from the culture medium of Bordetella pertussis. J. Biochem. **83:**305–312.
4. **Tamura M, Nogimori K, Murai S, Yajima M, Ito K, Katada T, Ui M, Ishii S.** 1982. Subunit structure of islet-activating protein, pertussis toxin, in conformity with the A-B model. Biochemistry **21:**5516–5522. http://dx.doi.org/10.1021/bi00265a021.
5. **Hoshino S, Kikkawa S, Takahashi K, Itoh H, Kaziro Y, Kawasaki H, Suzuki K, Katada T, Ui M.** 1990. Identification of sites for alkylation by *N*-ethylmaleimide and pertussis toxin-catalyzed ADP-ribosylation on GTP-binding proteins. FEBS Lett. **276:**227–231. http://dx.doi.org/10.1016/0014-5793(90)80548-W.
6. **Katada T, Oinuma M, Ui M.** 1986. Two guanine nucleotide-binding proteins in rat brain serving as the specific substrate of islet-activating protein, pertussis toxin. Interaction of the alpha-subunits with beta gamma-subunits in development of their biological activities. J. Biol. Chem. **261:**8182–8191.
7. **Kaslow HR, Burns DL.** 1992. Pertussis toxin and target eukaryotic cells: binding, entry, and activation. FASEB J. **6:**2684–2690.
8. **Morse SI, Morse JH.** 1976. Isolation and properties of the leukocytosis- and lymphocytosis-promoting factor of Bordetella pertussis. J. Exp. Med. **143:**1483–1502. http://dx.doi.org/10.1084/jem.143.6.1483.
9. **Furman BL, Walker E, Sidey FM, Smith M.** 1988. Metabolic disturbances produced by pertussis toxin, p 147–172. *In* Wardlaw AC, Parton R (ed), Pathogenesis and immunity in pertussis. John Wiley and Sons, Chichester, United Kingdom.
10. **Baxter AG.** 2007. The origin and application of experimental autoimmune encephalomyelitis. Nat. Rev. Immunol. **7:**904–912. http://dx.doi.org/10.1038/nri2190.
11. **Ma RZ, Gao J, Meeker ND, Fillmore PD, Tung KS, Watanabe T, Zachary JF, Offner H, Blankenhorn EP, Teuscher C.** 2002. Identification of Bphs, an autoimmune disease locus, as histamine receptor H1. Science **297:**620–623. http://dx.doi.org/10.1126/science.1072810.

12. **Sudweeks JD, Todd JA, Blankenhorn EP, Wardell BB, Woodward SR, Meeker ND, Estes SS, Teuscher C.** 1993. Locus controlling Bordetella pertussis-induced histamine sensitization (Bphs), an autoimmune disease-susceptibility gene, maps distal to T-cell receptor beta-chain gene on mouse chromosome 6. Proc. Natl. Acad. Sci. U. S. A. **90:**3700–3704. http://dx.doi.org/10.1073/pnas.90.8.3700.
13. **Walker ME, Hatfield JK, Brown MA.** 2012. New insights into the role of mast cells in autoimmunity: evidence for a common mechanism of action? Biochim. Biophys. Acta **1822:**57–65. http://dx.doi.org/10.1016/j.bbadis.2011.02.009.
14. **Oschatz C, Maas C, Lecher B, Jansen T, Bjorkqvist J, Tradler T, Sedlmeier R, Burfeind P, Cichon S, Hammerschmidt S, Muller-Esterl W, Wuillemin WA, Nilsson G, Renne T.** 2011. Mast cells increase vascular permeability by heparin-initiated bradykinin formation in vivo. Immunity **34:**258–268. http://dx.doi.org/10.1016/j.immuni.2011.02.008.
15. **Noubade R, Milligan G, Zachary JF, Blankenhorn EP, del Rio R, Rincon M, Teuscher C.** 2007. Histamine receptor H1 is required for TCR-mediated p38 MAPK activation and optimal IFN-gamma production in mice. J. Clin. Invest. **117:**3507–3518. http://dx.doi.org/10.1172/JCI32792.
16. **Lu C, Diehl SA, Noubade R, Ledoux J, Nelson MT, Spach K, Zachary JF, Blankenhorn EP, Teuscher C.** 2010. Endothelial histamine H1 receptor signaling reduces blood-brain barrier permeability and susceptibility to autoimmune encephalomyelitis. Proc. Natl. Acad. Sci. U. S. A. **107:**18967–18972. http://dx.doi.org/10.1073/pnas.1008816107.
17. **Gao JF, Call SB, Fillmore PD, Watanabe T, Meeker ND, Teuscher C.** 2003. Analysis of the role of Bphs/Hrh1 in the genetic control of responsiveness to pertussis toxin. Infect. Immun. **71:**1281–1287. http://dx.doi.org/10.1128/IAI.71.3.1281-1287.2003.
18. **Black WJ, Munoz JJ, Peacock MG, Schad PA, Cowell JL, Burchall JJ, Lim M, Kent A, Steinman L, Falkow S.** 1988. ADP-ribosyltransferase activity of pertussis toxin and immunomodulation by Bordetella pertussis. Science **240:**656–659. http://dx.doi.org/10.1126/science.2896387.
19. **Milligan G, Carr C, Gould GW, Mullaney I, Lavan BE.** 1991. Agonist-dependent, cholera toxin-catalyzed ADP-ribosylation of pertussis toxin-sensitive G-proteins following transfection of the human alpha 2-C10 adrenergic receptor into rat 1 fibroblasts. Evidence for the direct interaction of a single receptor with two pertussis toxin-sensitive G-proteins, Gi2 and Gi3. J. Biol. Chem. **266:**6447–6455.
20. **Inoue I, Yanai K, Kitamura D, Taniuchi I, Kobayashi T, Niimura K, Watanabe T.** 1996. Impaired locomotor activity and exploratory behavior in mice lacking histamine H1 receptors. Proc. Natl. Acad. Sci. U. S. A. **93:**13316–13320. http://dx.doi.org/10.1073/pnas.93.23.13316.
21. **Borkowski JA, Ransom RW, Seabrook GR, Trumbauer M, Chen H, Hill RG, Strader CD, Hess JF.** 1995. Targeted disruption of a B2 bradykinin receptor gene in mice eliminates bradykinin action in smooth muscle and neurons. J. Biol. Chem. **270:**13706–13710. http://dx.doi.org/10.1074/jbc.270.23.13706.
22. **Shesely EG, Maeda N, Kim HS, Desai KM, Krege JH, Laubach VE, Sherman PA, Sessa WC, Smithies O.** 1996. Elevated blood pressures in mice lacking endothelial nitric oxide synthase. Proc. Natl. Acad. Sci. U. S. A. **93:**13176–13181. http://dx.doi.org/10.1073/pnas.93.23.13176.
23. **Razani B, Engelman JA, Wang XB, Schubert W, Zhang XL, Marks CB, Macaluso F, Russell RG, Li M, Pestell RG, Di Vizio D, Hou H, Jr, Kneitz B, Lagaud G, Christ GJ, Edelmann W, Lisanti MP.** 2001. Caveolin-1 null mice are viable but show evidence of hyperproliferative and vascular abnormalities. J. Biol. Chem. **276:**38121–38138. http://dx.doi.org/10.1074/jbc.M105408200.
24. **Poltorak A, He X, Smirnova I, Liu MY, Van Huffel C, Du X, Birdwell D, Alejos E, Silva M, Galanos C, Freudenberg M, Ricciardi-Castagnoli P, Layton B, Beutler B.** 1998. Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. Science **282:**2085–2088. http://dx.doi.org/10.1126/science.282.5396.2085.
25. **Braz JC, Gregory K, Pathak A, Zhao W, Sahin B, Klevitsky R, Kimball TF, Lorenz JN, Nairn AC, Liggett SB, Bodi I, Wang S, Schwartz A, Lakatta EG, DePaoli-Roach AA, Robbins J, Hewett TE, Bibb JA, Westfall MV, Kranias EG, Molkentin JD.** 2004. PKC-alpha regulates cardiac contractility and propensity toward heart failure. Nat. Med. **10:**248–254. http://dx.doi.org/10.1038/nm1000.
26. **West RE, Jr, Moss J, Vaughan M, Liu T, Liu TY.** 1985. Pertussis toxin-catalyzed ADP-ribosylation of transducin. Cysteine 347 is the ADP-ribose acceptor site. J. Biol. Chem. **260:**14428–14430.
27. **Abeliovich A, Chen C, Goda Y, Silva AJ, Stevens CF, Tonegawa S.** 1993. Modified hippocampal long-term potentiation in PKC gamma-mutant mice. Cell **75:**1253–1262. http://dx.doi.org/10.1016/0092-8674(93)90613-U.
28. **Wettschureck N, Offermanns S.** 2005. Mammalian G proteins and their cell type specific functions. Physiol. Rev. **85:**1159–1204. http://dx.doi.org/10.1152/physrev.00003.2005.
29. **Wilkie TM, Gilbert DJ, Olsen AS, Chen XN, Amatruda TT, Korenberg JR, Trask BJ, de Jong P, Reed RR, Simon MI, Jenkins NA, Copeland NG.** 1992. Evolution of the mammalian G protein alpha subunit multigene family. Nat. Genet. **1:**85–91. http://dx.doi.org/10.1038/ng0592-85.
30. **Jiang M, Gold MS, Boulay G, Spicher K, Peyton M, Brabet P, Srinivasan Y, Rudolph U, Ellison G, Birnbaumer L.** 1998. Multiple neurological abnormalities in mice deficient in the G protein Go. Proc. Natl. Acad. Sci. U. S. A. **95:**3269–3274. http://dx.doi.org/10.1073/pnas.95.6.3269.
31. **Jiang M, Spicher K, Boulay G, Martin-Requero A, Dye CA, Rudolph U, Birnbaumer L.** 2002. Mouse gene knockout and knockin strategies in application to alpha subunits of Gi/Go family of G proteins. Methods Enzymol. **344:**277–298. http://dx.doi.org/10.1016/S0076-6879(02)44721-0.
32. **Pineda VV, Athos JI, Wang H, Celver J, Ippolito D, Boulay G, Birnbaumer L, Storm DR.** 2004. Removal of G(ialpha1) constraints on adenylyl cyclase in the hippocampus enhances LTP and impairs memory formation. Neuron **41:**153–163. http://dx.doi.org/10.1016/S0896-6273(03)00813-4.
33. **Rudolph U, Finegold MJ, Rich SS, Harriman GR, Srinivasan Y, Brabet P, Boulay G, Bradley A, Birnbaumer L.** 1995. Ulcerative colitis and adenocarcinoma of the colon in G alpha i2-deficient mice. Nat. Genet. **10:**143–150. http://dx.doi.org/10.1038/ng0695-143.
34. **Butterfield RJ, Sudweeks JD, Blankenhorn EP, Korngold R, Marini JC, Todd JA, Roper RJ, Teuscher C.** 1998. New genetic loci that control susceptibility and symptoms of experimental allergic encephalomyelitis in inbred mice. J. Immunol. **161:**1860–1867.
35. **Blankenhorn EP, Butterfield R, Case LK, Wall EH, del Rio R, Diehl SA, Krementsov DN, Saligrama N, Teuscher C.** 2011. Genetics of experimental allergic encephalomyelitis supports the role of T helper cells in multiple sclerosis pathogenesis. Ann. Neurol. **70:**887–896. http://dx.doi.org/10.1002/ana.22642.
36. **Wardlaw AC.** 1970. Inheritance of responsiveness to pertussis HSF in mice. Int. Arch. Allergy Appl. Immunol. **38:**573–589. http://dx.doi.org/10.1159/000230313.
37. **Weydert A, Cloez-Tayarani I, Fillion MP, Simon-Chazottes D, Guenet JL, Fillion G.** 1992. Molecular cloning of two partial serotonin 5-HT1D receptor sequences in mouse and one in guinea pig. C. R. Acad. Sci. III **314:**429–435.
38. **Das S, Kumar KN.** 1995. Nitric oxide: its identity and role in blood pressure control. Life Sci. **57:**1547–1556. http://dx.doi.org/10.1016/0024-3205(95)02130-B.
39. **Francis SH, Corbin JD.** 1999. Cyclic nucleotide-dependent protein kinases: intracellular receptors for cAMP and cGMP action. Crit. Rev. Clin. Lab Sci. **36:**275–328. http://dx.doi.org/10.1080/10408369991239213.
40. **Hussain MB, Hobbs AJ, MacAllister RJ.** 1999. Autoregulation of nitric oxide-soluble guanylate cyclase-cyclic GMP signalling in mouse thoracic aorta. Br. J. Pharmacol. **128:**1082–1088. http://dx.doi.org/10.1038/sj.bjp.0702874.
41. **Lin S, Fagan KA, Li KX, Shaul PW, Cooper DM, Rodman DM.** 2000. Sustained endothelial nitric-oxide synthase activation requires capacitative Ca^{2+} entry. J. Biol. Chem. **275:**17979–17985. http://dx.doi.org/10.1074/jbc.275.24.17979.
42. **Bongers G, de Esch I, Leurs R.** 2010. Molecular pharmacology of the four histamine receptors. Adv. Exp. Med. Biol. **709:**11–19. http://dx.doi.org/10.1007/978-1-4419-8056-4_2.
43. **Hill SJ, Ganellin CR, Timmerman H, Schwartz JC, Shankley NP, Young JM, Schunack W, Levi R, Haas HL.** 1997. International Union of Pharmacology. XIII. Classification of histamine receptors. Pharmacol. Rev. **49:**253–278.
44. **Leurs R, Brozius MM, Jansen W, Bast A, Timmerman H.** 1991. Histamine H1-receptor-mediated cyclic GMP production in guinea-pig lung tissue is an L-arginine-dependent process. Biochem. Pharmacol. **42:**271–277. http://dx.doi.org/10.1016/0006-2952(91)90713-F.
45. **Morais C, Ebrahem Q, Anand-Apte B, Parat MO.** 2012. Altered angiogenesis in caveolin-1 gene-deficient mice is restored by ablation of endothelial nitric oxide synthase. Am. J. Pathol. **180:**1702–1714. http://dx.doi.org/10.1016/j.ajpath.2011.12.018.
46. **Mellor H, Parker PJ.** 1998. The extended protein kinase C superfamily. Biochem. J. **332**(Pt 2)**:**281–292.

47. **Lei MG, Morrison DC.** 1993. Evidence that lipopolysaccharide and pertussis toxin bind to different domains on the same p73 receptor on murine splenocytes. Infect. Immun. **61:**1359–1364.
48. **Kerfoot SM, Long EM, Hickey MJ, Andonegui G, Lapointe BM, Zanardo RC, Bonder C, James WG, Robbins SM, Kubes P.** 2004. TLR4 contributes to disease-inducing mechanisms resulting in central nervous system autoimmune disease. J. Immunol. **173:**7070–7077. http://www.jimmunol.org/content/173/11/7070.long.
49. **Bache C, Spreitzer I, Becker B, Loeschner B, Rosskopf U, Hanschmann KM, Schwanig M, Schneider CK, Lieb B, Montag T.** 2012. Bordetella pertussis toxin does not induce the release of pro-inflammatory cytokines in human whole blood. Med. Microbiol. Immunol. **201:**327–335. http://dx.doi.org/10.1007/s00430-012-0238-1.
50. **Malyala P, Singh M.** 2008. Endotoxin limits in formulations for preclinical research. J. Pharm. Sci. **97:**2041–2044. http://dx.doi.org/10.1002/jps.21152.
51. **Lu C, Pelech S, Zhang H, Bond J, Spach K, Noubade R, Blankenhorn EP, Teuscher C.** 2008. Pertussis toxin induces angiogenesis in brain microvascular endothelial cells. J. Neurosci. Res. **86:**2624–2640. http://dx.doi.org/10.1002/jnr.21716.
52. **Katada T, Ui M.** 1982. Direct modification of the membrane adenylate cyclase system by islet-activating protein due to ADP-ribosylation of a membrane protein. Proc. Natl. Acad. Sci. U. S. A. **79:**3129–3133. http://dx.doi.org/10.1073/pnas.79.10.3129.
53. **Linthicum DS, Munoz JJ, Blaskett A.** 1982. Acute experimental autoimmune encephalomyelitis in mice. I Adjuvant action of Bordetella pertussis is due to vasoactive amine sensitization and increased vascular permeability of the central nervous system. Cell. Immunol. **73:**299–310.
54. **Abbott NJ.** 2000. Inflammatory mediators and modulation of blood-brain barrier permeability. Cell. Mol. Neurobiol. **20:**131–147. http://dx.doi.org/10.1023/A:1007074420772.
55. **Kalesnikoff J, Galli SJ.** 2008. New developments in mast cell biology. Nat. Immunol. **9:**1215–1223. http://dx.doi.org/10.1038/ni.f.216.
56. **Becker M, Reuter S, Friedrich P, Doener F, Michel A, Bopp T, Klein M, Schmitt E, Schild H, Radsak MP, Echtenacher B, Taube C, Stassen M.** 2011. Genetic variation determines mast cell functions in experimental asthma. J. Immunol. **186:**7225–7231. http://dx.doi.org/10.4049/jimmunol.1100676.
57. **Kitamura Y, Go S, Hatanaka K.** 1978. Decrease of mast cells in W/Wv mice and their increase by bone marrow transplantation. Blood **52:**447–452.
58. **Munoz J, Bergman RK.** 1968. Histamine-sensitizing factors from microbial agents, with special reference to *Bordetella pertussis*. Bacteriol. Rev. **32:**103–126.
59. **Oh H, Joung J, Kim BG, Nam KT, Hong SH, Song HC, Lee HL, Ahn BY.** 2012. Improved protocols for histamine sensitization testing of acellular pertussis vaccines. Vaccine **30:**7246–7252. http://dx.doi.org/10.1016/j.vaccine.2012.10.005.
60. **Danson EJ, Zhang YH, Sears CE, Edwards AR, Casadei B, Paterson DJ.** 2005. Disruption of inhibitory G-proteins mediates a reduction in atrial beta-adrenergic signaling by enhancing eNOS expression. Cardiovasc. Res. **67:**613–623. http://dx.doi.org/10.1016/j.cardiores.2005.04.034.
61. **Camerer E, Regard JB, Cornelissen I, Srinivasan Y, Duong DN, Palmer D, Pham TH, Wong JS, Pappu R, Coughlin SR.** 2009. Sphingosine-1-phosphate in the plasma compartment regulates basal and inflammation-induced vascular leak in mice. J. Clin. Invest. **119:**1871–1879. http://dx.doi.org/10.1172/JCI38575.
62. **Kim SF, Huang AS, Snowman AM, Teuscher C, Snyder SH.** 2007. From the cover: antipsychotic drug-induced weight gain mediated by histamine H1 receptor-linked activation of hypothalamic AMP-kinase. Proc. Natl. Acad. Sci. U. S. A. **104:**3456–3459. http://dx.doi.org/10.1073/pnas.0611417104.
63. **Parsons ME, Ganellin CR.** 2006. Histamine and its receptors. Br. J. Pharmacol. **147**(Suppl 1)**:**S127–S135. http://dx.doi.org/10.1038/sj.bjp.0706704.
64. **Girouard H, Bonev AD, Hannah RM, Meredith A, Aldrich RW, Nelson MT.** 2010. Astrocytic endfoot Ca^{2+} and BK channels determine both arteriolar dilation and constriction. Proc. Natl. Acad. Sci. U. S. A. **107:**3811–3816. http://dx.doi.org/10.1073/pnas.0914722107.
65. **Theoharides TC, Kalogeromitros D.** 2006. The critical role of mast cells in allergy and inflammation. Ann. N. Y. Acad. Sci. **1088:**78–99. http://dx.doi.org/10.1196/annals.1366.025.
66. **Bergman RK, Munoz J.** 1968. Efficacy of beta-adrenergic blocking agents in inducing histamine sensitivity in mice. Nature **217:**1173–1174. http://dx.doi.org/10.1038/2171173a0.
67. **Hein L.** 2008. Alpha adrenergic system, p 42–45. *In* Offermanns S, Rosenthal W (ed), Encyclopedia of molecular pharmacology, 2nd ed. Springer, Berlin, Germany.
68. **Korhonen H, Fisslthaler B, Moers A, Wirth A, Habermehl D, Wieland T, Schutz G, Wettschureck N, Fleming I, Offermanns S.** 2009. Anaphylactic shock depends on endothelial Gq/G11. J. Exp. Med. **206:**411–420. http://dx.doi.org/10.1084/jem.20082150.
69. **Lovenberg TW, Roland BL, Wilson SJ, Jiang X, Pyati J, Huvar A, Jackson MR, Erlander MG.** 1999. Cloning and functional expression of the human histamine H3 receptor. Mol. Pharmacol. **55:**1101–1107.
70. **Teuscher C, Subramanian M, Noubade R, Gao JF, Offner H, Zachary JF, Blankenhorn EP.** 2007. Central histamine H3 receptor signaling negatively regulates susceptibility to autoimmune inflammatory disease of the CNS. Proc. Natl. Acad. Sci. U. S. A. **104:**10146–10151. http://dx.doi.org/10.1073/pnas.0702291104.
71. **Beghdadi W, Porcherie A, Schneider BS, Morisset S, Dubayle D, Peronet R, Dy M, Louis J, Arrang JM, Mecheri S.** 2009. Histamine H(3) receptor-mediated signaling protects mice from cerebral malaria. PLoS One **4:**e6004. http://dx.doi.org/10.1371/journal.pone.0006004.
72. **Pero RS, Borchers MT, Spicher K, Ochkur SI, Sikora L, Rao SP, Abdala-Valencia H, O'Neill KR, Shen H, McGarry MP, Lee NA, Cook-Mills JM, Sriramarao P, Simon MI, Birnbaumer L, Lee JJ.** 2007. Galphai2-mediated signaling events in the endothelium are involved in controlling leukocyte extravasation. Proc. Natl. Acad. Sci. U. S. A. **104:**4371–4376. http://dx.doi.org/10.1073/pnas.0700185104.
73. **Ransohoff RM.** 2009. Chemokines and chemokine receptors: standing at the crossroads of immunobiology and neurobiology. Immunity **31:**711–721. http://dx.doi.org/10.1016/j.immuni.2009.09.010.
74. **Han SB, Moratz C, Huang NN, Kelsall B, Cho H, Shi CS, Schwartz O, Kehrl JH.** 2005. Rgs1 and Gnai2 regulate the entrance of B lymphocytes into lymph nodes and B cell motility within lymph node follicles. Immunity **22:**343–354. http://dx.doi.org/10.1016/j.immuni.2005.01.017.
75. **Hwang IY, Park C, Kehrl JH.** 2007. Impaired trafficking of $Gnai2^{+/-}$ and $Gnai2^{-/-}$ T lymphocytes: implications for T cell movement within lymph nodes. J. Immunol. **179:**439–448. http://http//www.jimmunol.org/content/179/1/439.
76. **Huang TT, Zong Y, Dalwadi H, Chung C, Miceli MC, Spicher K, Birnbaumer L, Braun J, Aranda R.** 2003. TCR-mediated hyperresponsiveness of autoimmune Galphai2(−/−) mice is an intrinsic naive CD4(+) T cell disorder selective for the Galphai2 subunit. Int. Immunol. **15:**1359–1367. http://dx.doi.org/10.1093/intimm/dxg135.
77. **Thompson BD, Jin Y, Wu KH, Colvin RA, Luster AD, Birnbaumer L, Wu MX.** 2007. Inhibition of G alpha i2 activation by G alpha i3 in CXCR3-mediated signaling. J. Biol. Chem. **282:**9547–9555. http://dx.doi.org/10.1074/jbc.M610931200.
78. **Bartholomäus I, Kawakami N, Odoardi F, Schlager C, Miljkovic D, Ellwart JW, Klinkert WE, Flugel-Koch C, Issekutz TB, Wekerle H, Flugel A.** 2009. Effector T cell interactions with meningeal vascular structures in nascent autoimmune CNS lesions. Nature **462:**94–98. http://dx.doi.org/10.1038/nature08478.
79. **Reboldi A, Coisne C, Baumjohann D, Benvenuto F, Bottinelli D, Lira S, Uccelli A, Lanzavecchia A, Engelhardt B, Sallusto F.** 2009. C-C chemokine receptor 6-regulated entry of TH-17 cells into the CNS through the choroid plexus is required for the initiation of EAE. Nat. Immunol. **10:**514–523. http://dx.doi.org/10.1038/ni.1716.
80. **Witt MA, Arias L, Katz PH, Truong ET, Witt DJ.** 2013. Reduced risk of pertussis among persons ever vaccinated with whole cell pertussis vaccine compared to recipients of acellular pertussis vaccines in a large US cohort. Clin. Infect. Dis. **56:**1248–1254. http://dx.doi.org/10.1093/cid/cit046.
81. **Fan H, Zingarelli B, Peck OM, Teti G, Tempel GE, Halushka PV, Spicher K, Boulay G, Birnbaumer L, Cook JA.** 2005. Lipopolysaccharide- and Gram-positive bacteria-induced cellular inflammatory responses: role of heterotrimeric Galpha (i) proteins. Am. J. Physiol. Cell Physiol. **289:**C293–C301. http://dx.doi.org/10.1152/ajpcell.00394.2004.