

Online supplement Table 1. Effects of NGF on the total histamine contents or amounts of histamine released due to stimulation by calcium ionophore, A23187, in NGF-cultured mast cells^a.

Histamine release (ng/1 x 10 ⁶ cells)								
Total			A23187 (M)					
			0.1			0.5		
HMC-1 cells								
(-) NGF	330.3	14.28		217.5	2.42 (65.8) ^c	255.7	5.13	(77.4) ^c
(+) NGF	458.7	6.93 (38.9) ^{b,**}		262.7	5.12 (57.3) ^{c,*}	348.5	17.23 (75.0) ^{c,**}	
BMMCs								
(-) NGF	659.2	11.21		85.7	16.96 (13.0) ^c	210.7	43.42 (32.0) ^c	
(+) NGF	1,005.7	24.73 (52.6) ^{b,***}		140.8	0.50 (14.0) ^{c,**}	314.0	38.37 (31.2) ^{c,**}	

^aHMC-1 cells or BMMCs (1 x 10⁶ cells) cultured in NGF (10 ng/ml) for 10 days or for 5 weeks were lysed with 0.4% triton X-100 or stimulated by A23187 (0.1 or 0.3 M) as described in “Materials and Methods”. Histamine was determined using an automated fluorometer. (-) NGF or (+) NGF, cells cultured without or with NGF, respectively.

^bParentheses represent percentage increased in NGF-cultured cells, compared to control media. ^cParentheses represent percentage released by A23187, compared to total contents. Spontaneous histamine release for HMC-1 cells or for BMMCs was 8–12% and 2–5% of total histamine content, respectively. **P*<0.05; ***P*<0.01; ****P*<0.01 compared with control media.

Online supplement Table 2. Effects of NGF on the total content and amounts released of tryptase due to stimulation by calcium ionophore (A23187) in NGF-cultured HMC-1 cells^a.

	Tryptase (ng/1 x 10 ⁶ cells)					
	Total		A23187 (M)			
			0.1		0.3	
(-) NGF	152	11.8	15	2.1 (9.8) ^c	25	1.8 (16.4) ^c
(+) NGF	204	10.6 (25.5) ^{b,*}	21	1.5 (10.3) ^c	33	2.6 (16.2) ^{c,*}

^aHMC-1 cells (1 x 10⁶ cells) cultured in NGF (10 ng/ml) for 10 days were lysed with 0.4% triton X-100 or stimulated by A23187 (0.1 or 0.3 M) as described in “Materials and Methods”. Tryptase were determined using ELISA. (-) NGF or (+) NGF, cells cultured without or with NGF, respectively. ^bParentheses represent percentage increased in NGF-cultured cells, compared to control media. ^cParentheses represent percentage released by A23187, compared to total contents. Spontaneous tryptase release for (-) NGF was 6.5 ± 2.46 ng/1 x 10⁶cells, for (+) NGF was 6.8 ± 3.60 ng/1 x 10⁶cells (N = 6).

**P*<0.05 compared with (-) NGF.

Online supplement Table 3. Total contents and amounts released of histamine and tryptase in NGF-cultured bone marrow-derived mast cells activated with antigen/antibody reaction^a.

		Histamine (ng/1 × 10 ⁶ cells) ^b			Tryptase (ng/1 × 10 ⁶ cells) ^c		
		Total	HSA-DNP/anti-DNP IgE (10ng/0.1 g) ^d		Total	HSA-DNP/anti-DNP IgE (10ng/0.1 g) ^d	
(-)	644.4 18.45	174.3 14.93 (27.0) ^f			252.2 25.77	44.7 ± 1.79 (17.7) ^f	
(+)	954.7 35.43 (48.2) ^{e,***}	305.5 22.43 (32.0) ^{f,***}			410.9 20.22 (62.7) ^{e,***}	80.1 ± 4.06 (19.5) ^{f,***}	

^aNGF-cultured BMMCs (1 × 10⁶ cells) for 5 weeks were stimulated with HSA-DNP/anti-DNP IgE antibody reaction (10 ng/0.1 g). Histamine contents were analyzed using an automated fluorometer, and tryptase by ELISA as described in “Materials and Methods”.

^bBMMCs cultured with WEHI-3B media. ^cBMMCs cultured with hrIL-3 media. ^dWe used various concentrations of antigen and antibody in BMMCs stimulation, but showed only mediator content in single dose of antigen and antibody. ^eParentheses represent percentage increased in NGF-cultured cells, compared to control. ^fParentheses represent percentage released by HSA-DNP/anti-DNP IgE, compared with total mediator contents. Spontaneous histamine and tryptase releases for (-) NGF were 32.2 ± 2.43 ng and 5.6 ± 0.66 ng/1 × 10⁶ cells; for (+) NGF were 26.1 ± 2.94 ng and 6.5 ± 0.43 ng/1 × 10⁶ cells, respectively (N = 6). ****P*<0.001 compared with (-) NGF. (-) or (+), cells cultured without or with NGF, respectively.

Online supplement Table 4. Total amounts of mediators in NGF-cultured bone marrow-derived mast cells in mice^a.

Week		Histamine ^b		Tryptase ^c
		(ng/1 x 10 ⁶ cells)		(ng/1 x 10 ⁶ cells)
1	(-) NGF	23.4	2.18	- ^d
	(+) NGF	62.3	2.41 (2.7) ^{e, **}	-
2	(-) NGF	360.5	6.97	-
	(+) NGF	736.7	9.38 (2.0) [*]	-
3	(-) NGF	632.5	21.53	198.9 ± 18.91
	(+) NGF	986.3	40.41 (1.6) [*]	344.2 ± 23.99 (1.7) [*]
4	(-) NGF	652.7	5.54	225.5 ± 28.90
	(+) NGF	1019.2	33.05 (1.6) [*]	351.9 ± 16.06 (1.6) [*]
5	(-) NGF	662.9	5.82	252.2 ± 25.77
	(+) NGF	1060.8	20.20 (1.6) [*]	410.9 ± 10.22 (1.6) ^{d,*}

^aBMMCs (1 x 10⁶ cells) cultured in absence or presence of 10 ng/ml NGF were lysed with 0.4% triton X-100 at each week culture as described in “Materials and Methods”. Total histamine contents were analyzed using an automated fluorometer analyzer, and tryptase using ELISA method. ^bBMMCs cultured with WEHI-3B media. ^cBMMCs cultured with media containing hrIL-3. ^dno detection. ^eParentheses represent an increased ratio by NGF treatment. Total histamine contents for BMMCs cultured with hrIL-3 media were checked, but not shown to avoid complexity because they were gradually increased in the same way as tryptase and TNF- contents. ^{*}*P*<0.05; ^{**}*P*<0.01 compared with (-) NGF. (-)NGF or (+) NGF, cells cultured without or with NGF (10 ng/ml), respectively.

Online supplement Table 5. Effects of transfections or inhibitors on total histamine and tryptase contents in NGF-cultured HMC-1 cells^a.

	Total histamine (ng/1 x 10 ⁶ cells)		Total tryptase (ng/1 x 10 ⁶ cells)
NGF	453.5	10.34 [39.4] ^b	204.2 ± 10.59 [34.2] ^b
Pyruvate kinase	263.0	6.23 (42.0) ^{c,**}	143.6 ± 8.49 (29.7) ^{**}
Annexin I siRNA	249.4	5.91 (45.0) ^{**}	147.8 ± 15.64 (27.6) ^{**}
co-transfection	185.1	4.40 (59.2) ^{***}	125.1 ± 14.40 (38.7) ^{**}
PD98059 (μM)			
1	443.4	8.2 (2.4)	199.0 ± 13.75 (2.5)
10	387.7	1.42 (14.5) [*]	174.6 ± 9.67 (14.5) [*]
100	210.0	4.57 (53.7) ^{**}	144.5 ± 12.87 (29.2) ^{**}
K252a (nM) ^d			
10	417.1	10.58 (1.5)	200.5 ± 10.58 (1.8)
50	403.6	9.56 (11.0)	184.9 ± 17.02 (9.5)
100	340.6	5.00 (24.9) [*]	161.9 ± 13.54 (20.7) [*]
LY294002 (μM)			
100	448.1	20.42 (1.2)	203.2 ± 22.23 (0.5)
300	447.2	13.46 (1.4)	200.4 ± 10.54 (1.8)
Wortmannin (nM)			
5	422.3	17.23 (3.0)	203.5 ± 6.29 (0.3)
10	431.8	24.40 (1.2)	201.1 ± 22.10 (2.0)

^aHMC-1 cells or transfected HMC-1 cells (1×10^6) were cultured with NGF in WEHI-3B conditioned media for 10 days as described in “Materials and Methods”. Each inhibitor (K252a, PD98059, LY294002 or wortmannin) was added in HMC-1 cells 1 hr before NGF treatment. Histamine or tryptase levels were determined using an automated fluorometer analyzer and ELISA, respectively (N = 6). ^bA square bracket represents increased percentage, compared to control media (325.3 ± 33.54 ng/ 1×10^6 cells for histamine; 152.2 ± 11.77 ng/ml for tryptase). ^cParentheses represent decreased percentage, compared to NGF-cultured cells. ^dK252a could not use higher concentration than 100 nM because K252a induces apoptosis. Histamine and tryptase releases in non-relevant siRNA with NGF was 458.2 ± 5.35 ng/ 1×10^6 cells and 211.3 ± 15.42 ng/ 1×10^6 cells, respectively. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ compared with NGF treatment.