Table 1. Characteristics of patients transplanted in CR1, according to donor type

	Related (n = 310)		Unrelated (n = 331)		
No. of patients	**No.**	**%**	**No.**	**%**	***P***
Median WBC count at diagnosis/μL (range)	10 250 (109-328 000)		11 000 (700-892 000)		.43
Median patient age at allo-SCT, y (range)	30 (16-66)		31 (16-59)		.95
16-20	66	21.3	77	23.3	
21-30	93	30.0	82	24.8	
31-40	71	22.9	86	26.0	
41-50	58	18.7	68	20.5	
51 or older	22	7.1	18	5.4	
Sex					.09
Male	157	50.6	190	57.4	
Female	153	49.4	141	42.6	
Source					< .0001
BM	212	68.4	331	100.0	
PB	98	31.6	0	0.0	
Lineage					.01
T	50	16.1	54	16.3	
B	218	70.3	203	61.3	
Other	42	13.5	74	22.4	
Cytogenetics					.07
Normal	193	62.3	208	62.8	
t(4;11)	11	3.5	5	1.5	
Other MLL/11q23 translocations	1	0.3	3	0.9	
t(1;19)	10	3.2	6	1.8	
t(8;14)	3	1.0	3	0.9	
14q32 translocations	1	0.3	0	0.0	
del(6q)	3	1.0	1	0.3	
del(7p)	2	0.6	1	0.3	
−7*	5	1.6	2	0.6	
+8*	2	0.6	0	0.0	
+X*	0	0.0	1	0.3	
del(9p)	3	1.0	9	2.7	
abnormality of 11q	0	0.0	3	0.9	
del(12p)	2	0.6	1	0.3	
del(13q)/−13	1	0.3	2	0.6	
del(17p)	0	0.0	1	0.3	
Complex	10	3.2	15	4.5	
Low hypodiploidy/near triploidy	2	0.6	0	0.0	
High hyperdiploidy	16	5.2	12	3.6	
Other abnormality (no t(9;22))†	45	14.5	58	17.5	
JALSG risk stratification					.21
Low	39	12.6	45	13.6	
Intermediate	163	52.6	192	58.0	
High	108	34.8	94	28.4	
HLA matching					< .0001
Match	285	91.9	192	58.0	
Class I 1 locus-mismatch	18	5.8	53	16.0	
Class II 1 locus-mismatch	7	2.3	32	9.7	
2 or more loci mismatch	0	0.0	54	16.3	
Time from diagnosis to transplantation, mo (range)	5.7 (1.9-36.6)		10.0 (4.0-43.0)		< .0001
< 6	169	54.5	23	6.9	
6-9	109	35.2	143	43.2	
10 or longer	32	10.3	165	49.8	
Preparative regimen					.004
CY + TBI	140	45.2	156	47.1	
CA + CY + TBI	66	21.3	84	25.4	
BU + CY + TBI	17	5.5	15	4.5	
VP + CY + TBI	23	7.4	32	9.7	
Other TBI myeloablative regimens	39	12.6	32	9.7	
BU + CY	17	5.5	12	3.6	
Other non-TBI myeloablative regimens	8	2.6	0	0.0	
GVHD prophylaxis					< .0001
Cyclosporine A with or without other	283	91.3	171	51.7	
Tacrolimus with or without other	27	8.7	160	48.3	

Table 1. Characteristics of patients transplanted in CR1, according to donor type (continued)

	Related (n = 310)		Unrelated (n = 331)		
No. of patients	**No.**	**%**	**No.**	**%**	***P***
Years of allo-SCT					.26
1993-1997	48	15.5	55	16.6	
1998-2002	132	42.6	120	36.3	
2003-2007	130	41.9	156	47.1	

WBC indicates white blood cell; BM, bone marrow; PB, peripheral blood; related HLA match, identical HLA-A, -B, and -DRB1 loci; unrelated HLA match, HLA-A, -B, -Cw, and -DRB1 loci; HLA mismatch, at least one disparity at one of these loci; CY, cyclophosphamide; TBI, total body irradiation; CA, cytarabine; BU, busulfan; and VP, etoposide.

*These groups exclude cases with low hypodiploidy and high hyperdiploidy.

†Abnormal karyotypes excluding those with any of the aforementioned abnormalities.

Among evaluable patients who survived at least 100 days after allo-SCT, no significant difference was observed between related and unrelated allo-SCTs in the incidence of chronic GVHD (related vs unrelated: 41% vs 41% at 2 years; $P = .76$). Extensive disease was observed in 60 (55%) of 109 with chronic GVHD after related allo-SCT and in 80 (74%) of 118 after unrelated allo-SCT ($P = .048$).

Causes of death among patients transplanted in CR1

Although relapse was the leading cause of death in both related and unrelated allo-SCTs, the proportion of relapse was significantly lower in those transplanted from unrelated donors ($P = .01$). Infection, GVHD, and organ failure were the major causes of NRM, and the incidence of interstitial pneumonia was higher in patients transplanted from unrelated donors ($P = .06$; Table 5).

Discussion

This study reports the largest series of adult Ph⁻ ALL patients who underwent allo-SCT. There was no significant survival difference between related and unrelated allo-SCTs in any disease stage. Among patients who underwent a related allo-SCT in CR1, a shorter interval from diagnosis to allo-SCT was associated with relapse, and age at allo-SCT was associated with NRM. On the other hand, among patients who underwent an unrelated allo-SCT, abnormal karyotype was associated with both relapse and NRM, and a longer interval from diagnosis to allo-SCT and HLA mismatch were associated with NRM. These results indicated that factors affecting transplantation outcomes were different according to donor type.

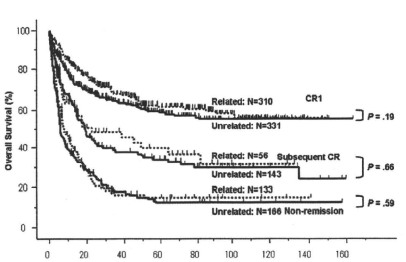

Figure 1. OS according to disease status and donor type. OS rates were significantly superior among patients transplanted in CR1. There was no significant difference between related and unrelated allo-SCTs (related vs unrelated: 65% vs 62% in CR1, $P = .19$; 44% vs 38% in subsequent CR, $P = .66$; and 17% vs 16% in nonremission, $P = .59$; respectively).

In our study, unrelated allo-SCT resulted in OS rates similar to those from related allo-SCT, which was compatible with the result of one prospective study for standard-risk hematologic malignancies.[14] The rates of OS, relapse, and NRM among patients who underwent a related allo-SCT in CR1 were 65%, 32%, and 14%, respectively, which were compatible with those observed in the United Kingdom Medical Research Council UKALL XII/Eastern Cooperative Oncology Group E2993 trial (53%, 24%, and 18%, respectively).[3] Some patients were transplanted from a 1-locus mismatched related donor because it was reported that the outcome of allo-SCT from a 1 locus-mismatched related donor was similar to that of matched unrelated allo-SCT in the Japanese population.[15] On the other hand, the rates of OS, relapse, and NRM among patients who underwent an unrelated allo-SCT were 62%, 22%, and 27%, respectively, which were better than those reported from the Center for International Blood and Marrow Transplant Research (39%, 20%, and 42%, respectively).[4] These differences in NRM could be explained by the lower incidence of acute GVHD in our population, which possibly resulted from the genetic homogeneity in the Japanese population.[16,17] Interestingly, abnormal karyotype was associated with NRM. This could be explained by the possibility that patients with abnormal karyotype received intensive chemotherapy before allo-SCT because of persistent minimal residual disease, which might result in increased NRM rates. Another possibility is that rapid taper of immunosuppressive treatment might also cause GVHD leading to NRM.

In this study, NRM rates were higher in unrelated allo-SCT compared with related allo-SCT, whereas comparable NRM rates were reported in some recent reports,[18] suggesting that NRM rates after unrelated allo-SCT could be reduced with further efforts, such as better HLA matching. Because HLA-C was not routinely typed before 2003, most of the HLA-C data in this study were examined retrospectively, revealing that considerable numbers of patients had received class I allele-mismatched unrelated allo-SCT. Better HLA matching might reduce NRM after unrelated allo-SCT in the future. Although slower hematopoietic recovery after bone marrow transplantation compared with peripheral blood stem cell transplantation might affect the timing of deaths, there was no statistical difference in early mortality between the grafts (data not shown).

There was no statistical difference in the incidence of chronic GVHD between related and unrelated allo-SCTs, although acute GVHD was observed more frequently in unrelated allo-SCT. This was compatible with a past report in which the incidence of chronic GVHD was similar between related and unrelated allo-SCTs, whereas acute GVHD was observed more frequently in related allo-SCT.[14]

It was noteworthy that the interval from diagnosis to allo-SCT revealed a different effect on related and unrelated allo-SCTs. In Japanese clinical practice, the JALSG protocols have been common, where 1.5-month induction chemotherapy was followed by

Table 2. Univariate and multivariate analyses of factors influencing OS among patients transplanted in CR1, according to donor type

	Related (n = 310)					Unrelated (n = 331)				
		Univariate		Multivariate			Univariate		Multivariate	
Covariates	N	HR (95% CI)	P	HR (95% CI)	P	N	HR (95% CI)	P	HR (95% CI)	P
WBC count at diagnosis										
< 30 000/μL	224	1.00		—		230	1.00		—	
30 000/μL or more at diagnosis	86	1.19 (0.78-1.82)	.42	—		101	0.83 (0.56-1.25)	.38	—	
Lineage										
B	218	1.00		—		203	1.00		—	
T	50	0.73 (0.34-1.77)	.52	—		54	0.81 (0.44-1.48)	.35	—	
Other	42	0.94 (0.54-1.64)	.84	—		74	1.08 (0.70-1.67)	.72	—	
Karyotype										
Normal	193	1.00		—		208	1.00		—	
t(4;11) or t(1;19)	21	0.51 (0.14-1.54)	.19	—		11	1.49 (0.54-4.09)	.44	1.59 (0.58-4.36)	.37
Other (no t(9;22))	96	1.03 (0.67-7.14)	.89	—		112	1.49 (1.03-2.17)	.04	1.43 (1.13-2.40)	.01
JALSG risk stratification										
Low	39	1.00		—		45	1.00		—	
Intermediate	163	1.36 (0.87-2.12)	.18	—		192	1.06 (0.71-1.59)	.77	—	
High	108	1.77 (0.95-3.31)	.07	—		94	1.02 (0.56-1.88)	.94	—	
Age at allo-SCT										
< 45 y old	255	1.00		—		281	1.00		—	
45 y old or older at allo-SCT	55	2.04 (1.30-3.13)	.002	2.13 (1.36-3.34)	.0009	50	1.05 (0.63-1.73)	.86		
HLA										
Match	285	1.00		—		192	1.00		—	
Mismatch	25	0.95 (0.46-1.96)	.90	—		139	1.44 (1.01-2.06)	.04	1.45 (1.01-2.07)	.04
Stem cell source										
Bone marrow	212	1.00		—					—	
Peripheral blood	98	1.43 (0.94-2.13)	.09	1.40 (0.93-2.11)	.11					
Time from diagnosis to allo-SCT										
6 mo or longer	169	1.00		—		23	1.00		—	
< 6 mo	141	1.75 (1.16-2.63)	.007	1.80 (1.19-2.71)	.005	308	0.33 (0.10-1.04)	.06	—	
< 10 mo	278	1.00		—		166	1.00		—	
10 mo or longer	32	0.56 (0.26-1.20)	.14	—		165	1.54 (1.07-2.21)	.02	1.62 (1.12-2.34)	.01
Preparative regimen										
Non-TBI regimens	25	1.00		—		12	1.00		—	
TBI regimens	285	0.72 (0.38-1.35)	.30	—		319	0.59 (0.27-1.26)	.17	—	
GVHD prophylaxis										
Cyclosporine A with or without other	283	1.00		—		171	1.00		—	
Tacrolimus with or without other	27	2.02 (1.15-3.56)	.01	—		160	1.38 (0.96-1.97)	.08	—	

HR indicates hazard ratio; CI, confidence interval; WBC, white blood cell; —, not applicable; and TBI, total body irradiation.

6-month consolidation chemotherapy and 16-month maintenance chemotherapy.[8] Therefore, a shorter interval from diagnosis to allo-SCT, which was more common in related cases, might result in insufficient consolidation chemotherapy and worse survival because of increased relapse rates in related allo-SCT. Alternatively, effects from insufficient consolidation chemotherapy might be more prominent in related allo-SCT because graft-versus-leukemia effects might be weaker after related allo-SCT than unrelated allo-SCT.[19] On the other hand, a longer interval from diagnosis to allo-SCT, which was more common in unrelated cases, might result in the cumulative toxic sequelae of chemotherapy responsible for interstitial pneumonia indicated in the past reports.[20-25] Because the JALSG protocols do not define the timing of allo-SCT, it is possible that chemotherapy before allo-SCT might be prolonged because of persistent minimal residual disease. However, we could not confirm this because there were no data concerning minimal residual disease in the registry database.

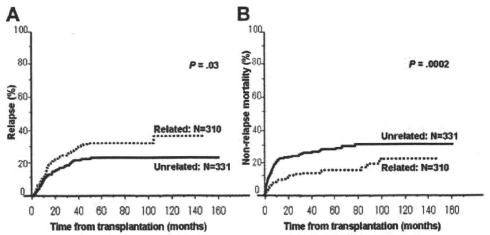

Figure 2. Cumulative incidence of relapse and NRM in patients transplanted in CR1 according to donor type. (A) Cumulative incidence of relapse among patients transplanted in CR1 was significantly higher in patients who underwent a related allo-SCT compared with those who underwent an unrelated allo-SCT (related vs unrelated: 32% vs 22% at 4 years, $P = .03$). (B) Cumulative incidence of NRM among patients transplanted in CR1 was significantly higher in patients who underwent an unrelated allo-SCT compared with those who underwent a related allo-SCT (related vs unrelated: 14% vs 27% at 4 years, $P = .0002$).

Table 3. Univariate and multivariate analyses of factors influencing relapse among patients transplanted in CR1, according to donor type

	Related (n = 310)					Unrelated (n = 331)				
		Univariate		Multivariate			Univariate		Multivariate	
Covariates	N	HR (95% CI)	P	HR (95% CI)	P	N	HR (95% CI)	P	HR (95% CI)	P
WBC count at diagnosis										
< 30 000/μL	224	1.00		—		230	1.00		—	
30 000/μL or more at diagnosis	86	0.88 (0.52-1.47)	.62	—		101	1.11 (0.62-1.98)	.72	—	
Lineage										
B	218	1.00		—		203	1.00		—	
T	50	0.54 (0.22-1.37)	.09	—		54	1.31 (0.57-3.02)	.62	—	
Other	42	1.21 (0.66-2.21)	.54	—		74	1.06 (0.53-2.11)	.87	—	
Karyotype										
Normal	193	1.00		—		208	1.00		—	
t(4;11) or t(1;19)	21	0.64 (0.19-2.12)	.36	—		11	1.97 (0.46-8.35)	.91	—	
Other (no t(9;22))	96	1.11 (0.68-1.82)	.67	—		112	2.15 (1.24-3.73)	.01	2.15 (1.24-3.73)	.01
JALSG risk stratification										
Low	39	1.00		—		45	1.00		—	
Intermediate	163	0.96 (0.59-1.55)	.87	—		192	1.04 (0.57-1.91)	.90	—	
High	108	0.81 (0.35-1.84)	.61	—		94	1.04 (0.43-2.52)	.94	—	
Age at allo-SCT										
< 45 y old	255	1.00		—		281	1.00		—	
45 y old or older at allo-SCT	55	0.82 (0.41-1.64)	.57	—		50	0.74 (0.42-1.32)	.08	—	
HLA										
Match	285	1.00		—		192	1.00		—	
Mismatch	25	0.82 (0.33-2.02)	.66	—		139	0.74 (0.42-1.32)	.31	—	
Stem cell source*										
Bone marrow	212	1.00		—					—	
Peripheral blood	98	1.07 (0.65-1.76)	.79	—					—	
Time from diagnosis to allo-SCT										
6 mo or longer	169	1.00		—		23	1.00		—	
< 6 mo	141	1.68 (1.05-2.69)	.03	1.68 (1.05-2.69)	.03	308	0.47 (0.11-1.92)	.29	—	
< 10 mo	278	1.00		—		166	1.00		—	
10 mo or longer	32	0.49 (0.18-1.34)	.16	—		165	0.92 (0.54-1.58)	.76	—	
Preparative regimen										
Non-TBI regimens	25	1.00		—		12	1.00		—	
TBI regimens	285	0.62 (0.31-1.25)	.18	—		319	0.47 (0.15-1.52)	.21	—	
GVHD prophylaxis										
Cyclosporine A with or without other	283	1.00		—		171	1.00		—	
Tacrolimus with or without other	27	1.62 (0.81-3.26)	.18	—		160	1.39 (0.81-2.38)	.24	—	

HR indicates hazard ratio; CI, confidence interval; WBC, white blood cell; —, not applicable; and TBI, total body irradiation.
*Stem cell source (peripheral blood) was not a significant risk factor for relapse in the multivariate analysis.

Although we mainly focused on patients in CR1, our results also indicated that some, but not all, patients with refractory disease could be rescued by allo-SCT. These patients could not have survived long with chemotherapy alone, and complete unresponsiveness, even to allo-SCT, was often assumed. These results were compatible with some reports showing that long-term survival could be achieved for patients receiving allo-SCT, even in refractory disease.[26-28]

Our study has several limitations. First, there might be some selection biases between related and unrelated allo-SCTs. It was possible that eligibility was more stringent in patients who received unrelated allo-SCT, and they might have had better pretransplantation conditions. Second, a time-censoring effect might impact the outcome. The longer interval from diagnosis to unrelated allo-SCT eliminates the effect of patients who die during that period. This bias might improve the outcome of unrelated allo-SCT. Third, we could not make the comparison between chemotherapy and allo-SCT in this study.

The time-censoring effect could be the major bias in this study, which resulted in lower relapse rates, especially in patients transplanted from unrelated donors. We tried to correct this bias by the previously described method.[29] In the JALSG ALL study, it was reported that approximately 80% and 75% of patients were alive 6 months and 10 months after enrollment, respectively.[8] Because 6 months and 10 months were the median interval from diagnosis to related and unrelated allo-SCTs, respectively, a crude way to apply a correction factor for the survival seen in our study is to lower the survival estimate at any given time point by 20% for related allo-SCT and 25% for unrelated allo-SCT, respectively. Thus, the corrected OS rates at 4 years were 52% ± 5% for related allo-SCT and 47% ± 4% for unrelated allo-SCT, which showed no statistical difference between related and unrelated allo-SCTs. Time-censoring effects would not change the results.

The change of transplantation indication for adolescents through the observation period might affect the outcome. In the JALSG protocol ALL202 (from September 2002), we treated patients less than 25 years old with a similar protocol performed for pediatric patients. Because allo-SCT was recommended only for high-risk patients, such as those with t(4;11) or MLL-rearrangement in the pediatric protocol, the outcome of young patients might be affected by the difference in the indication for allo-SCT between pediatric and adult protocols after 2002. However, the effect of this small population would not be so large.

Table 4. Univariate and multivariate analyses of factors influencing NRM among patients transplanted in CR1, according to donor type

	Related (n = 310)					Unrelated (n = 331)				
		Univariate		Multivariate			Univariate		Multivariate	
Covariates	n	HR (95% CI)	P	HR (95% CI)	P	N	HR (95% CI)	P	HR (95% CI)	P
WBC count at diagnosis										
< 30 000/μL	224	1.00		—		230	1.00		—	
30 000/μL or more at diagnosis	86	1.21 (0.63-2.34)	.57	—		101	0.79 (0.48-1.30)	.35	—	
Lineage										
B	218	1.00		—		203	1.00		—	
T	50	1.25 (0.41-3.81)	.53	—		54	0.62 (0.29-1.38)	.17	—	
Other	42	0.87 (0.34-2.26)	.78	—		74	1.08 (0.65-1.81)	.76	—	
Karyotype										
Normal	193	1.00		—		208	1.00		—	
t(4;11) or t(1;19)	21	0.77 (0.16-3.17)	.73	—		11	1.03 (0.25-4.30)	.63	1.11 (0.27-4.64)	.57
Other (no t(9;22))	96	0.92 (0.47-1.81)	.81	—		112	1.47 (0.94-2.29)	.09	1.67 (1.06-2.64)	.03
JALSG risk stratification										
Low	39	1.00		—		45	1.00		—	
Intermediate	163	1.85 (0.86-3.97)	.12	—		192	1.01 (0.62-1.65)	.96	—	
High	108	2.82 (1.09-7.31)	.03	—		94	1.03 (0.50-2.10)	.94	—	
Age at allo-SCT										
< 45 y old	255	1.00		—		281	1.00		—	
45 y old or older at allo-SCT	55	3.90 (2.09-7.25)	< .0001	3.90 (2.09-7.25)	< .0001	50	1.26 (0.72-2.20)		.42	
HLA										
Match	285	1.00		—		192	1.00		—	
Mismatch	25	1.64 (0.64-4.18)	.30	—		139	1.69 (1.10-2.60)	.02	1.69 (1.10-2.61)	.02
Stem cell source										
Bone marrow	212	1.00		—					—	
Peripheral blood	98	1.75 (0.94-3.28)	.08	—					—	
Time from diagnosis to allo-SCT										
6 mo or longer	169	1.00		—		23	1.00		—	
< 6 mo	141	1.64 (0.87-3.11)	.13	—		308	0.31 (0.08-1.25)	.10	—	
< 10 mo	278	1.00		—		166	1.00		—	
10 mo or longer	32	1.07 (0.42-2.72)	.89	—		165	1.90 (1.21-2.99)	.01	1.98 (1.26-3.13)	.003
Preparative regimen										
Non-TBI regimens	25	1.00		—		12	1.00		—	
TBI regimens	285	0.63 (0.25-1.61)	.34	—		319	0.67 (0.25-1.85)	.44	—	
GVHD prophylaxis										
Cyclosporine A with or without other	283	1.00		—		171	1.00		—	
Tacrolimus with or without other	27	1.66 (0.65-3.80)	.29	—		160	1.33 (0.86-2.05)	.52	—	

HR indicates hazard ratio; CI, confidence interval; WBC, white blood cell; —, not applicable; and TBI, total body irradiation.

In conclusion, comparable survival rates were observed between adult Ph⁻ ALL patients who underwent related and unrelated allo-SCTs in CR1, although relapse rates, incidences of NRM, and risk factors for transplantation outcomes were different between them. Better outcomes could be achieved by performing allo-SCT at an appropriate timing and HLA compatibility according to donor type.

Table 5. Causes of death among patients transplanted in CR1, according to donor type

	Related (n = 310)		Unrelated (n = 331)		
	n	%	n	%	P
Relapse	44	44	32	26	.01
Infection	12	12	23	19	.20
Organ failure	12	12	17	14	.83
GVHD	9	8.9	16	13	.40
Interstitial pneumonia	5	5.0	15	12	.06
Hemorrhage	3	3.0	6	5.0	.52
Graft failure	2	2.0	3	2.5	1.0
ARDS	1	1.0	3	2.5	.63
Other	8	7.9	6	5.0	.42
Unknown	5	5.0	0	0.0	.02
Total	101	100	121	100	

ARDS indicates acute respiratory distress syndrome.

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Authorship

Contribution: S.N., Y.I., and K.M. designed the research and wrote the manuscript; S.N. and Y.I. performed the statistical analysis and interpreted the data; H.S., M. Kurokawa, H.I., H.O., T.F., Y.O., N.K., M. Kasai., T.M., K.I., T.Y., M.O., and

K.M. provided the patient data; and K.K., Y.M., R.S., and Y.A. collected the patient data.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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Notch2 signaling is required for proper mast cell distribution and mucosal immunity in the intestine

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Notch2 signaling is required for proper mast cell distribution and mucosal immunity in the intestine

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Notch receptor-mediated signaling is involved in the developmental process and functional modulation of lymphocytes, as well as in mast cell differentiation. Here, we investigated whether Notch signaling is required for antipathogen host defense regulated by mast cells. Mast cells were rarely found in the small intestine of wild-type C57BL/6 mice but accumulated abnormally in the lamina propria of the small-intestinal mucosa of the *Notch2*-conditional knockout mice in naive status. When transplanted into mast cell–deficient W^{sh}/W^{sh} mice, *Notch2*-null bone marrow-derived mast cells were rarely found within the epithelial layer but abnormally localized to the lamina propria, whereas control bone marrow-derived mast cells were mainly found within the epithelial layer. After the infection of *Notch2* knockout and control mice with L3 larvae of *Strongyloides venezuelensis*, the abundant number of mast cells was rapidly mobilized to the epithelial layer in the control mice. In contrast, mast cells were massively accumulated in the lamina propria of the small intestinal mucosa in *Notch2*-conditional knockout mice, accompanied by impaired eradication of *Strongyloides venezuelensis*. These findings indicate that cell-autonomous Notch2 signaling in mast cells is required for proper localization of intestinal mast cells and further imply a critical role of Notch signaling in the host-pathogen interface in the small intestine. (*Blood*. 2011;117(1):128-134)

Introduction

Mast cells are important in a wide variety of physiologic and pathologic processes, including protective immune responses to parasites and allergic disorders.[1,2] In intestinal parasite infection, mast cells play a central role in the immune response.[3] During the induction phase of parasite-induced inflammation, mast cells move from the submucosa to the tip of the villi, accompanying the serial changes in the protease expression pattern. Initially, they are positive for mouse mast cell protease-5 (mMCP-5) but negative for mMCP-1 and mMCP-2; eventually, they become positive for mMCP-1 and mMCP-2 but negative for mMCP-5, demonstrating convergence from connective tissue–type mast cells (CTMCs) to mature mucosal-type mast cells (MTMCs).[4] The parasite-infected mice consequently experience jejunal mast cell hyperplasia,[5] and the serum concentration of mMCP-1, an activation marker of small intestinal mast cells, is increased by $>$ 1000-fold compared with that in the naive status.[5]

In the mammalian immune system, we and other groups have demonstrated that Notch signaling is involved in the commitment and differentiation of T cells, the development of splenic marginal zone B cells, and the differentiation and functional modulation of mature T cells, including T-helper type I (Th1)/Th2 polarization[6,7] and differentiation of CD8-positive cytotoxic T cells.[8] Regarding the Notch signaling in mast cells, bone marrow-derived mast cells (BMMCs) highly express Jagged1[9] and Notch2[10] among the Notch ligands and the receptors, respectively. We have previously shown that signaling through the Notch2 receptor induces mast cell development from myeloid progenitors by transcriptional up-regulation of hairy and enhancer of split homolog-1 (Hes-1) and transacting T cell–specific transcription factor GATA-3 (GATA3).[11] Induction of antigen-presenting potential of mast cells by Notch signaling is also demonstrated.[12] A question yet to be solved is how Notch signaling affects mast cell properties in vivo.

In this report, we examined the effect of Notch2 signaling in in vivo mast cells using *Notch2*-conditional knockout mice.[13] We show that Notch2 signaling is specifically required for intraepithelial localization of intestinal mast cells and antiparasite immunity. In contrast, Notch2 is dispensable for either distribution or development of CTMCs.

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Methods

Mice

The generation of *Notch2*$^{flox/flox}$ mice was described previously.[13] Mx-*Cre* transgenic mice[14] were crossed with *Notch2*$^{flox/flox}$ mice (*N2*-MxcKO mice) and the progeny were injected with polyinosinic-polycytidylic acid (pIpC; Sigma-Aldrich) 7 times every other day from 3 days after birth (25 μg/g body weight) or 3 times between 4 and 6 weeks of age (20 μg/g body weight). *N2*-MxcKO mice were further crossed with C57BL/6-Ly5.1 mice (a kind gift from Dr H. Nakauchi, University of Tokyo) to generate Ly5.1-*N2*-MxcKO mice. *Notch2* deletion in bone marrow was examined by polymerase chain reaction and 3% agarose gel electrophoresis[13] (supplemental Figure 1, available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article). *W*^{sh}/*W*^{sh} mice were purchased from The Jackson Laboratory. All experiments were done with approval from the University of Tsukuba Institutional Review Board.

Staining

Sections, fixed with Carnoid fluid, were stained with 0.5% toluidine blue (Sigma-Aldrich), pH 0.3, followed by eosin. Small intestine was embedded in optimal cutting temperature (OCT) compound (TissueTek) and cut with cryostat (Leica CM1850). The section was fixed with 4% paraformaldehyde, washed with phosphate-buffered saline (PBS), blocked in 10% horse serum and 0.1% Triton-PBS, and then stained with either 1:100 goat anti-Jagged1 antibody (C-20; Santa Cruz Biotechnology), goat anti-Delta1 antibody (Genzyme Tech), or control goat immunoglobulin G (IgG; Santa Cruz Biotechnology) overnight at 4°C. The sections were washed with PBS and stained with anti–goat Alexa 594 (Invitrogen). Sections were analyzed by fluorescence microscope (Zeiss; Axioplan2), original magnification ×200.

BMMCs

Bone marrow cells from each mouse strain were cultured in RPMI 1640 medium (Sigma-Aldrich) supplemented with 10% fetal bovine serum (FBS), 50 ng/mL stem cell factor (SCF; PeproTech), and 10 ng/mL interleukin-3 (IL-3; PeproTech) for 4 weeks. Generation of BMMCs was confirmed by staining with lineage markers, c-Kit and IgE, as previously described.[11] Briefly, the cells were incubated with purified IgE (BD Biosciences) after blocking the Fcγ receptors with purified anti-CD16/32 antibody (BD Biosciences), stained with anti-IgE–fluorescein isothiocyanate (FITC; BD Biosciences), anti–Gr-1–phycoerythrin (PE), anti-Mac1–PE (eBioscience), and anti–c-Kit–allophycocyanin (APC; eBioscience), and then analyzed by FACSCalibur (BD Biosciences).

Peritoneal mast cells

Five milliliters ice-cold PBS was injected into the peritoneal cavity, and then 3 mL PBS was recovered. c-Kit and IgE receptor (FcεRI) expression was used to define the cells as peritoneal mast cells. Ly5.1 and Notch2 were stained with anti-Ly5.1–PE (BD Biosciences) or biotinylated anti-Notch2 antibody (clone HMN2-35)[8] followed by streptavidin PE (eBioscience), respectively.

Bone marrow transplantation

C57BL/6 mice and *W*^{sh}/*W*^{sh} mice were lethally irradiated with a total dose of 9.5 Gy and then transplanted with 1×10^7 whole bone marrow cells from either *N2*-MxcKO-Ly5.1 mice or *Notch2*$^{flox/flox}$-Ly5.1 mice from the tail vein. Tissues of transplanted mice were assessed at 3 to 4 months after transplantation. Donor-cell engraftment was assessed by fluorescence-activated cell sorting (FACS) analysis of peripheral blood, which was stained by anti-Ly5.2–FITC (BD Biosciences) and anti-Ly5.1–PE.

S venezuelensis infection

Mice were infected by subcutaneous injection of third-stage infective larvae of *Strongyloides venezuelensis*. The degree of infection was monitored by counting the number of eggs per gram of feces. Mast cells were counted and presented as the number per 10 villus crypt units. BMMCs were washed with PBS twice and then cultured with 10 ng/mL IL-4 and 10 ng/mL IL-10 for 3 days. These Th2-conditioned BMMCs were injected at day 3 and day 6 of experiments.[15] In contrast to the bone marrow transplantation, mice were not irradiated before BMMC injection.

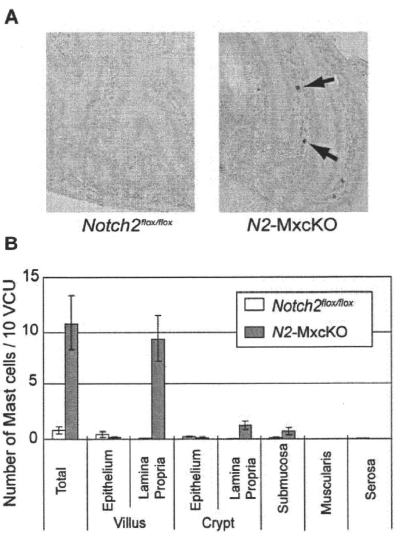

Figure 1. Mature mast cells were abnormally accumulated in the lamina propria of the small intestine of *Notch2*-deficient mice. (A) Sections of the small intestine of *N2*-MxcKO or littermate control *Notch2*$^{flox/flox}$ mice. Toluidine blue staining, followed by eosin. Original magnification ×200. (B) The numbers of mast cells per 10 villus crypt units (vcus) distributing to various layers of the small intestine. Data are presented as means ± SEM; *Notch2*$^{flox/flox}$ ($n = 10$) versus *N2*-MxcKO ($n = 8$); $P = .000461$ (total), $P = .000261$ (villus, lamina propria), $P = .001918$ (crypt, lamina propria), $P = .046874$ (submucosa).

Statistical analysis

The data for the number of mast cells and the *S venezuelensis* infection data were analyzed by the *t* test. *P* values < .05 were considered significant.

Results

Notch signaling affects the number and localization of mast cells in the small intestine

We have previously reported that Notch2 regulates mast cell differentiation in vitro.[11] To examine whether Notch2 controls the differentiation or development of MTMCs in vivo, we examined intestinal mast cells by toluidine blue staining in C57BL/6 mice carrying the *Notch2*$^{flox/flox}$ allele with or without the Mx1-*Cre* transgene (*N2*-MxcKO mice or *Notch2*$^{flox/flox}$ mice, respectively) after pIpC treatment.[13] Mast cells were only sparsely detected in the small intestine of *Notch2*$^{flox/flox}$ mice, mainly within the epithelium. However, the total number of mast cells in the small intestine of *N2*-MxcKO mice was unanticipatedly greater than that of *Notch2*$^{flox/flox}$ mice. Furthermore, those mast cells were mainly

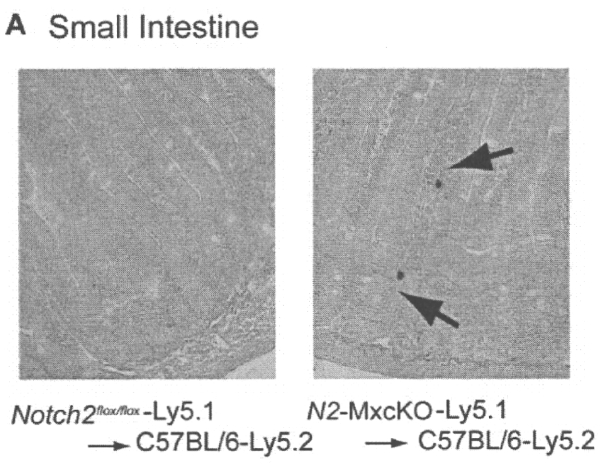

Figure 2. Localization of intestinal mast cells is abnormal in wild-type mice transplanted with *N2*-MxcKO-Ly5.1 bone marrow cells, reminiscent of that in *N2*-MxcKO mice. (A) Bone marrow cells from either *N2*-MxcKO- Ly5.1 mice or littermate *Notch2*$^{flox/flox}$- Ly5.1 mice were transplanted into lethally irradiated (9.5 Gy) C57BL/6-Ly5.2 mice. Toluidine blue staining, followed by eosin. Original magnification ×200. (B) The numbers of mast cells per 10 vcus distributing to various layers of the small intestine. Data are presented as means ± SEM; Mast cells in C57BL/6-Ly5.2 mice transplanted with *Notch2*$^{flox/flox}$-Ly5.1 (n = 3) versus *N2*-MxcKO-Ly5.1 (n = 3). P = .020594 (total) and P = .030123 (villus, lamina propria).

localized to the lamina propria, and very few mast cells were found within the epithelium (Figure 1A-B).

Localization of MTMCs is abnormal in wild-type mice transplanted with *N2*-MxcKO bone marrow cells, reminiscent of that in *N2*-MxcKO mice

Because the Mx-*Cre*–based conditional knockout system deletes target genes not only in the bone marrow cells but also, albeit partially, in the intestinal cells,[14] there was a possibility that *Notch2* deletion in the intestinal cells was responsible for the distinct distribution pattern or increased number of mast cells in *N2*-MxcKO mice compared with control mice. To exclude this possibility, we transplanted *Notch2*-null bone marrow cells carrying the Ly5.1 marker to irradiated wild-type C57BL/6-Ly5.2 mice. A chimerism of donor-derived Ly5.1-positive fraction accounted for more than 70% in the peripheral blood (data not shown). The recipients of bone marrow cells from *Notch2*$^{flox/flox}$ mice showed that the intestinal mast cell distribution was virtually the same as that in wild-type mice, whereas the recipients of *Notch2*-null bone marrow cells showed an increase in mast cells mainly in the lamina propria in an indistinguishable manner from the *N2*-MxcKO mice (Figure 2A-B). This result indicates that deletion of *Notch2* in bone marrow-derived cells alters the distribution pattern and increases the number of mast cells in the small intestine.

Notch-ligand expression in the small intestine

Notch signaling is known to be activated through Notch ligand-receptor binding.[16] We examined the expression pattern of Notch ligands in the small intestine with antibodies against Notch ligands Jagged1 and Delta1 and found that the epithelial layer was clearly stained with anti-Jagged1 but not with anti-Delta1 antibody (Figure 3). The staining with the anti-Jagged1 antibody was confined to the surface of epithelial cells, especially at their basal side rather than the apical side (Figure 3). The Jagged1 expression pattern suggests a possibility that Jagged1-Notch2 interaction between the basal side of the epithelial cells and mast cells has an important role for mast cell migration from the lamina propria across the basement membrane toward the epithelium (Figure 3). Furthermore, the ligand-receptor binding itself might contribute to mast cell–epithelial cell adhesion to some extent, based on our observation that *Notch2*-expressing BMMCs attached to the Jagged1-expressing Chinese hamster ovary (CHO) cells, while *Notch2*-null BMMCs did not (supplemental Figure 2).

Notch2 is dispensable for the CTMC development and distribution

We next investigated the roles of Notch2 in the development of CTMCs. The localization and the number of CTMCs in the skin and peritoneal cavity were not significantly different between *N2*-MxcKO and littermate *Notch2*$^{flox/flox}$ mice more than 4 weeks after the treatment with pIpC (data not shown). This observation might simply indicate that the Mx-*Cre* system was inefficient in the tissue-resident mast cells, as a great majority of peritoneal

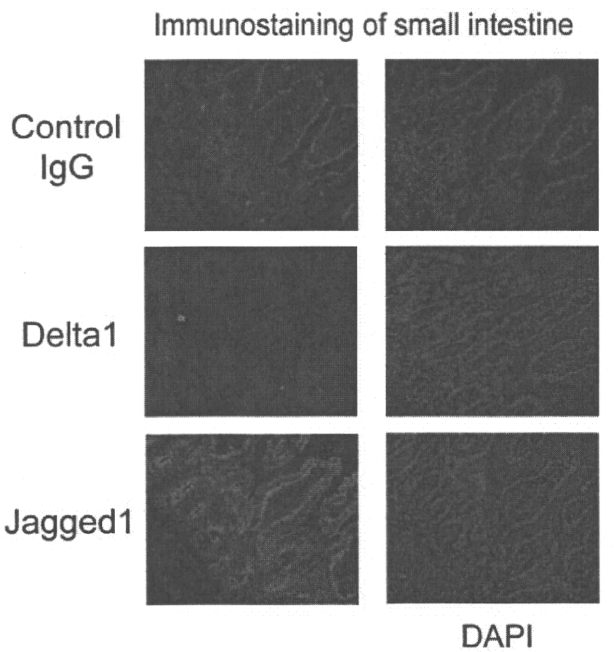

Figure 3. Jagged1 is strongly expressed on the surface of the epithelial cells, especially at their basal side. A section of small intestine prepared using cryostat was stained with goat anti-Jagged1 and goat anti-Delta1 antibodies followed by anti–goat Alexa594. Original magnification ×200.

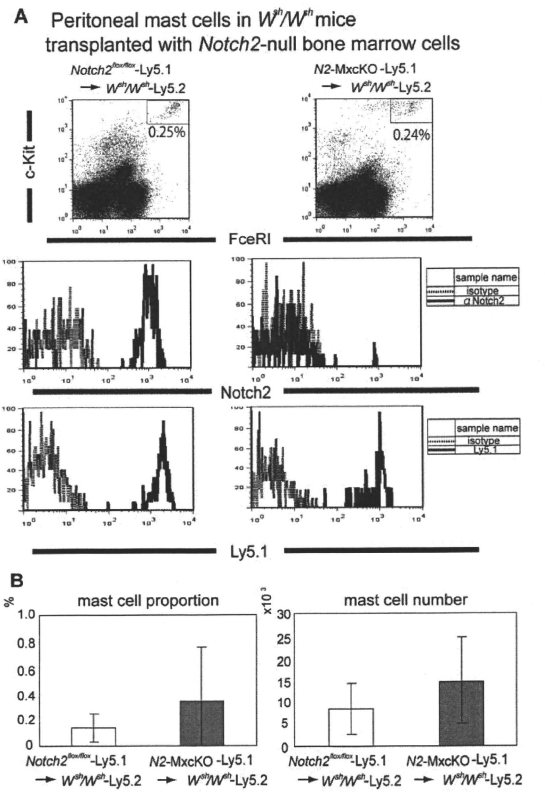

Figure 4. Notch2 is not required for peritoneal mast cell development. (A) Bone marrow cells from *N2*-MxcKO-Ly5.1 mice or control *Notch2*$^{flox/flox}$-Ly5.1 mice were transplanted into lethally irradiated *W*^{sh}/*W*^{sh} mice. Peritoneal mast cells were stained with anti–c-Kit–APC, IgE, and biotinylated anti-Notch2 antibody (HMN2-35), followed by anti-IgE–FITC and streptavidin-PE, or they were stained with anti–c-Kit–APC, IgE, and anti-Ly 5.1–PE, followed by anti-IgE–FITC; they were then analyzed by FACSCalibur (BD Biosciences). (B) The proportion (left) and the absolute number (right) of peritoneal mast cells were not significantly different between *W*^{sh}/*W*^{sh} mice transplanted with *Notch2*-WT bone marrow cells and those transplanted with *Notch2*-null bone marrow cells. $P = .210642$ (mast cell proportion) and $P = .196045$ (mast cell number).

mast cells of pIpC-treated *N2*-MxcKO mice still expressed Notch2 (data not shown). Therefore, to clarify the requirement of *Notch2* in the CTMC development, we examined peritoneal mast cells in mast cell-deficient *W*^{sh}/*W*^{sh} mice after transplantion of *Notch2*-null bone marrow cells carrying the Ly5.1 marker. In this system, mast cells exclusively develop from transplanted bone marrow progenitors, in which the *Cre* recombinase under the Mx-promoter is quite effective [14] (supplemental Figure 1). In this experiment, we found that the proportion and absolute number of peritoneal mast cells was not significantly different between those developed from the *N2*-MxcKO-Ly5.1 bone marrow cells and those developed from littermate *Notch2*$^{flox/flox}$-Ly5.1 bone marrow cells (Figure 4A-B). Notch2 was not expressed in the peritoneal mast cells derived from *N2*-MxcKO-Ly5.1 bone marrow cells but was expressed in those derived from littermate *Notch2*$^{flox/flox}$-Ly5.1 bone marrow cells (Figure 4A middle), indicating that *Notch2* was deleted efficiently. These results suggest that Notch2 is dispensable for the development and distribution of CTMCs.

Cell-autonomous Notch2 signaling in mast cells is important for mast cell migration across the basement membrane in the small intestine

We then asked a question whether aberrant mast cell migration in the small intestine in *N2*-MxcKO mice is dependent on Notch2 signaling in mast cells per se. We intravenously infused *Notch2*-null or control BMMCs into nonirradiated *W*^{sh}/*W*^{sh} mice after *S venezuelensis* infection, because it is reported that BMMCs could only transiently reconstitute intestinal mast cells in mast-cell deficient mice if these recipient mice are in naive status.[17] In tissue sections, we found that the distribution of mast cells in the small intestine was different between control BMMCs-reconstituted mice and *Notch2*-null BMMCs-reconstituted mice; control BMMCs were mainly migrated into the epithelial layer, while a majority of *Notch2*-null BMMCs remained in the lamina propria. This observation indicates that mast cell-autonomous Notch2 expression contributes to mast cell migration across the basement membrane from lamina propria into the epithelial layer (Figure 5A-B). Even in the control BMMC-infused mice, however, a substantial proportion of

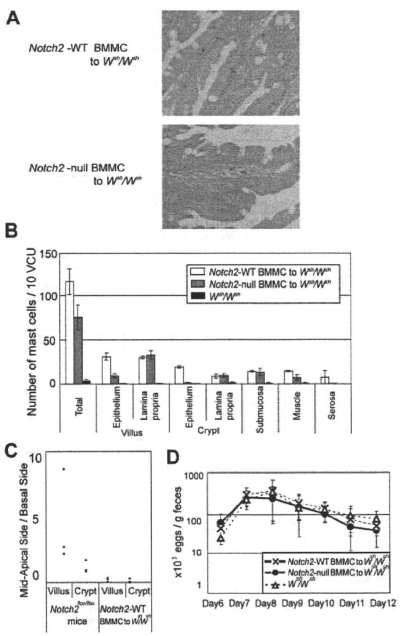

Figure 5. Mast cell–autonomous Notch2 expression is required for mast cell migration toward the epithelium. W^{sh}/W^{sh} mice infected with *S venezuelensis* were intravenously infused with Th2-conditioned *Notch2*-null or control BMMCs on days 3 and 6 of infection. (A) *Notch2*-null BMMCs poorly migrated toward the epithelium compared with control BMMCs. Toluidine blue staining followed by eosin staining. Original magnification ×200. (Top) Control BMMCs; (Bottom) *Notch2*-null BMMCs. (B) The number of mast cells per 10 vcus in the small intestine on day 12 after *S venezuelensis* infection in W^{sh}/W^{sh} mice, without BMMC infusion, with control BMMC infusion, and with *Notch2*-null BMMC infusion. Data are presented as means ± SEM; n = 3 (control BMMC infusion) and n = 4 (*Notch2*-null BMMC infusion), $P = .004080$ (villus, epithelium) and $P = .000020$ (crypt, epithelium). Note that mast cells in W^{sh}/W^{sh} mice infused with *Notch2*-null BMMCs abnormally resided in the lamina propria, whereas most of those in W^{sh}/W^{sh} mice infused with control BMMCs had intraepithelially migrated. (C) Mast cell number in mid to apical side of the epithelial layer was divided with that in the basal side of the epithelial layer. (D) Time course of *S venezuelensis* egg numbers in the stool. The number of excreted eggs was not significantly different between W^{sh}/W^{sh} mice infused with *Notch2*-null and control BMMCs. Data are presented as means ± SEM.

mast cells still remained in the lamina propria, submucosa, and smooth muscle layers, and the distribution of mast cells within the epithelium was confined to the basement membrane side of the epithelial layer (Figure 5B-C). This mast cell localization pattern was different from that in the $Notch2^{flox/flox}$ mice with *S venezuelensis* infection, in which mast cells were present mainly at the mid to apical side of the epithelial layer (Figure 5C). The numbers of *S venezuelensis* eggs in the stool were virtually the same in the *S venezuelensis*–infected W^{sh}/W^{sh} mice infused with *Notch2*-null and control BMMCs and in the *S venezuelensis*–infected W^{sh}/W^{sh} mice without any BMMC infusion throughout the period after infection (Figure 5D).

Taken together, the BMMC-W^{sh}/W^{sh} transplantation model demonstrated that Notch2 in the mast cells indeed determines their intraepithelial migration from lamina propria; nevertheless, this model was not adequate to examine the physiologic mast cell distribution pattern and subsequent parasite expulsion that depends on mast cells.

Notch2 signaling regulates antiparasite immunity of mast cells in the intestine

The BMMC-W^{sh}/W^{sh} reconstitution model could not completely reflect physiologic mast cell distribution pattern in the small intestine. Therefore, to further assess the effect of Notch2 signaling on the mucosal immune response of intestinal mast cells under a pathologic condition, *N2*-MxcKO or control $Notch2^{flox/flox}$ mice were infected with *S venezuelensis*. Total mast cell number was increased in $Notch2^{flox/flox}$ mice much more than in *N2*-MxcKO mice, especially in the epithelium in both crypts and villi 8 days after infection (Figure 6A-B). Thirteen days after infection, mast cells in the epithelium in $Notch2^{flox/flox}$ mice were still more abundant than those in *N2*-MxcKO mice (Figure 6C-D), while mast cell accumulation in the lamina propria in *N2*-MxcKO mice was more prominent in both villi and crypt than that in the earlier stage of infection (Figure 6A,C). In particular, dense aggregation of mast cells was prominent in the lamina propria of *N2*-MxcKO mice at the tip of the villi (Figure 6D). As a consequence, the total number of mast cells in the intestine of *N2*-MxcKO mice became equivalent to those of $Notch2^{flox/flox}$ mice 13 days after infection (Figure 6C,E). The number of *S venezuelensis* eggs in the stool was gradually decreased during day 8 to 10 in control $Notch2^{flox/flox}$ mice but not in *N2*-MxcKO mice (Figure 6F). Furthermore, the worms were still observed in *N2*-MxcKO mice but not in $Notch2^{flox/flox}$ mice 12 days after infection (Figure 6G). These data suggest that Notch2 deficiency alters the distinct distribution pattern of mast cells in the small intestine, which is responsible for the defective eradication of *S venezuelensis*.

Discussion

There is a growing body of evidence that Notch signaling modulates cellular migration and adhesion in endothelial, neural, and lymphoid lineage cells, as well as cancer cells.[18] We have shown that Notch2 signaling induces the development of mast cells.[11] However, it has remained unclear whether Notch2 signaling is involved in the distribution of mast cells in the intestinal mucosa or connective tissues or in controlling the functions of mast cells against microorganisms. Here, we investigated the role of Notch2 signaling in mast cells in terms of their distribution and functions using cell-specific *Notch2*-deficient mice. We found that in *N2*-MxcKO mice, mast cells were abnormally accumulated in the lamina propria of the small intestine, suggesting that *Notch2*-null mast cells have some defect in the migration toward the epithelium. Furthermore, *N2*-MxcKO mice failed to eradicate *S venezuelensis* and exhibited a distinct mast cell migration pattern in the intestine compared with control mice, suggesting that mast cells regulate the host-microbial interface in the intestine through Notch2 signaling.

Mast cell number was rather increased in the intestinal mucosa of *N2*-MxcKO mice compared with control mice in naive status. Mast cell progenitors were supposed to reside in the submucosa and gradually move toward the villi, accompanied by their differentiation into mature mast cells. Based on our observation in

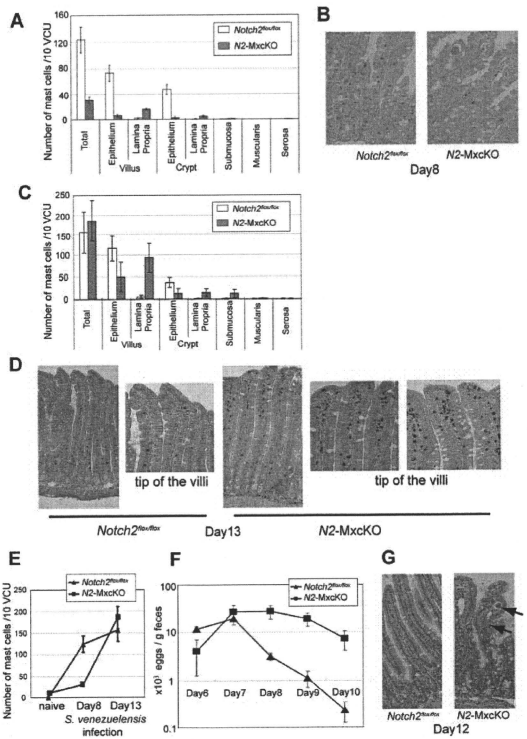

Figure 6. Notch2 is essential for antiparasite immunity of mast cells in the intestine. *N2*-MxcKO or control *Notch2*$^{flox/flox}$ mice were subcutaneously injected with third-stage infective larvae of *S venezuelensis*. (A) The number of mast cells per 10 vcus in the small intestine on day 8 after *S venezuelensis* infection. Data are presented as means ± SEM. The number of mast cells was much less in *N2*-MxcKO mice; n = 3, P = .008592 (total), P = .005695 (villus, epithelium), P = .000715 (villus, lamina propria), P = .005245 (crypt, epithelium), and P = .045466 (crypt, lamina propria). Note that mast cells in *N2*-MxcKO mice were abnormally clustered in the lamina propria, whereas most of those in the control *Notch2*$^{flox/flox}$ mice were intraepithelially migrated. (B) Toluidine blue staining followed by eosin staining of the small intestine on day 8; original magnification ×200. (C) The number of mast cells per 10 vcus in the small intestine on day 13 after *S venezuelensis* infection. Data are presented as means ± SEM; n = 3, P = .026076 (villus, epithelium), P = .00194 (villus, lamina propria), P = .021177 (crypt, epithelium), and P = .019324 (crypt, lamina propria), P = .047445 (submucosa). (D) Toluidine blue staining followed by eosin staining of the small intestine on day 13. Original magnification ×200. (E) The total number of mast cells per 10 vcus on day 0, day 8, and day 13 of infection. The total number of mast cells was significantly lower in *N2*-MxcKO mice at the early phase (day 8) and almost equal at the later phase (day 13) to that of control mice. Data are presented as means ± SEM; n = 10 and 8 (day 0, *Notch2*$^{flox/flox}$ and *N2*-MxcKO); n = 3 and 3 (day 8, *Notch2*$^{flox/flox}$ and *N2*-MxcKO); n = 4 and 4 (day 13, *Notch2*$^{flox/flox}$ and *N2*-MxcKO). (F) Time course of egg number in the stool. The number of excreted eggs was significantly greater in *N2*-MxcKO mice compared with those in *Notch2*$^{flox/flox}$ mice. Data are represented as means ± SEM; n = 4; P = .0291 (day 8) and P = .0219 (day 9). (G) Hematoxylin-eosin staining of the small intestine on day 12. Original magnification ×200. Arrows indicate worms. Worms were still observed in the villi in the jejunum of *N2*-MxcKO, but not of *Notch2*$^{flox/flox}$ mice.

an *S venezuelensis*–infection model, mast cells increase in number in the epithelium in control *Notch2*$^{flox/flox}$ mice, while they abnormally aggregate in lamina propria in *N2*-MxcKO mice, especially in the later stage of infection. This suggests that mast cell migration from lamina propria toward the epithelium across the basement membrane is impaired in *N2*-MxcKO mice. Consequently, mast cell turnover might be prolonged in *N2*-MxcKO mice. Given that the mechanism of mast cell migration from lamina propria toward the epithelium is common in naive status and infection status, such migration defect may also explain the mast cell increase in *N2*-MxcKO mice in naive status that we observed.

The defect of mast cell migration toward intraepithelium of the small intestine in *N2*-MxcKO mice is very similar to that in integrin β6-deficient mice,[19] in which activation of transforming growth factor (TGF)–β signaling is impaired.[20] A crosstalk between Notch signaling and TGF-β signaling might occur in intestinal mast cells as well as the cases of other cell types.[21] Alternatively, Notch signaling might directly regulate a downstream target of TGF-β1 in intestinal mast cell migration (eg, the induction of integrin αE expression).[19,22] Integrin αE, forming an integrin αEβ7 complex on mast cells, binds to E-cadherin on epithelial cells and is involved in mast cell localization in the epithelium.[22] The expression level of integrin αEβ7, measured by flow cytometric analysis, however, was not affected by Notch-ligand stimulation in BMMCs (unpublished data).

In the previous paper we showed that Notch signaling facilitates mast cell lineage development at the expense of granulocyte/macrophage development from both common myeloid progenitors (CMPs) and granulocyte-macrophage progenitors (GMPs) in vitro.[11] Mast cells, however, were not depleted in *N2*-MxcKO mice in naive status in vivo, but rather slightly increased in the small intestine of *N2*-MxcKO mice. This clearly indicates that Notch2 signaling is dispensable for steady-state mast cell generation in vivo. However, the dynamic increase of mast cells during the early phase of intestinal parasite infection was markedly impaired in *N2*-MxcKO mice. The mechanisms underlying the Notch2 signaling requirement only in parasite-infected mice remain to be clarified. Nevertheless, rapidly increasing intestinal mast cells have to be supplied by mast cell progenitors. The pathways and mechanisms responsible for mast cell progenitor recruitment and trafficking are likely to be dynamic and susceptible to modification during inflammation.[1] Such a modulation of the mast cell generation pathway during intestinal infection might underlie the requirement of *Notch2* only during parasite infection. This is similar to

IL-3–deficient mice. IL-3 is essential for mast cell differentiation in vitro; however, IL-3–deficient mice have the normal number of mast cells at the steady state, whereas mast cell hyperplasia is impaired upon intestinal parasite infection.[23]

Our data showed that parasite expulsion was impaired in *N2*-MxcKO mice. We could not exclude the possibility that the *Notch2* deletion in immune cells other than mast cells modulate the response against the nematode infection. If we could show that Th2-conditioned wild-type BMMCs successfully eradicate *S venezuelensis* in W^{sh}/W^{sh} mice and that *Notch2*-null BMMCs do not, it would be clearer that Notch2 signaling in mast cells per se but not in other immune cells should be critically important for defense against *S venezuelensis* infection. The failure of rescue experiments may be caused by the abnormal mast cell distribution pattern of wild-type BMMCs in W^{sh}/W^{sh} mice. Nevertheless, the result of this experiment supported the previous finding that the proper epithelial migration of mast cells is required for efficient expulsion of *S venezuelensis*[24] and thus provides an insight that the impaired *S venezuelensis* expulsion in *N2*-MxcKO mice is attributed to the mast cell-autonomous deletion of *Notch2*.

In conclusion, our data clearly indicate that Notch2 receptor signaling is specifically required for proper intestinal mast cell distribution in a cell-autonomous manner. Furthermore, involvement of Notch2 signaling in mucosal immunity was proven, particularly for eradication of infected parasites, although whether this is due to the Notch2 signaling in mast cells is yet to be elucidated.

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Authorship

Contribution: M.S.-Y. designed and performed the research, analyzed the data, and wrote the paper; T.S., Y. Miyake, and Y. Morishita performed the research; T.I.S., H.M., and H.Y. contributed new reagents; E.N.-Y., K.K., M.F., S.O., and M.K. provided vital discussion; K.Y. designed the research; and S.C. designed the research, analyzed the data, and wrote the paper.

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