Appendix 1-1 Clinical signs in mice during quarantine and acclimation periods

Individual No.	Animal[1)] No.	Receipt day (Day 1)					Quarantine and acclimation day						
		Behavior	Fur	Nose, ears, eyes, oral	Tail, limbs	Others	2	3	4	5	6	7	8
1	2501	-	-	-	-	-	-	-	-	-	-	-	-
2	2601	-	-	-	-	-	-	-	-	-	-	-	-
3	2401	-	-	-	-	-	-	-	-	-	-	-	-
4	exc.	-	-	-	-	-	-	-	-	-	-	-	-
5	2201	-	-	-	-	-	-	-	-	-	-	-	-
6	2202	-	-	-	-	-	-	-	-	-	-	-	-
7	exc.	-	-	-	-	-	-	-	-	-	-	-	-
8	2301	-	-	-	-	-	-	-	-	-	-	-	-
9	2102	-	-	-	-	-	-	-	-	-	-	-	-
10	2701	-	-	-	-	-	-	-	-	-	-	-	-
11	2702	-	-	-	-	-	-	-	-	-	-	-	-
12	2101	-	-	-	-	-	-	-	-	-	-	-	-
13	2703	-	-	-	-	-	-	-	-	-	-	-	-
14	2302	-	-	-	-	-	-	-	-	-	-	-	-
15	2402	-	-	-	-	-	-	-	-	-	-	-	-
16	exc.	-	-	-	-	-	-	-	-	-	-	-	-
17	exc.	-	-	-	-	-	-	-	-	-	-	-	-
18	2403	-	-	-	-	-	-	-	-	-	-	-	-
19	2103	-	-	-	-	-	-	-	-	-	-	-	-
20	2602	-	-	-	-	-	-	-	-	-	-	-	-
21	2303	-	-	-	-	-	-	-	-	-	-	-	-
22	2502	-	-	-	-	-	-	-	-	-	-	-	-
23	2203	-	-	-	-	-	-	-	-	-	-	-	-
24	2104	-	-	-	-	-	-	-	-	-	-	-	-
25	2204	-	-	-	-	-	-	-	-	-	-	-	-
26	2704	-	-	-	-	-	-	-	-	-	-	-	-
27	exc.	-	-	-	-	-	-	-	-	-	-	-	-
28	2404	-	-	-	-	-	-	-	-	-	-	-	-
29	2603	-	-	-	-	-	-	-	-	-	-	-	-
30	2504	-	-	-	-	-	-	-	-	-	-	-	-
31	exc.	-	-	-	-	-	-	-	-	-	-	-	-
32	exc.	-	-	-	-	-	-	-	-	-	-	-	-
33	2503	-	-	-	-	-	-	-	-	-	-	-	-
34	2304	-	-	-	-	-	-	-	-	-	-	-	-
35	2306	-	-	-	-	-	-	-	-	-	-	-	-
36	exc.	-	-	-	-	-	-	-	-	-	-	-	-
37	2206	-	-	-	-	-	-	-	-	-	-	-	-
38	2605	-	-	-	-	-	-	-	-	-	-	-	-
39	2406	-	-	-	-	-	-	-	-	-	-	-	-
40	2106	-	-	-	-	-	-	-	-	-	-	-	-
41	2305	-	-	-	-	-	-	-	-	-	-	-	-
42	2105	-	-	-	-	-	-	-	-	-	-	-	-
43	2606	-	-	-	-	-	-	-	-	-	-	-	-
44	2506	-	-	-	-	-	-	-	-	-	-	-	-
45	2706	-	-	-	-	-	-	-	-	-	-	-	-
46	2604	-	-	-	-	-	-	-	-	-	-	-	-
47	2405	-	-	-	-	-	-	-	-	-	-	-	-
48	2205	-	-	-	-	-	-	-	-	-	-	-	-
49	2705	-	-	-	-	-	-	-	-	-	-	-	-
50	2505	-	-	-	-	-	-	-	-	-	-	-	-

-: No abnormalities.

1) Identification numbers after allocation to treatment groups (exc.: excluded from this study).

Appendix 1-2 Body weights in mice during quarantine and acclimation periods

Individual No.	Animal[1)] No.	Receipt day	Final day of quarantine	Body weight gain
1	2501	19.4	18.7	-0.7
2	2601	17.7	19.4	1.7
3	2401	20.0	22.0	2.0
4	exc.	21.5	22.3	0.8
5	2201	20.5	22.6	2.1
6	2202	19.3	21.1	1.8
7	exc.	19.2	20.0	0.8
8	2301	18.4	20.7	2.3
9	2102	19.2	21.1	1.9
10	2701	18.8	21.0	2.2
11	2702	18.6	21.2	2.6
12	2101	18.5	19.7	1.2
13	2703	17.3	19.7	2.4
14	2302	19.0	22.0	3.0
15	2402	19.8	22.1	2.3
16	exc.	19.3	21.2	1.9
17	exc.	18.6	20.9	2.3
18	2403	18.6	21.7	3.1
19	2103	18.8	21.3	2.5
20	2602	18.2	19.0	0.8
21	2303	19.1	21.4	2.3
22	2502	18.7	20.4	1.7
23	2203	20.3	22.2	1.9
24	2104	18.1	20.3	2.2
25	2204	18.2	20.7	2.5
26	2704	20.8	23.1	2.3
27	exc.	21.0	23.4	2.4
28	2404	20.6	22.6	2.0
29	2603	19.1	21.2	2.1
30	2504	18.3	20.3	2.0
31	exc.	20.7	22.1	1.4
32	exc.	20.4	21.7	1.3
33	2503	18.3	19.8	1.5
34	2304	21.4	23.4	2.0
35	2306	21.1	24.3	3.2
36	exc.	21.5	23.2	1.7
37	2206	18.9	19.8	0.9
38	2605	17.6	19.7	2.1
39	2406	21.3	23.2	1.9
40	2106	18.2	19.7	1.5
41	2305	20.5	23.1	2.6
42	2105	21.1	22.6	1.5
43	2606	21.1	23.0	1.9
44	2506	19.9	20.7	0.8
45	2706	18.8	21.6	2.8
46	2604	18.8	21.2	2.4
47	2405	20.3	22.2	1.9
48	2205	19.2	19.8	0.6
49	2705	20.6	23.0	2.4
50	2505	21.3	22.6	1.3
Mean		19.5	21.4	1.9
S.E.		0.2	0.2	0.1

Unit: g.

1) Identification numbers after allocation to treatment groups (exc.: excluded from this study).

Body weight gain = Body weight difference between the final day and receipt day during 8 days.

Appendix 2 Body weights in mice treated with RK-569 and Dexamethasone - The details of statistical analysis

Comparison with the non-challenge and the control groups

Day	F test *p* value	*t*-test	*t*-test *p* value
1	0.6710	Student	0.8422
8	0.7320	Student	0.5223
15	0.4953	Student	0.8309
22	0.3106	Student	0.5285
29	0.3094	Student	0.3702
36	0.6602	Student	0.6895
42	0.8945	Student	0.5872
43	0.9021	Student	0.9276

Comparison with the control and the RK-569 groups

Day	Bartlett's test *p* value	Multiple comparison	*p* value of multiple comparison			
			Comparison group with the control			
			0.1 ng/head	1 ng/head	10 ng/head	100 ng/head
1	0.5614	Dunnett	0.2291	0.1780	0.8730	0.9066
8	0.3285	Dunnett	0.4594	0.2850	0.5993	0.7776
15	0.5996	Dunnett	0.8328	0.7047	0.1932	0.4202
22	0.2855	Dunnett	0.9922	0.9332	0.0589	0.1416
29	**0.0418**	Steel	0.9694	0.9931	0.2307	0.7890
36	0.3372	Dunnett	0.9989	0.6855	0.9672	0.4588
42	0.7982	Dunnett	0.8610	0.5500	0.9618	0.4675
43	0.7726	Dunnett	0.9486	0.5700	0.9842	0.5879

Comparison with the control and the dexamethasone groups

Day	F test *p* value	*t*-test	*t*-test *p* value
1	0.9676	Student	0.5763
8	0.4073	Student	0.8592
15	0.7578	Student	0.6729
22	0.1709	Student	0.3104
29	**0.0272**	Aspin-Welch	0.7399
36	0.3807	Student	**0.0205**
42	0.2914	Student	**0.0220**
43	0.5708	Student	**0.0444**

Bold: Statistically significant at significant level 0.05.

Appendix 3-1 Effects of RK-569 and Dexamethasone on airway hyperresponsiveness induced by ovalbumin in mice

Test substance	Route	Dose (ng/head)	Animal No.	Specific airway resistance ($cmH_2O \cdot sec$) MCh concentration (mg/mL)					
				0 (Saline)	1.56	3.125	6.25	12.5	25
Non-challenge	i.v.	0	2101	4.559	5.225	5.348	5.586	6.552	9.525
			2102	4.653	4.656	4.967	6.163	7.155	8.432
			2103	5.125	5.919	6.721	7.149	7.832	10.111
			2104	6.280	6.730	7.791	7.621	8.468	10.514
			2105	5.363	6.069	5.927	7.059	7.731	8.556
			2106	6.547	7.016	7.003	7.136	8.575	9.121
			N	6	6	6	6	6	6
			Mean	5.421	5.936	6.293	6.786	7.719	9.377
			S.E.	0.338	0.363	0.437	0.308	0.316	0.341
Control	i.v.	0	2201	4.891	6.419	6.664	8.215	9.257	11.240
			2202	6.371	7.228	7.988	8.668	11.281	14.738
			2203	6.591	7.081	7.610	9.108	11.026	16.477
			2204	6.956	7.637	8.353	10.347	14.126	13.699
			2205	6.162	8.555	8.138	9.206	11.587	14.521
			2206	5.813	6.797	7.517	7.877	11.625	14.205
			N	6	6	6	6	6	6
			Mean	6.131	7.286	7.712	8.904	11.484	14.147
			S.E.	0.294	0.304	0.246	0.356	0.638	0.697
RK-569	i.v.	0.1	2301	4.985	6.217	7.244	8.085	8.443	14.915
			2302	6.463	7.260	7.631	8.765	9.304	10.592
			2303	6.833	6.983	8.354	9.586	10.082	12.035
			2304	6.638	7.359	8.561	8.647	11.924	12.810
			2305	5.664	6.011	7.296	7.241	9.494	14.596
			2306	5.783	6.036	6.693	7.970	10.045	12.654
			N	6	6	6	6	6	6
			Mean	6.061	6.644	7.630	8.382	9.882	12.934
			S.E.	0.288	0.255	0.290	0.328	0.476	0.660
	i.v.	1	2401	5.319	5.587	6.994	7.033	9.443	10.289
			2402	6.458	6.879	7.974	8.604	9.978	12.994
			2403	6.582	7.129	7.557	8.215	9.589	12.178
			2404	6.582	7.219	9.021	8.687	11.346	10.947
			2405	5.968	6.468	7.422	7.485	8.966	12.058
			2406	5.636	5.755	7.462	7.669	11.961	11.781
			N	6	6	6	6	6	6
			Mean	6.091	6.506	7.738	7.949	10.214	11.708
			S.E.	0.219	0.286	0.287	0.269	0.481	0.391
	i.v.	10	2501	5.390	7.124	7.043	7.058	10.323	14.758
			2502	6.719	7.204	8.699	8.980	9.944	11.500
			2503	6.813	7.412	7.441	9.274	9.210	10.373
			2504	6.477	7.306	7.648	8.478	8.627	9.860
			2505	6.192	6.638	7.104	7.764	11.333	11.130
			2506	5.780	6.696	7.762	8.557	8.576	10.825
			N	6	6	6	6	6	6
			Mean	6.229	7.063	7.616	8.352	9.669	11.408
			S.E.	0.227	0.132	0.246	0.333	0.438	0.710
	i.v.	100	2601	5.511	5.981	6.601	8.234	9.595	11.270
			2602	6.277	6.855	8.834	8.626	9.235	10.653
			2603	6.509	7.110	7.451	8.237	9.282	10.846
			2604	6.233	6.566	7.048	7.548	9.071	10.030
			2605	5.699	6.563	6.564	6.922	7.577	9.955
			2606	5.920	6.598	7.196	7.829	9.094	13.524
			N	6	6	6	6	6	6
			Mean	6.025	6.612	7.282	7.899	8.976	11.046
			S.E.	0.155	0.154	0.341	0.247	0.290	0.536

Appendix 3-2 Effects of RK-569 and Dexamethasone on airway hyperresponsiveness induced by ovalbumin in mice

Test substance	Route	Dose (mg/kg)	Animal No.	Specific airway resistance ($cmH_2O \cdot sec$)					
				MCh concentration (mg/mL)					
				0 (Saline)	1.56	3.125	6.25	12.5	25
Dexamethasone	p.o.	3	2701	3.886	4.574	4.780	5.238	6.475	8.191
			2702	4.023	4.539	4.888	5.267	5.602	6.358
			2703	5.751	6.144	6.059	7.153	9.293	10.026
			2704	6.280	6.804	7.530	8.869	10.581	9.873
			2705	5.596	6.020	6.792	7.216	8.364	9.907
			2706	5.327	5.783	5.970	6.696	7.479	8.361
			N	6	6	6	6	6	6
			Mean	5.144	5.644	6.003	6.740	7.966	8.786
			S.E.	0.397	0.371	0.436	0.558	0.748	0.589

Appendix 3-3 Effects of RK-569 and Dexamethasone on airway hyperresponsiveness induced by ovalbumin in mice - The details of statistical analysis inn specific airway resistance

Comparison with the non-challenge and the control groups

MCh concentration (mg/mL)	F test *p* value	*t*-test	*t*-test *p* value
0 (Saline)	0.7654	Student	0.1443
1.56	0.7047	Student	**0.0172**
3.125	0.2337	Student	**0.0178**
6.25	0.7611	Student	**0.0011**
12.5	0.1482	Student	**0.0004**
25	0.1425	Student	**0.0001**

Comparison with the control and the RK-569 groups

MCh concentration (mg/mL)	Bartlett's test *p* value	Multiple comparison	*p* value of multiple comparison			
			Comparison group with the control			
			0.1 ng/head	1 ng/head	10 ng/head	100 ng/head
0 (Saline)	0.6950	Dunnett	0.9987	0.9999	0.9953	0.9936
1.56	0.3233	Dunnett	0.1964	0.0893	0.9064	0.1650
3.125	0.9515	Dunnett	0.9987	1.0000	0.9977	0.6675
6.25	0.9322	Dunnett	0.5839	0.1200	0.5370	0.0959
12.5	0.6067	Dunnett	0.0822	0.2100	**0.0421**	**0.0037**
25	0.7347	Dunnett	0.4448	**0.0310**	**0.0139**	**0.0051**

Comparison with the control and the dexamethasone groups

MCh concentration (mg/mL)	F test *p* value	*t*-test	*t*-test *p* value
0 (Saline)	0.5236	Student	0.0738
1.56	0.6732	Student	**0.0065**
3.125	0.2350	Student	**0.0066**
6.25	0.3452	Student	**0.0085**
12.5	0.7352	Student	**0.0050**
25	0.7207	Student	**0.0002**

Bold: Statistically significant at significant level 0.05.

Appendix 4-1 Effects of RK-569 and Dexamethasone on the changes of bronchoalveolar lavage fluid induced by ovalbumin in mice

Test substance	Route	Dose (ng/head)	Animal No.	Cell number (10^2 cells/BALF)				
				WBCs	Eosi	Neut	Lymp	Mono
Non-challenge	i.v.	0	2101	1025	0	4	6	1015
			2102	500	0	2	7	491
			2103	500	0	0	3	497
			2104	550	0	6	10	535
			2105	525	0	30	9	485
			2106	725	0	7	16	702
			N	6	6	6	6	6
			Mean	638	0	8	9	621
			S.E.	85	0	4	2	86
Control	i.v.	0	2201	625	360	65	63	138
			2202[a)]	6400	5069	269	538	525
			2203	800	272	35	88	405
			2204	1750	847	196	196	511
			2205	1425	502	191	228	504
			2206	1100	222	81	136	660
			N	5	5	5	5	5
			Mean	1140	441	114	142	444
			S.E.	204	112	33	31	87
RK-569	i.v.	0.1	2301	926	109	50	102	665
			2302	1283	259	216	95	713
			2303	1238	322	47	228	641
			2304	1025	88	340	107	490
			2305	925	24	298	109	494
			2306	1050	42	82	179	748
			N	6	6	6	6	6
			Mean	1075	141	172	137	625
			S.E.	63	50	53	22	45
	i.v.	1	2401	825	33	120	87	584
			2402	594	1	17	96	480
			2403	925	48	57	117	703
			2404	600	1	10	22	568
			2405	950	19	57	160	714
			2406	698	18	45	82	553
			N	6	6	6	6	6
			Mean	765	20	51	94	600
			S.E.	64	7	16	18	37
	i.v.	10	2501	653	37	30	82	504
			2502	563	52	99	84	328
			2503	689	12	54	112	511
			2504	975	4	21	43	907
			2505	700	13	38	119	531
			2506	850	27	24	75	724
			N	6	6	6	6	6
			Mean	738	24	44	86	584
			S.E.	61	7	12	11	82
	i.v.	100	2601	925	43	74	80	729
			2602	650	16	29	48	558
			2603	750	38	71	86	557
			2604	825	31	18	36	739
			2605	700	13	10	31	647
			2606	525	16	24	32	454
			N	6	6	6	6	6
			Mean	729	26	38	52	614
			S.E.	57	5	11	10	45

a) abnormal data, excluded from the statistical analysis.

BALF: bronchoalveolar lavage fluid.

Eosi: Eosinophil, Neut: Neutrophil, Lymp: Lymphocyte, Mono: Monocyte or Macrophage.

Appendix 4-2 Effects of RK-569 and Dexamethasone on the changes of bronchoalveolar lavage fluid induced by ovalbumin in mice

Test substance	Route	Dose (mg/kg)	Animal No.	Cell number (10^2 cells/BALF)				
				WBCs	Eosi	Neut	Lymp	Mono
Dexamethasone	p.o.	3	2701	500	5	66	24	405
			2702	650	0	25	20	606
			2703	750	5	29	26	692
			2704	625	0	48	28	550
			2705	725	6	71	19	629
			2706	775	0	16	17	742
			N	6	6	6	6	6
			Mean	671	3	43	22	604
			S.E.	42	1	9	2	48

BALF: bronchoalveolar lavage fluid.
Eosi: Eosinophil, Neut: Neutrophil, Lymp: Lymphocyte, Mono: Monocyte or Macrophage.

Appendix 4-3 Effects of RK-569 and Dexamethasone on the changes of bronchoalveolar lavage fluid induced by ovalbumin in mice

Test substance	Route	Dose (ng/head)	Animal No.	Volume of BALF (mL)	WBCs (cells/μL)	Differential leukocyte counts				
						Eosi	Neut	Lymp	Mono	Total
Non-challenge	i.v.	0	2101	1.00	102.5	0	2	3	495	500
			2102	1.00	50.0	0	2	7	491	500
			2103	1.00	50.0	0	0	3	497	500
			2104	1.00	55.0	0	5	9	486	500
			2105	1.00	52.5	0	29	9	462	500
			2106	1.00	72.5	0	5	11	484	500
			N	6	6	6	6	6	6	6
			Mean	1.00	64	0	7	7	486	500
			S.E.	0.00	8	0	4	1	5	0
Control	i.v.	0	2201	1.00	62.5	288	52	50	110	500
			2202[a)]	1.00	640.0	396	21	42	41	500
			2203	1.00	80.0	170	22	55	253	500
			2204	1.00	175.0	242	56	56	146	500
			2205	1.00	142.5	176	67	80	177	500
			2206	1.00	110.0	101	37	62	300	500
			N	5	5	5	5	5	5	5
			Mean	1.00	114	195	47	61	197	500
			S.E.	0.00	20	32	8	5	35	0
RK-569	i.v.	0.1	2301	0.95	97.5	59	27	55	359	500
			2302	0.95	135.0	101	84	37	278	500
			2303	0.90	137.5	130	19	92	259	500
			2304	1.00	102.5	43	166	52	239	500
			2305	1.00	92.5	13	161	59	267	500
			2306	1.00	105.0	20	39	85	356	500
			N	6	6	6	6	6	6	6
			Mean	0.97	112	61	83	63	293	500
			S.E.	0.02	8	19	27	9	21	0
	i.v.	1	2401	1.00	82.5	20	73	53	354	500
			2402	0.95	62.5	1	14	81	404	500
			2403	1.00	92.5	26	31	63	380	500
			2404	0.80	75.0	1	8	18	473	500
			2405	1.00	95.0	10	30	84	376	500
			2406	0.90	77.5	13	32	59	396	500
			N	6	6	6	6	6	6	6
			Mean	0.94	81	12	31	60	397	500
			S.E.	0.03	5	4	9	10	17	0
	i.v.	10	2501	0.90	72.5	28	23	63	386	500
			2502	0.90	62.5	46	88	75	291	500
			2503	0.95	72.5	9	39	81	371	500
			2504	1.00	97.5	2	11	22	465	500
			2505	1.00	70.0	9	27	85	379	500
			2506	1.00	85.0	16	14	44	426	500
			N	6	6	6	6	6	6	6
			Mean	0.96	77	18	34	62	386	500
			S.E.	0.02	5	7	12	10	24	0
	i.v.	100	2601	1.00	92.5	23	40	43	394	500
			2602	1.00	65.0	12	22	37	429	500
			2603	1.00	75.0	25	47	57	371	500
			2604	1.00	82.5	19	11	22	448	500
			2605	1.00	70.0	9	7	22	462	500
			2606	1.00	52.5	15	23	30	432	500
			N	6	6	6	6	6	6	6
			Mean	1.00	73	17	25	35	423	500
			S.E.	0.00	6	3	6	6	14	0

a) abnormal data, excluded from the statistical analysis.

BALF: bronchoalveolar lavage fluid.

Eosi: Eosinophil, Neut: Neutrophil, Lymp: Lymphocyte, Mono: Monocyte or Macrophage.

Appendix 4-4 Effects of RK-569 and Dexamethasone on the changes of bronchoalveolar lavage fluid induced by ovalbumin in mice

Test substance	Route	Dose (mg/kg)	Animal No.	Volume of BALF (mL)	WBCs (cells/μL)	Differential leukocyte counts				
						Eosi	Neut	Lymp	Mono	Total
Dexamethasone	p.o.	3	2701	1.00	50.0	5	66	24	405	500
			2702	1.00	65.0	0	19	15	466	500
			2703	1.00	75.0	3	19	17	461	500
			2704	1.00	62.5	0	38	22	440	500
			2705	1.00	72.5	4	49	13	434	500
			2706	1.00	77.5	0	10	11	479	500
			N	6	6	6	6	6	6	6
			Mean	1.00	67	2	34	17	448	500
			S.E.	0.00	4	1	9	2	11	0

BALF: bronchoalveolar lavage fluid.
Eosi: Eosinophil, Neut: Neutrophil, Lymp: Lymphocyte, Mono: Monocyte or Macrophage.

Appendix 4-5 Effects of RK-569 and Dexamethasone on the changes of bronchoalveolar lavage fluid induced by ovalbumin in mice - The details of statistical analysis

Comparison with the non-challenge and the control groups

Parameter	F test *p* value	*t*-test	*t*-test *p* value
WBCs	0.1141	Student	**0.0381**
Eosi	**<0.0001**	Aspin-Welch	**0.0171**
Neut	**0.0008**	Aspin-Welch	**0.0337**
Lymp	**<0.0001**	Aspin-Welch	**0.0127**
Mono	0.9018	Student	0.1827

Comparison with the control and the RK-569 groups

Parameter	Bartlett's test *p* value	Multiple comparison	*p* value of multiple comparison Comparison group with the control			
			0.1 ng/head	1 ng/head	10 ng/head	100 ng/head
WBCs	**0.0257**	Steel	0.9991	0.3791	0.3791	0.3791
Eosi	**<0.0001**	Steel	0.0902	**0.0211**	**0.0215**	**0.0211**
Neut	**0.0022**	Steel	0.7564	0.2769	0.1980	0.1359
Lymp	0.1799	Dunnett	0.9987	0.2669	0.1607	**0.0125**
Mono	0.3226	Dunnett	0.1524	0.2493	0.3338	0.1914

Comparison with the control and the dexamethasone groups

Parameter	F test *p* value	*t*-test	*t*-test *p* value
WBCs	**0.0055**	Aspin-Welch	0.0826
Eosi	**<0.0001**	Aspin-Welch	**0.0175**
Neut	**0.0224**	Aspin-Welch	0.1006
Lymp	**<0.0001**	Aspin-Welch	**0.0184**
Mono	0.3088	Student	0.1243

Bold: Statistically significant at significant level 0.05.

Appendix 5-1 Effects of RK-569 and Dexamethasone on the changes of OVA-IgE and OVA-IgG1 induced by ovalbumin in mice

Test substance	Route	Dose (ng/head)	Animal No.	OVA-IgE (U/mL)	OVA-IgG1 (U/mL)
Non-challenge	i.v.	0	2101	176.1	330.8
			2102	160.2	489.2
			2103	114.6	110.8
			2104	132.5	99.4
			2105	131.2	293.1
			2106	67.7	259.2
			N	6	6
			Mean	130.4	263.8
			S.E.	15.5	59.6
Control	i.v.	0	2201	90.0	113.5
			2202	188.3	909.5
			2203	198.9	423.8
			2204	121.4	575.6
			2205	111.8	397.6
			2206	171.6	708.9
			N	6	6
			Mean	147.0	521.5
			S.E.	18.4	112.5
RK-569	i.v.	0.1	2301	157.1	578.8
			2302	158.5	116.6
			2303	132.0	673.4
			2304	88.4	91.3
			2305	169.2	414.2
			2306	124.2	628.1
			N	6	6
			Mean	138.2	417.1
			S.E.	12.2	105.3
	i.v.	1	2401	156.2	727.2
			2402	87.6	98.7
			2403	108.3	616.6
			2404	159.1	516.6
			2405	149.2	530.6
			2406	156.3	424.1
			N	6	6
			Mean	136.1	485.6
			S.E.	12.4	87.9
	i.v.	10	2501	140.9	105.7
			2502	147.6	93.5
			2503	172.0	104.5
			2504	212.1	94.7
			2505	130.1	228.6
			2506	136.7	369.9
			N	6	6
			Mean	156.6	166.2
			S.E.	12.6	45.9
	i.v.	100	2601	58.6	50.0
			2602	156.9	109.3
			2603	175.8	99.7
			2604	164.6	588.1
			2605	152.3	448.8
			2606	128.2	461.1
			N	6	6
			Mean	139.4	292.8
			S.E.	17.4	94.8

Appendix 5-2 Effects of RK-569 and Dexamethasone on the changes of OVA-IgE and OVA-IgG1 induced by ovalbumin in mice

Test substance	Route	Dose (mg/kg)	Animal No.	OVA-IgE (U/mL)	OVA-IgG1 (U/mL)
Dexamethasone	p.o.	3	2701	80.3	92.1
			2702	89.6	381.3
			2703	98.3	62.6
			2704	76.8	73.7
			2705	115.8	290.5
			2706	117.4	316.9
			N	6	6
			Mean	96.4	202.9
			S.E.	7.1	58.1

Appendix 5-3 Effects of RK-569 and Dexamethasone on the changes of OVA-IgE and OVA-IgG1 induced by ovalbumin in mice - The details of statistical analysis

Comparison with the non-challenge and the control groups

Parameter	F test *p* value	*t*-test	*t*-test *p* value
OVA-IgE	0.7116	Student	0.5048
OVA-IgG1	0.1894	Student	0.0705

Comparison with the control and the RK-569 groups

Parameter	Bartlett's test *p* value	Multiple comparison	*p* value of multiple comparison			
			Comparison group with the control			
			0.1 ng/head	1 ng/head	10 ng/head	100 ng/head
OVA-IgE	0.8153	Dunnett	0.9807	0.9586	0.9736	0.9885
OVA-IgG1	0.4624	Dunnett	0.8391	0.9959	**0.0389**	0.2614

Comparison with the control and the dexamethasone groups

Parameter	F test *p* value	*t*-test	*t*-test *p* value
OVA-IgE	0.0568	Student	**0.0280**
OVA-IgG1	0.1727	Student	**0.0306**

Bold: Statistically significant at significant level 0.05.

Appendix 6-1 Effects of RK-569 and Dexamethasone on the changes of interleukin (IL) -5 and -13 induced by ovalbumin in mice

Test substance	Route	Dose (ng/head)	Animal No.	IL-5 (pg/mL)	IL-13 (pg/mL)
Non-challenge	i.v.	0	2101	3.674	N.D.
			2102	1.092	N.D.
			2103	0.942	N.D.
			2104	1.158	N.D.
			2105	1.380	N.D.
			2106	1.202	N.D.
			N	6	6
			Mean	1.575	0.000
			S.E.	0.424	0.000
Control	i.v.	0	2201	5.836	N.D.
			2202	11.174	N.D.
			2203	28.118	10.682
			2204	16.452	N.D.
			2205	8.542	N.D.
			2206	3.658	N.D.
			N	6	6
			Mean	12.297	1.780
			S.E.	3.648	1.780
RK-569	i.v.	0.1	2301	1.836	N.D.
			2302	9.552	N.D.
			2303	7.530	N.D.
			2304	21.078	N.D.
			2305	5.768	N.D.
			2306	5.938	N.D.
			N	6	6
			Mean	8.617	0.000
			S.E.	2.699	0.000
	i.v.	1	2401	4.596	N.D.
			2402	12.728	N.D.
			2403	5.516	N.D.
			2404	16.236	N.D.
			2405	25.148	10.368
			2406	4.038	N.D.
			N	6	6
			Mean	11.377	1.728
			S.E.	3.412	1.728
	i.v.	10	2501	0.754	N.D.
			2502	2.052	N.D.
			2503	2.348	N.D.
			2504	4.878	N.D.
			2505	8.564	N.D.
			2506	3.762	N.D.
			N	6	6
			Mean	3.726	0.000
			S.E.	1.129	0.000
	i.v.	100	2601	3.210	N.D.
			2602	0.152	N.D.
			2603	3.270	N.D.
			2604	N.D.	N.D.
			2605	0.208	N.D.
			2606	2.068	N.D.
			N	6	6
			Mean	1.485	0.000
			S.E.	0.635	0.000

N.D.: not detected, calculeted as 0 pg/mL.

Appendix 6-2 Effects of RK-569 and Dexamethasone on the changes of interleukin (IL) -5 and -13 induced by ovalbumin in mice

Test substance	Route	Dose (mg/kg)	Animal No.	IL-5 (pg/mL)	IL-13 (pg/mL)
Dexamethasone	p.o.	3	2701	N.D.	N.D.
			2702	N.D.	N.D.
			2703	N.D.	N.D.
			2704	7.452	N.D.
			2705	2.206	N.D.
			2706	1.686	N.D.
			N	6	6
			Mean	1.891	0.000
			S.E.	1.180	0.000

N.D.: not detected, calculeted as 0 pg/mL.

Appendix 6-3 Effects of RK-569 and Dexamethasone on the changes of interleukin (IL) -5 and -13 induced by ovalbumin in mice - The details of statistical analysis

Comparison with the non-challenge and the control groups

Parameter	F test *p* value	*t*-test	*t*-test *p* value
IL-5	**0.0002**	Aspin-Welch	**0.0320**
IL-13	not statistical analysis		

Comparison with the control and the RK-569 groups

Parameter	Bartlett's test *p* value	Multiple comparison	*p* value of multiple comparison			
			Comparison group with the control			
			0.1 ng/head	1 ng/head	10 ng/head	100 ng/head
IL-5	**0.0050**	Steel	0.8398	0.9932	0.1196	**0.0143**
IL-13	not statistical analysis					

Comparison with the control and the dexamethasone groups

Parameter	F test *p* value	*t*-test	*t*-test *p* value
IL-5	**0.0270**	Aspin-Welch	**0.0347**
IL-13	not statistical analysis		

Bold: Statistically significant at significant level 0.05.

Appendix 7 Organ weights in mice treated with RK-569 and Dexamethasone - The details of statistical analysis

Comparison with the non-challenge and the control groups

Organ	F test *p* value	*t*-test	*t*-test *p* value
Lung	0.0511	Student	**0.0047**
Spleen	0.5597	Student	0.6421
Kidneys	0.8369	Student	0.5424
Liver	0.9607	Student	0.1110

Comparison with the control and the RK-569 groups

Organ	Bartlett's test *p* value	Multiple comparison	*p* value of multiple comparison			
			Comparison group with the control			
			0.1 ng/head	1 ng/head	10 ng/head	100 ng/head
Lung	0.4883	Dunnett	0.9996	0.9242	0.2186	0.0503
Spleen	0.2724	Dunnett	**0.0423**	0.0736	**0.0058**	**<0.0001**
Kidneys	0.7016	Dunnett	0.9069	0.4265	0.9990	0.9990
Liver	0.2303	Dunnett	0.9578	0.8107	0.9971	0.9257

Comparison with the control and the dexamethasone groups

Organ	F test *p* value	*t*-test	*t*-test *p* value
Lung	**0.0496**	Aspin-Welch	**0.0202**
Spleen	0.1334	Student	**<0.0001**
Kidneys	0.4177	Student	0.7765
Liver	0.3929	Student	0.0613

Bold: Statistically significant at significant level 0.05.

Appendix 8 Histopathological findings in mice treated with RK-569 and Dexamethasone

	Test substance	Non-challenge						Control						RK-569 0.1 ng/head					
	Route	i.v.						i.v.						i.v.					
	Animal No.	2101	2102	2103	2104	2105	2106	2201	2202	2203	2204	2205	2206	2301	2302	2303	2304	2305	2306
Bronchus																			
Mononuclear cell infiltration		1	0	1	0	0	0	2	2	2	1	2	1	1	2	2	2	1	2
Hyperplasia, goblet cell		0	0	0	0	0	0	2	1	2	1	2	2	1	1	1	2	1	1
Lung																			
Mononuclear cell infiltration around the bronchioles and blood vessels		0	0	0	0	0	0	2	3	2	3	3	2	2	2	2	1	1	2
Hyperplasia, goblet cell		0	0	1	0	0	0	4	4	3	4	3	2	3	4	2	3	3	4

	Test substance	RK-569 1 ng/head						RK-569 10 ng/head						RK-569 100 ng/head					
	Route	i.v.						i.v.						i.v.					
	Animal No.	2401	2402	2403	2404	2405	2406	2501	2502	2503	2504	2505	2506	2601	2602	2603	2604	2605	2606
Bronchus																			
Mononuclear cell infiltration		0	1	1	1	2	1	1	2	1	1	2	1	0	2	1	2	1	1
Hyperplasia, goblet cell		1	0	1	0	1	1	1	1	1	0	1	2	1	1	1	1	1	1
Lung																			
Mononuclear cell infiltration around the bronchioles and blood vessels		1	1	2	1	1	1	1	1	1	0	2	2	0	1	1	2	1	1
Hyperplasia, goblet cell		3	3	3	2	2	2	3	3	3	0	2	2	3	2	2	2	2	2

	Test substance	Dexamethasone 3 mg/kg					
	Route	p.o.					
	Animal No.	2701	2702	2703	2704	2705	2706
Bronchus							
Mononuclear cell infiltration		1	0	1	1	0	0
Hyperplasia, goblet cell		0	0	1	0	0	0
Lung							
Mononuclear cell infiltration around the bronchioles and blood vessels		0	0	0	0	0	1
Hyperplasia, goblet cell		0	0	1	0	0	0

Grade 0: No change, 1: Very slight, 2: Slight, 3: Moderate, 4: Marked.

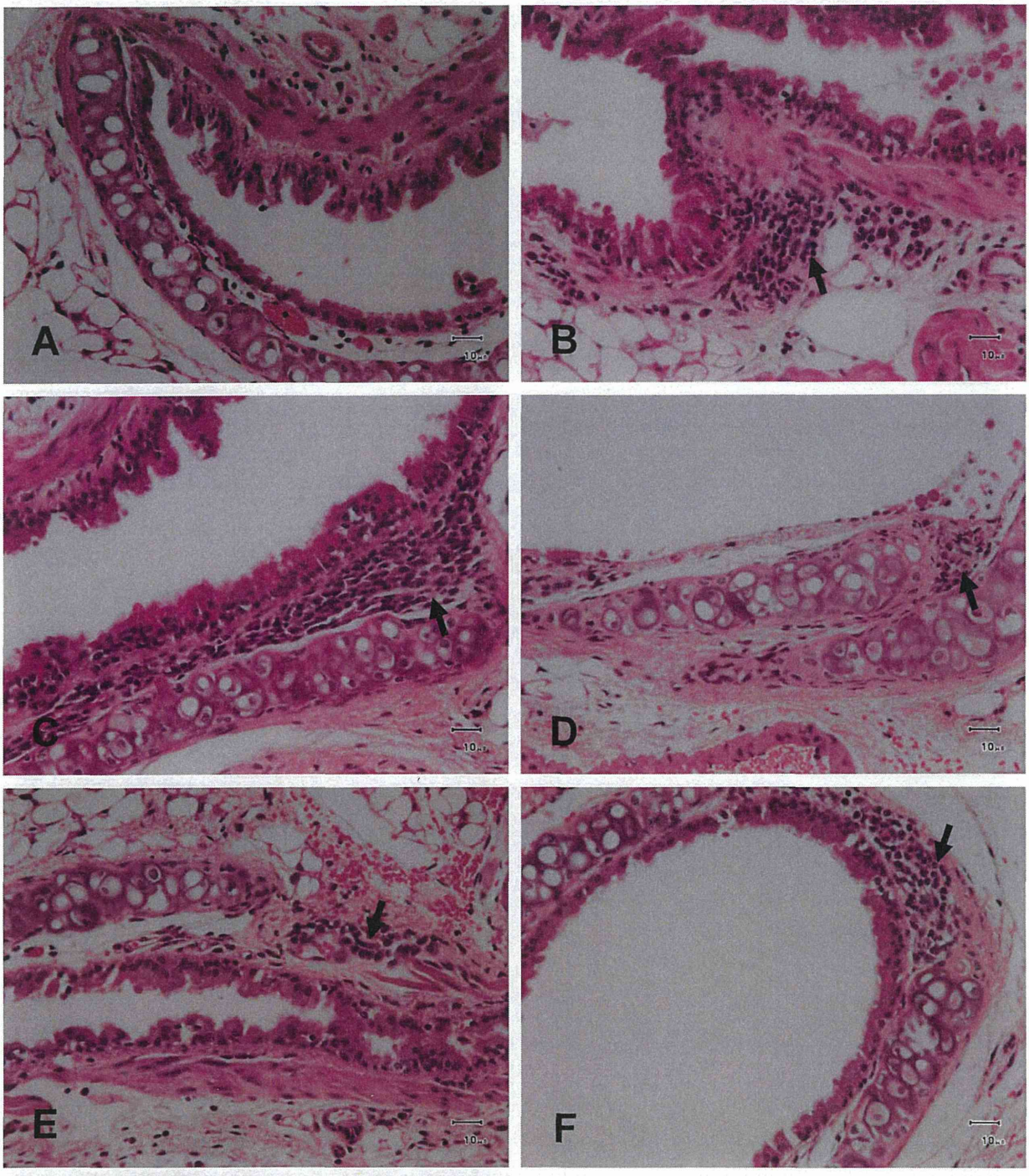

Photo.1-1 Histopathological features in mice treated with RK-569 and Dexamethasone

Bronchus: Mononuclear cell infiltration (HE stain)

A: No change (Non-challenge group, A No.2102)
B: Slight (Control group, A No.2203)
C: Slight (RK-569 5ng/kg group, A No.2302)
D: Very slight (RK-569 50ng/kg group, A No.2403)
E: Very slight (RK-569 500ng/kg group, A No.2506)
F: Very slight (RK-569 5000ng/kg group, A No.2603)
G: Very slight (Dexamethasone group, A No.2703)
Arrow: Mononuclear cell infiltration