Genetic Effects of Mutual Thymus Transplantation between Lines on Marek's Disease Resistance and Immunocompetences in Chickens

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Abstract The genetic effects of thymectomy and thymus transplantation on resistance to Marek's disease (MD) and immunocompetences were studied. Chicks from the IgG-H and IgG-L lines, selected for high and low levels of serum immunoglobulin G (IgG), were thymectomized and some of them were mutually transplanted with the thymus from those of the other line. One group of chicks was inoculated with the JM strain of MD virus and another group was examined for immunocompetences. MD incidence, MD mortality, graft-versus-host reaction (GVHR) competence and antibody titers to sheep red blood cells (SRBC) and *Brucella abortus* were compared among three groups; control, thymectomized and thymus transplanted. The IgG-L line was more resistant to MD than the IgG-H line. In the thymectomized groups, MD incidence and MD mortality increased in both lines. Mean days at MD incidence and MD death showed a decreasing trend. In the thymus transplanted groups, MD incidence and mortality were decreased in the IgG-H line which received thymus from the resistant L line and increased in the IgG-L line which received susceptible H line's thymus. A reverse trend was also observed in the mean days at MD incidence and MD death. The GVHR competence was little affected by thymectomy or thymus transplantation. Immune responses to SRBC and *Brucella abortus* were also not affected by thymectomy. However, thymus transplantation caused a significant decrease of MD resistance in the IgG-H line which received thymus from the IgG-L line, although almost no effect was observed in the IgG-L line which received the IgG-H thymus. It was suggested that MD resistance and immune response competence could be transferred by thymus grafting.

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The finding that Marek's disease (MD) resistance in chickens was transplantable by thymus transplantation was first described by SHEIH and SEVOIAN¹⁰⁾. They examined the effect of transplantation of thymus between MD resistant JM-N and susceptible JM-P lines. When thymectomized JM-P line received JM-N thymus, their MD resistance increased significantly, although the reverse treatment showed

no effect on MD resistance. On the contrary, GALLATIN and LONGENECKER²⁾ reported that mortality from MD was significantly increased in resistant line chicks following neonatal transplantation of thymus of susceptible chicken line. POWELL *et al.*⁸⁾ have also confirmed GALLATIN and LONGENECKER's results. That is to say, the transfer of MD susceptibility by thymus grafting from the susceptible line into

the resistant line was possible, but not in the reverse procedure. Thus, the effect of thymus transplantation is still a controversial problem.

In the previous study the authors also tested the genetic effects of thymus transplantation on MD resistance, using the GVHR-H and L lines⁶⁾. However, the results were not so conclusive, since the difference in MD resistance between the two lines was small¹⁾. Therefore, further experiments were needed to examine the effects of thymus transplantation.

On the other hand, VAN DER ZIJPP *et al.*¹²⁾ reported that genetic resistance to MD was associated with immune response to sheep red blood cells (SRBC). High responders to SRBC were more resistant to MD than low responders.

The IgG-H and L lines, developed by selection for high and low immunoglobulin G (IgG) levels¹¹⁾, are highly different in MD resistance and immune responses⁶⁾. Then, the present study was planned to examine the genetic effects of thymus transplantation on MD resistance and also on immune responses, using the IgG-H and L lines.

Materials and Methods

Chickens

Chickens were obtained from two White Rock lines, IgG-H and IgG-L, which had been established by eight generations of selection for high and low levels of serum IgG at 10 weeks of age¹¹⁾. After that, the lines were maintained by random mating.

Hatched chicks were then wing-banded and used for the experiments. Chicks were kept in batteries during the whole experimental period in an isolated condition.

Surgical procedures

The detailed procedures of thymectomy and thymus grafting have previously described by AFRAZ *et al.*¹⁾.

Challenge experiment of Marek's disease virus

The JM strain of MD virus was supplied from Kanonji Institute, Research Foundation for Microbial Diseases of Osaka University. Chicks of each line were divided into three groups; control, thymectomized and thymus transplanted. All chicks were inoculated intramuscularly with MD virus of a quantity of 3.9×10^3 plaque-forming units at two days of age after finishing the transplantation operation. The chicks were raised together in an isolation room for 68 days, and MD incidence and MD death were checked daily. The day of MD incidence indicates the day that the chick showed characteristic MD symptoms such as paralysis, and the day of MD death shows the day on which the chick fell down with severe paralysis. Autopsies were performed on each dead chick. The experiment was conducted in two replications.

Graft-versus-host reaction

The graft-versus-host reaction (GVHR) competence was measured by splenomegaly. To avoid immunological immaturity, blood was obtained from chickens older than 11 weeks of age. Lymphocytes separated from blood samples were suspended in a density of 10^6 cells/0.1 ml of phosphate buffered saline. A dose of 0.1 ml of the suspension was injected into the chorioallantoic vein of 14-day embryos, and 5 days later spleen weight was measured. Usually, five hosts from a strain with different genotypes were used for each donor. The degree of GVHR was expressed as an index called the splenomegaly index (SI). The SI was calculated by dividing the spleen weight of the embryo in milligrams by body weight in grams.

Immune response

Washed SRBC and *Brucella abortus* were suspended in the same physiological saline at concentrations of 10% and 10^9 cells per ml, respectively. Chickens were injected intravenously with 1 ml/kg body weight of the antigens at 12 weeks of age. The chickens were bled one and two weeks after injection. Antibody titers to SRBC and *Brucella abortus*

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were determined by agglutination test.

Statistical analyses

For total MD incidence and MD mortality, comparisons among groups were conducted by χ^2 tests. Other data were analyzed by the analysis of variance.

Results

Table 1 summarizes the total MD incidence and MD mortality in each treated or non-treated group. In the non-treated control, MD incidence and mortality in the IgG-H line were as high as 17.95 and 7.69%, respectively, although the IgG-L control showed no incidence and no mortality. The line difference of MD incidence was almost significant statistically ($P < 0.1$).

After thymectomy both MD incidence and MD mortality were increased in both the H and L lines, although the difference from the control was significant statistically only for the incidence in the H line.

In thymus transplantation groups, total MD incidence reduced in the IgG-H, when they were compared with the thymectomized group. On the contrary, MD incidence increased in the IgG-L line which received H line's thymus, and the difference from the control was significant statistically. MD mortality in the IgG-H line was lower than that in the control, but that in the L line stayed at the same level as the

thymectomized group, although the differences from the control were not significant in either lines.

Mean days at MD incidence and MD death are shown in Table 2. The data also confirm the results given in Table 1. In thymus grafted groups, the mean days at MD incidence and MD death were delayed in the H line which received the L line's thymus, but became earlier in the L line which received the H line's thymus, although the differences were not significant.

The GVHR competence in each treated group is shown in Table 3. In the non-treated control groups, the SI value of the IgG-H was significantly higher than the L line. Thymectomy treatment brought about a little increase of GVHR competence in the IgG-L line, but not in the IgG-H line. Thymus transplantation caused almost no effect in either lines.

Table 4 shows antibody titers to SRBC and *Brucella abortus* in each treated group. In the untreated control groups the IgG-H line showed significantly higher immune responses to both antigens than the IgG-L line. Thymectomy brought about almost no effect on antibody titers in either lines. On the other hand, thymus transplantation reduced significantly antibody titers in the IgG-H line grafted with the IgG-L thymus, but not in the L line which received the H line's thymus. The effects of

Table 1. Total MD incidence and MD mortality in each experimental group at 68 days of age

Line	Thymectomy	Thymus transplanted from	No. of chicks tested	Total MD incidence(%)		MD mortality(%)	
IgG-H	-	-	39	(7)	17.95 ^a	(3)	7.69 ^a
	+	-	29	(15)	51.72 ^b	(5)	17.24 ^a
	+	IgG-L	27	(11)	40.74 ^b	(1)	3.70 ^a
IgG-L	-	-	11	(0)	0.00 ^A	(0)	0.00 ^A
	+	-	21	(5)	23.61 ^{AB}	(1)	4.76 ^A
	+	IgG-H	21	(7)	33.33 ^B	(1)	4.76 ^A

^{a-b, A-B} Means with different superscripts within each column for each line are significantly different ($P < 0.05$).

() Number of birds attacked or died.

Table 2. Means and standard deviations of age at MD incidence and MD death

Line	Thymectomy	Thymus transplanted from	No. of birds ¹⁾	Mean days at MD incidence	No. of birds ¹⁾	Mean days at MD death
IgG-H	-	-	7	57.00 ± 9.27 ^a	3	63.67 ± 5.88 ^a
	+	-	15	49.54 ± 14.95 ^a	5	50.20 ± 14.31 ^a
	+	IgG-L	11	57.91 ± 13.80 ^a	1	58.00 ± 0.00 ^a
IgG-L	-	-	0	>68 ^A	0	>68 ^A
	+	-	5	67.40 ± 1.34 ^{AB}	1	65.00 ± 0.00 ^A
	+	IgG-H	7	53.71 ± 16.37 ^B	1	65.00 ± 0.00 ^A

¹⁾ Chickens for which the date of MD incidence or death was not recorded were excluded from the data.

^a-^b, ^A-^B Means with different superscripts within each column for each line are significantly different ($P < 0.05$).

Table 3. Splenomegaly index (SI) of donor lymphocytes in each experimental group

Line	Thymectomy	Thymus transplanted from	GVHR	Line difference
IgG-H	-	-	7.56 ± 1.59 ^a (10)	*
	+	-	7.74 ± 2.21 ^a (13)	
	+	IgG-L	7.80 ± 1.60 ^a (14)	
IgG-L	-	-	5.93 ± 1.56 ^A (10)	*
	+	-	6.70 ± 1.86 ^A (5)	
	+	IgG-H	6.48 ± 1.49 ^A (8)	

^a, ^A No significant differences between groups within each line.

() Number of birds used as donor.

* The difference between the IgG-H and IgG-L lines in the non-treated controls is significant at $P < 0.05$ level.

thymus transplantation were almost the same for both SRBC and *Brucella abortus* antigens.

Discussion

OKADA and YAMAMOTO⁶⁾ reported that the IgG-L line was more resistant to MD than the IgG-H line. The present study confirms their results, although the line difference in the present results was not significant statistically. It seems to be due to the limited number of birds used in the present experiment,

especially in the IgG-L line.

After thymectomy, total MD incidence and mortality increased in both IgG-H and L lines. These data support the important role of cell-mediated immunity in MD resistance^{1,3,8-10)}.

GALLATIN and LONGENECKER²⁾ and POWELL *et al.*⁸⁾ indicated that thymus transplantation from susceptible chickens brought about an increase of the MD susceptibility in resistant chickens. However, the reverse procedure, *i.e.*, grafting the thymi of the resistant line into

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Table 4. Comparison of antibody responses to SRBC and *Brucella abortus* between experimental groups

Line	Thymectomy	Thymus transplanted from	SRBC		<i>Brucella abortus</i>	
			1 wk	2 wk	1 wk	2 wk
IgG-H	-	-	11.40±1.06 ^{ab**} (15)	9.13±1.06 ^{a**} (15)	11.73±0.88 ^{a**} (15)	8.60±1.06 ^{a**} (15)
	+	-	11.92±1.50 ^a (13)	9.46±1.80 ^a (13)	11.69±1.18 ^a (13)	9.15±1.72 ^a (13)
	+	IgG-L	10.73±1.16 ^a (15)	7.57±0.76 ^b (14)	10.20±0.94 ^b (15)	8.07±0.47 ^a (14)
IgG-L	-	-	9.10±1.29 ^A (10)	5.00±0.67 ^A (10)	9.80±1.39 ^A (10)	6.10±0.74 ^A (10)
	+	-	9.67±0.82 ^A (6)	5.50±0.55 ^A (6)	9.83±0.75 ^A (6)	6.17±1.83 ^A (6)
	+	IgG-H	8.75±1.28 ^A (8)	5.00±0.76 ^A (8)	9.38±1.06 ^A (8)	6.50±0.76 ^A (8)

^{a-b}, ^A Means with different superscripts within each column for each line are significantly different ($P<0.05$).

() Number of birds tested.

** The differences between the IgG-H and IgG-L lines in the non-treated controls are significant at $P<0.01$ level.

susceptible chickens, did not affect MD resistance of the susceptible recipients. Our previous study was also consistent with their results, although such trend was observed only in MD mortality¹⁾. However, SHEIH and SEVOIAN¹⁰⁾ reported different results, namely, that susceptible chicks became more resistant as a result of thymus transplantation from resistant chicks, but no effect in the reverse grafting. In the present study, although the effects of thymus transplantation were observed in both resistant and susceptible lines, MD incidence of the transplanted group in the H line was still considerably higher than in the control (Table 1). However, MD mortality of the transplanted group was lower than the control. This difference between MD incidence and mortality seemed to be due to the fact that some period was requested for the working of transplanted thymus and also for recovering from surgical injury. Concerning the effect of thymus transplantation, GALLATIN and LONGENECKER²⁾ suggested two possibilities (1)

thymus transplants simply provide more susceptible target cells and (2) a transplanted thymus was able to influence the T-cell phenotype in the process when the host's stem cells differentiate into mature T-cells in the thymus gland. The present data support the second hypothesis, since the grafting effect was observed also in the susceptible line which received the resistant line's thymus. If this hypothesis is true, the effect of thymus transplantation should be also observed in GVHR and immune responses. However, the results in GVHR and immune responses did not necessarily support the hypothesis.

Some workers^{4,7)} suggested that the low GVHR competence is attributed to the restraint of proliferation of T lymphocyte which are target cells of MD virus, and it may lead to MD resistance. Although the line difference in the control groups is in accordance with the hypothesis, the effect of thymus transplantation could not be explained by this.

Effects of thymus transplantation were

also found on immune responses in the IgG-H line grafted thymus from the IgG-L line. Significant decreases in antibody titers were observed in immune responses to both T cell-dependent antigen SRBC and T cell-independent antigen *Brucella abortus*. The result, therefore, suggests that this decrease is not due to the direct effect of T cells. On the other hand, the IgG-L line which received thymus from the IgG-H line showed no significant change in antibody responses. However, since the number of birds tested in the L line was comparatively small, further experiments might be needed to confirm the results.

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胸腺の系統間相互移植が鶏のマレック病抵抗性 および免疫能力に及ぼす遺伝的效果

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胸腺摘除並びに胸腺移植が鶏のマレック病抵抗性および免疫能力に及ぼす遺伝的效果について分析した。免疫グロブリン G (IgG) 量について高低 2 方向に選抜した IgG-H および L 系統のヒナを用いて、胸腺摘除並びに胸腺移植を行なった。胸腺の移植は H, L 両系統間で相互交換することにより行なった。胸腺摘除、胸腺移植および対照群間でマレック病抵抗性、移植片対宿主反応能並びにヒツジ赤血球とブルセラ菌に対する免疫応答能について比較を行なった。マレック病抵抗性は H 系より L 系が大であったが、胸腺摘除群では両系統とも抵抗性の低下がみられた。胸腺移植群では、抵抗性系統である L 系の胸腺を移植された H 系のヒナは抵抗性を回復したが、感受性系統である H 系の胸腺を移植された L 系のヒナでは胸腺摘除群よりも更に抵抗性が低下した。移植片対宿主反応能については、胸腺摘除も胸腺移植もほとんど効果を示さなかった。免疫応答では、L 系の胸腺を移植された H 系においてのみ抗体価の有意な減少が観察された。これらの結果から、マレック病抵抗性および免疫応答については、胸腺移植によって移植された胸腺のもつ遺伝的效果が移植されたヒナに移されることが推測された。

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