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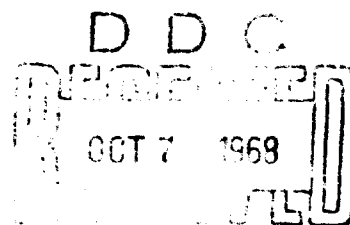
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IMPORTANCE OF THYMUS IN THE ONCOGENE OF ANTIBODY PRODUCTION

ANTIBODY PRODUCTION DURING AND AFTER POX VIRUS

(Following is the translation of an article by U. Bundein, Institute of Animal Hygiene and Zoonotic Diseases, Veterinary College, Hannover, published in the German language periodical *Dtsch. Vet.* 112, pages 143-154 (65). Translation performed by Constance E. Lust.)

For some time it has been assumed that the thymus influences antibody formation. Numerous trials to test this hypothesis have so far all been negative. Warner (1918), Maclean (1954) and Harris (1958) found no difference in antibody formation against several different antigens between thymectomized and normal animals. In the serum of immunized animals no elevated antibody content was found, which was the case for spleen and lymph nodes. Bjornboe 1947, Wagners 1948, Harris 1954, Trowell 1956. In thymocytes Harris et al (1958) or in their tissue culture (Horstbecke and Koenig 1959) no antibodies were found.

In connection between thymic function and antibody syntheses appears probable if one considers the reports of various autoimmune illnesses of humans. If the thymus is lacking, or contains a tumor, there is a loss, or lack, of circulating gamma globulin (Good 1954, Maclean 1956, Ramos 1956, Martin 1957, Boder 1958, Gitlin 1959, Gelfi 1960.)

Finally it was possible to show that early thymectomy in mice, rats, rabbits and guinea pigs is associated with a loss (total or partial) in formation of agglutinating, precipitating and hemagglutinating antibody. After skin testing with antigens as *S. typhimurium*, influenza virus, T2 phage, sheep erythrocytes and BSA no antibodies were formed (Archer 1961, Richtelins 1961, Miller 1961, 1962 a,b, 1963, Archer 1962, Jankovic 1962, Papernaster 1962a, Miller 1962). The host reaction to a transplant (graft) was also slowed, or abolished (Miller 1961 1962 a, c, Arnason 1962, Arnason 1962, Salmasco 1962 a, b, Martison 1962). In the natural erythrocytosis occurs in blood as well as in the lymphatic organs (Paton 1964, Papernaster 1964, Hesse 1964, Cornea 1957, Nakamoto 1957, Moteale 1960, Miller 1961, Schooley 1961, Martison 1961, Parrot 1962, Arnason 1962, Nakamoto 1962).

It was assumed that in the bird the function of the thymic thymus is divided into the thymus and the Bursa Fabricii which is also designated as "cloacal thymus" by Jolly (1928) (Barnes 1962, Papernaster 1962, Warner 1961). When the Bursa Fabricii is removed surgically in early life (few days) an insufficient amount of antibody results (syndrome). This was reported by Chang, 1951, 57, 58, 59, Ghick 1958, Jankovic 1962, Meyer 1962, Furek 1962, Grastner 1963, Kenner 1963).

After injecting testosterone into incubating chicken eggs (between

the 5th and 12th day of incubation) the Bursa Fabricii did not form (Meyer 1959, Porok 1960) and no antibody synthesis followed (Muller 1960, Warner 1961, Muller 1962, Papenmaster 1962 b, Kennes 1963). Ten percent of the animals showed atrophy of thymus along with no bursa. These animals lost the ability to reject skin grafts. Sixty percent had a normally developed thymus, but these animals made no antibodies against a group of antigens (Szenberg and Warner 1962).

Removal of thymus surgically had no clear influence on antibody synthesis. All thymectomized as well as all normal animals formed antibodies after stimulation with human gamma globulin (HGG) (Warner 1962). Other antigens eg. BSA gave the same result (Wolf 1963, Miller 1964, Graetzer 1963, Kennes 1963).

Contrary to the results described above with bacterial antigens are BSA and HGG, if the bursa was removed in chickens, and then infected with NDV (B₁ strain) normal antibody formation was seen. Cho (1963) concluded that the bursa fabricii is not always involved in antibody formation, but that this depends on the type of antigen. It may be possible that in fowl the thymus is responsible for the synthesis of certain antibodies.

In extensive trials the effect of thymectomy of chickens on antibody formation was investigated during atypic NDV and chicken pox infection.

Methods and Materials

The whole experiments were performed with 124 HNL chicks. The eggs were obtained from NDV -and pox free colony and were hatched in the institute. For the NDV trial 60 chicks were used in groups of 10. For the pox trial 64 chicks were grouped per 16 animals. Half of the chicks were thymectomized at 3-6 days; the rest were controls. All chicks were kept in incubators and fed pellets of food.

Operation: Anesthetic after Freytag (1963) with nembutal diluted 1:6 with saline; dose of 0,005 ml per gram body weight IM. Median cut dorsal from atlas to chest, the thymus knots were removed with a bent forceps. Suture with 4 stitches (not removed).

Newcastle Disease Virus: The Hitchner B₁ strain of ND water vaccine (Behring Co.) was used. The dose for 100 animals was dissolved in 100 ml H₂O and every animal received 1 ml by pipette into the gullet. The chicks were challenged with highly virulent field virus. Every chick was injected with 1 ml of 1:50 dilution (10⁶ egg LD 50).

Chicken Pox: Chicks were vaccinated into wings with type H. Challenge with strain C 360, 1 ml 1:50 dilution IV. Titer was 10⁵ egg LD corresponding 320 cell agglutination units (Kanguike and Hanson 1961).

Serology: Blood was obtained by left-side heart puncture 1 week post vaccination and two weeks post infection. Serum was stored at -20°C and used as: 1) hemagglutination (HI) inhibition diluted 1:5 - 1:20, 840,

2) Agar gel precipitation (Meyers and Smith 1950), 3) Neutralization test (Meyers 1950). The neutralization test was chosen because it conserves serum and because it shows differences in the antibody concentration directly (Meyers and Smith 1950).

A single vaccination with Hitchner B₁ was done at 3 weeks of age, infection 3 months later. This illness without great danger of illness occurred.

Statistics: The neutralization or HI titers of control and thyrosectomized groups were compared by the t-test. The median value and standard deviation and significance were calculated according to Mudra (1950).

Since no control HI titration was there the statistical could not be compared to the initial data. For a dilution of 1:5 SM₂ or HI a value of 1; 1:10 = 2,) 20 = 3. IIF a positive value = 1 negative = 0; they were calculated with the "chi" square test after Yates (1940).

Results

Nonfatal Disease: Without vaccine no differences in clinical course after infection with field virus was seen between the normals and thyrosectomized. Illness was severest at day 3 and 6 in both groups, (loss of appetite, dyspnea, diarrhea). In the following 3 days all but one chick in each group died.

After infection after vaccination, likewise no difference was seen. Incubation and course of illness correspond to the unvaccinated group. Mortality was clearly lowered. Forty-five per cent of 20 Hitchner B₁ vaccinated animals (5 thyrosectomized and 4 controls) died in the first week after infection.

During the pathology study all dead animals showed typical signs. In most chicks hyperemic and bleeding of the organs was observed.

A table of the serologic results is presented in table 3 (statistical analysis in table 4).

The following conclusions are made:

In controls not vaccinated, not infected, no positive IIF or HI or SM₂ tests.

Of those vaccinated with Hitchner B₁ not all precipitated sera, except one thyrosectomized chick 4 weeks post vaccination.

HI titers were positive in 4 thyrosectomized animals (1:40, 1:10, 1:10, 1:10) and in 3 normals. Neutralizing antibodies were demonstrated for 1 (1:20) thyrosectomized and 1 normal (1:5). The elevated antibody content in the normal animals 4 weeks post vaccination is not statistically

significant. Fifteen weeks after vaccination all sera were negative in the precipitation test.

HI titers were positive in 4 of 5 sera in both groups. Generally the small differences seen were not significant.

The difference in formation of neutralizing antibodies was clearer. In the normal group all 5 animals were positive (1:10, 1:5, 1:5, 1:5, 1:5); in the thymectomized only 2 of 5 (1:5, 1:5). This difference is statistically weakly significant. Two weeks after HI infection with virus, after previous vaccination with Hitchner B₁, no significant differences were seen.

Nine thymectomized and 4 of 5 normals gave positive sera in MPP. Sera of all chickens -also the thymectomized- had high NT and HI titers (between 1:640 and 1:5120).

It was assumed that full immunity against NDV is probable so long the HI titer is at a level of 1:4. (Bungeledorff 1963). However, 15 weeks after vaccination of chicken which had HI titers of 1:10, experimental infections resulted in a high mortality and illness in almost all. To be sure these animals were infected parenterally with high doses of virus. In the infected group which had not been vaccinated (high mortality) no serologic studies were done.

Pox

No differences were seen in the formation of a local reaction between normals and thymectomized animals. In all cases the reactions could be read easily on day 7 post vaccination. The clinical picture also looked the same. The first pox appeared on the comb on day 5 in the vaccinated group, on day 16 the normal signs could be seen in all 16 animals. One thymectomized chicken died on day 13. None of the vaccinated chickens became ill after infection with pox.

A review of the serologic results are presented in table 5. The following can be concluded.

The precipitation tests were negative in controls (neither vaccinated nor infected); also in the vaccinated animals this was as expected.

Two weeks after the IV infection with field virus (after vaccination) all sera of thymectomized animals gave negative precipitative test. In controls 3 of 8 chicks -sera precipitated. But, this difference is not significant. Smaller still was the difference in the non-vaccinated chickens; 2 weeks post infection precipitative tests were positive in sera of 4 of 8 thymectomized and 5 of 8 normal chickens.

It must be definite that neither the bursa fabricii nor the thymus plays a dominant role in humoral antibody formation against atypical chicken NDV. The thymus also does not influence immunity in chicken pox. It should be mentioned that even with much care during thymectomy of chickens

small pieces of thymus may remain in the animal (Warner 1968, Aspinall 1969). These small bits may exert an influence even though it be reduced. Warner et al (1968) and Aspinall (1969) used this concept to explain their data concerning protracted, incomplete rejection of skin grafts in chickens.

Because of this the differences we report must be considered, even though they may not be convincing. Therefore the differences in antibody formation 15 weeks post vaccination with Hitchner E₁ virus, or infection with pox, should be considered even though they are not statistically significant.

The possibility does exist that this small difference in antibody titer against Hitchner E₁, is not due to a protracted formation, but due to an increased removal of the antibody level after thymectomy.

Since the local vaccine-reaction, the level of protection, the clinical picture was the same, or only slightly different (not statistically significant), one cannot conclude unequivocally that the thymus has an important function in antibody formation in chicks against MDV or chicken pox.

Summary

After inoculation and infection with the virus of MD and FP no significant differences were detected between thymectomized and not-thymectomized chickens in respect of the reaction to vaccination, formation of humoral antibodies, degree of immunity, disease pattern and mortality.

It is worth pointing out that in the thymectomized chickens infected with FP the number of positive precipitation tests, particularly in the vaccinated birds, was somewhat less than in the control birds and that 15 weeks after vaccination with Hitchner E₁ MD virus the neutralization (0.05-0.01) and HI titers were also lower in the thymectomized birds.

Figure 1

Chicks before thymectomy. Both thymus glands visible



Table 1

Experiment with Newcastle Disease Virus - Number and age in selected

		(1)	(2)	(3)	(4)	(5)	(6)
	Tiere pro Gruppe	thymek- tomisiert im Alter von	vacciniert im Alter von	infiziert im Alter von	Anzahl der Blut- entnah- men	Blutentnah- men im Alter von	
Kontrolle	nicht thymektomisiert	5			5	2 W 18 W	
	thymektomisiert	5	3-6 T		5	2 W 7 W 18 W	
Hühner 3:	nicht thymektomisiert	5		3 W	5	7 W 18 W	
	thymektomisiert	5	3-6 T	3 W	5	7 W 18 W	
Hühner 3: Feldvirus	nicht thymektomisiert	10		3 W	5	18 W	
	thymektomisiert	10	3-6 T	3 W	5	18 W	
Feldvirus	nicht thymektomisiert	10		16 W	5		
	thymektomisiert	10	3-6 T	16 W	5		

T=Tage W=Wochen

(1)-animals per group (2)-thymectomized at age (3)-vaccinated age
 (4)-infected age (5)-blood taken (6)-age

Table 2

Experiment with pox Virus

		(1)	(2)	(3)	(4)	(5)	(6)
	Tiere pro Gruppe	thymek- tomisiert im Alter von	vacciniert im Alter von	infiziert im Alter von	Anzahl der Blut- entnah- men	Blutentnah- men im Alter von	
Kontrolle	nicht thymektomisiert	5			5	2 W 5 W 14 W	
	thymektomisiert	5	3-6 T		5	2 W 5 W 14 W	
Pocken- Vaccine	nicht thymektomisiert	5		3 W	5	5 W 14 W	
	thymektomisiert	5	3-6 T	3 W	5	5 W 14 W	
Pocken- Vaccine Feldvirus	nicht thymektomisiert	5		3 W	5	14 W	
	thymektomisiert	5	3-6 T	3 W	5	14 W	
Feldvirus	nicht thymektomisiert	5		12 W	5	14 W	
	thymektomisiert	5	3-6 T	12 W	5	14 W	

T=Tage W=Wochen

(1)-animals per group (2)-thymectomized at age (3)-vaccinated age
 (4)-infected age (5)-blood taken (6)-age

Table 3

Antibody formation and virulence in thymectomized and control animals
 (after vaccination with virulent B₁ and infection with virulent B₂
 100% virulent, 100% virulent, 100% virulent, 100% virulent, 100% virulent
 100% virulent, 100% virulent, 100% virulent, 100% virulent, 100% virulent

			1	2	3	4	5	Mortality in % of the p.p.		
Non-vaccinated	nicht thymektomisiert	N-Test	—	—	—	—	—			
		NAH	—	—	—	—	—			
		AGP	—	—	—	—	—			
	thymektomisiert	N-Test	—	—	—	—	—			
		NAH	—	—	—	—	—			
		AGP	—	—	—	—	—			
Feldvax B ₁ + Woodcock p.p.	nicht thymektomisiert	N-Test	1: 5	—	—	—	—			
		NAH	1: 10	1: 10	1: 5	—	—			
		AGP	—	—	—	—	—			
	thymektomisiert	N-Test	1: 20	—	—	—	—			
		NAH	1: 40	1: 10	1: 10	1: 10	—			
		AGP	+	—	—	—	—			
Feldvax B ₂ + Woodcock p.p.	nicht thymektomisiert	N-Test	1: 10	1: 5	1: 5	1: 5	1: 5			
		NAH	1: 10	1: 10	1: 10	1: 10	—			
		AGP	—	—	—	—	—			
	thymektomisiert	N-Test	1: 5	1: 5	—	—	—			
		NAH	1: 5	1: 5	1: 5	1: 5	—			
		AGP	—	—	—	—	—			
Feldvax B ₂ Feldvax	nicht thymektomisiert	N-Test	1:1280	1:2560	1:2560	1:2560	1:1280	50%		
		NAH	1:2560	1:1280	1:1280	1:1280	1: 640			
		AGP	+	+	+	+	—			
	thymektomisiert	N-Test	1:1280	1:2560	1:2560	1:1280	1: 640	40%		
		NAH	1:2560	1:1280	1:1280	1:1280	1:1280			
		AGP	+	+	+	+	+			
Feldvax	nicht thymektomisiert	N-Test							93%	
		NAH								
		AGP								
	thymektomisiert	N-Test							93%	
		NAH								
		AGP								

Table 4

Statistical evaluation of the results in table 3

			1	2	3	4	5	6	7	8	9
								Mean- wert	Stan- dard- Abwei- chung	T-Test χ^2 -Wert	Statist- ische Signi- fikanz (%)
Kontrolle	nicht	N-Test	—	—	—	—	—	0	0	0	—
	thymektomiert	HAH	—	—	—	—	—	0	0	0	—
		AGP	—	—	—	—	—	0	0	$\chi^2 = 0$	—
	thymektomiert	N-Test	—	—	—	—	—	0	0	—	—
		HAH	—	—	—	—	—	0	0	—	—
		AGP	—	—	—	—	—	0	0	—	—
Hitchner B 1 4 Wochen p.i.	nicht	N-Test	1	—	—	—	—	0,00	$\pm 0,45$	0,63	—
	thymektomiert	HAH	2	2	1	—	—	1,00	$\pm 1,00$	1,29	—
		AGP	—	—	—	—	—	—	—	$\chi^2 = 0$	—
	thymektomiert	N-Test	3	—	—	—	—	0,60	$\pm 1,34$	—	—
		HAH	2	2	2	2	—	2,00	$\pm 1,41$	—	—
		AGP	—	—	—	—	—	—	—	—	—
Hitchner B 1 15 Wochen p.i.	nicht	N-Test	2	1	1	1	1	1,20	$\pm 0,45$	2,52	—
	thymektomiert	HAH	2	2	2	2	—	1,00	$\pm 0,80$	1,78	—
		AGP	—	—	—	—	—	—	—	$\chi^2 = 0$	—
	thymektomiert	N-Test	1	1	—	—	—	0,40	$\pm 0,55$	—	—
		HAH	1	1	1	1	—	0,80	$\pm 0,45$	—	—
		AGP	—	—	—	—	—	—	—	—	—
Hitchner B 1 ND-Feldvirus	nicht	N-Test	11	10	10	10	9	10,00	$\pm 0,71$	0,67	—
	thymektomiert	HAH	10	9	9	9	8	9,00	$\pm 0,71$	0,56	—
		AGP	1	1	1	1	—	—	—	$\chi^2 = 0$	—
	thymektomiert	N-Test	11	10	10	9	8	9,60	$\pm 1,28$	—	—
		HAH	10	9	9	9	—	9,20	$\pm 0,39$	—	—
		AGP	1	1	1	1	1	—	—	—	—
ND-Feldvirus	nicht	N-Test	—	—	—	—	—	—	—	—	—
	thymektomiert	HAH	—	—	—	—	—	—	—	—	—
		AGP	—	—	—	—	—	—	—	—	—
	thymektomiert	N-Test	—	—	—	—	—	—	—	—	—
		HAH	—	—	—	—	—	—	—	—	—
		AGP	—	—	—	—	—	—	—	—	—

6-mean 7-standard deviation 8- χ^2 9-significance

N=neutralization, HAH=HI AGP=agar gel precipitation test

		1	2	3	4	5	6	7	8	Positive SGP-H-4	2°	Antibody titer
Rabbits	Thymectomized	—	—	—	—	—	—	—	—	0	—	—
	Thymectomized	—	—	—	—	—	—	—	—	0	—	—
Mice	Thymectomized	—	—	—	—	—	—	—	—	0	—	—
	Thymectomized	—	—	—	—	—	—	—	—	0	—	—
Guinea Pigs	Thymectomized	—	—	—	—	—	—	—	—	37.5	1,64	—
	Thymectomized	—	—	—	—	—	—	—	—	0	—	—
Rats	Thymectomized	—	—	—	—	—	—	—	—	62.5	0.05	—
	Thymectomized	—	—	—	—	—	—	—	—	50.0	—	—

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