World Academy of Science, Engineering and Technology
International Journal of Animal and Veterinary Sciences
Vol:7, No:7, 2013

Tuberculin, Tetanus Immunoglobulin and DPT Vaccine as an Avian *in vivo* T- Lymphocyte Mitogens

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***Abstract*—**The avian phytohaemagglutinin skin test is being proved as an in vivo system for the evaluation an avian *in vivo* T cell mitogenicity. The test system was one week old *Gallus domesticus* broiler Chickens. Five replicates were done for each of the whole, 1:10 dilutions of each of 0.05 IU tuberculin, tetanus immunoglobulin and DPT vaccine as test materials. The evaluation parameters were the skin indurations and lymphoblast percentages in bone marrow lymphocytes.

Tuberculin indurations were 2.06 and 1.26mm for 0.05 IU respectively while lymphoblast percent were 0.234 and 0.1 accordingly.

The skin indurations of 135mg/ml and 1.35mg/ml tetanus immunoglobulin were 4.86 and 3.96mm while lymphoblast percentages were 0.3 and 0.14 respectively.

The whole DPT and 1:10 concentration were with 4.5 and 3.2mm while their lymphoblast percentages were 0.28 and 0.12 accordingly.

Thus the mitogenicity of the test materials was of dependant type.

***Keywords*—**DPT, Mitogenicity, Tetenus, immunoglobulin, Tubercular.

I. Introduction

THE phytohaemagglutinin in (PHA) skin testing is helpful in studing the avian immune competence [1]-[7].

Injection of PHA into patagium of captive green finches (*Carduelis chrlorids*) increases the concentration of heterphils the phagocytic cells in peripherial blood for at least 30 days [7]. Shnawa and Albyatee [8] have been modified the test check mitogenicity of some plant seed lectins to the avian T-lymphocytes.

The objective of the present work was to investigate T cell mitogenicity of some standard biological products through scoring skin indurations and lymphoblast percent in bone marrow smears.

II. Materials and Methods

A. Test Mitogens

Tubercullin: Atubersol, "tuberculin", the purified protein derivation (PPD), mode by Aventis Pasteur limited, the product was diluted to 0.05 and 0.05 IU.

Standard Tetanus immune globulin: It is Tetagam® with the active ingredient of human tetanus immunoglobulin. The product was used as whole 135mg/ml and 1.35mg/ml. It was made by CSL Behring each ampoule contains one milliliter.

DPT vaccine: It is DTCDQ/ DPT for intramuscular rout. The vaccine meets W.H.O. requirements.

The ampoule size was five milliliter given as ten doses. Lot number E7010. Expiration date April 2012 should be kept between 2-8°C when stored. The vaccine was diluted to 1:10 in addition to whole preparation. It is a product of Sanofi Pasteur.

B. Control Mitogens

Vibrio sp. LPS prepared as in Kwapinski [9] and Standard PHA made of Iraqi Human Genetic Center.

C. Test System

One week old *Gallus domesticus* chicken weighing 20-25mg each were adapted for housing conditions for 48hr, Then 0.1ml of each of the three test materials and their dilutions was injected through subcutaneous rout in patagium area of the wing .The indurations were measured 18 hrs post injection. To stop cell cycle, 100mg/ml cholchicine in a rate of 0.25 ml per each animal was injected intramuscularly. One hour later, femur bone was trimmed from both ends and 5mls of sterile saline injected for bone marrow collection [8], [10].

D. T cell Mitogenicity

Skin indurations, using vernia, skin of wing of non-treated chickens was calibrated, test chickens showing indurations were also calibrated in same way. The actual in duration is the subtraction of after injection from before injection.

Lymphoblast Percentage:

Thick bone marrow smears were made and Giemsa stained for each chick showing positive indurations as in Dacie [10]. Thousand lymphocytes were counted for presence of lymphoblast percentages [11]-[12]. The percentages were calculated as iD the following formula.

$$\text{LB\%} = \frac{No\ of\ lymphblast}{1000} \text{ X } 100$$

The LB% was made for control chickens in same way of the test materials.

Scores for skin indurations and LB% for vibrio sp. LPS and standard PHA was made as for test materials.

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III. Result

A. Tuberculin:

The tuberculin dilutions of 0.05 and 0.005 IU were found with skin indurations means of 1.84 and 1.09 mms while the lymphoblast percentage were 0.234 and 0.1 respectively.

B. Tetanus Immunoglobulin:

The 135mg/ml and 1.35mg/ml of tetanus immunoglobulin were showing in duration of 4.88and 3.84mm. As well as bone marrow lymphoblast percentage were 0.3 and 0.14 respectively (Table II).

C. DPT Vaccine:

The whole and $1/10^{th}$ dilution of the vaccine was within duration of 1.89mm and 1.09mm while the lymphoblast percentages were 0.26 and 0.11 (Table III).

D. T cell Response Nature:

The skin indurations as well as lymphoblast percentages were found of dose dependent type (Table I-III).

IV. Discussion

Phytohaemagglutinin PHA skin testing had been originally evolved for the evaluation of the avian immune competence in normal state as well as in the cases of parasite infestation or toxicosis [1]-[6].

The test is only skin in duration reaction [1]-[6] Shnawa cmd. Al Byatte [8] has been using it for the in vivo mitogenicity testings of lectin solutions, tuberculin as well as LPS. The aim of the present work was to prove the possible mitogenic action of Tetanus immunoglobulin, DPT and preapproval of tuberculin in lower concentration spectrum [8].

Tetanus immunoglobulin is an immune reagent for passive immunization against accidental tetanus and supposed to be safe [12]. The present result proposed it as an avian T Cell mitogen.

The classical version of DPT vaccine was Th2 response inducer in mammalian host. However when the conjugate substituted by subunit moiety of pertusses it becomes Th1 and Th2 response inducer [13]. Now it is proved as Th1 inducer in an avian lymphocyte in vivo.

Thus, results presented in Table I-III were showing that the testing biological products were showing an in vivo T cell mitogenicity in an avian system. These mitogenic potentials were of dose dependent type and reflect lights on the possible cell mediated immune reactions including delayed type hypersensitivity. Since it is proved in avian vertebrate might works in mammalian vertebrate and man due to the evident presence of homology in the elements of lympho reticular tissue as well as the uniformity of plane of cellular and molecular organization of the components of adaptive immunity, which they are strikingly conserved among chordates including man [14].

The mitogenicity proved for standard biological products (Tables I-III), approve the previous recommendation [8].

TABLE I
The Tuberculin Mitogenicity in Week Old Chicken

Chicken repeals	Indurations reaction		Lymphoblast percentage	
	After	Before	Test	control
	0.05	0.005	0.15	0.005
C1	2.0	1.5	0.19	0.09
C2	2.1	1.1	0.21	0.1
C3	2.2	1.7	0.18	0.099
C4	1.9	0.9	0.19	0.1
C5	2.1	1.6	0.2	0.12
C⁻	2.06	1.26	0.234	0.1
PHA 0.25mg/ml		2.0	0.37	
LPS 0.25mg/ml		1.9	0.59	

TABLE II
Tetanus Immunoglobulin Mitogenicity in One Week Old Chickens

Chicken repeals	Indurations reaction		Lymphoblast percentage	
	Test	control	Test	Control
	135mg	1.35mg	135mg	1.35mg
C1	5.0	4.1	0.3	0.1
C2	4.9	3.5	0.32	0.16
C3	5.10	4.2	0.29	0.18
C4	4.50	4.0	0.31	0.10
C5	4.8	4.0	0.28	0.19
C⁻	4.86	3.96	0.3	0.144
PHA 0.25mg/ml		2.0	0.37	
LPS 0.25mg/ml		1.9	0.59	

TABLE III
The Mitogenicity of DPT Vaccine in One Week Old Chickens

Chicken repeals	Indurations reaction		Lymphoblast percentage	
	Test	control	Test	Control
	W	1/10	W	1/10
C1	3.5	3.0	0.3	0.005
C2	3.9	2.8	0.25	0.1
C3	6.1	4.6	0.35	0.15
C4	6.0	3.1	0.2	0.15
C5	3.0	3.0	0.2	0.1
C⁻	3.0	3.0	0.28	0.12
PHA 0.25mg/ml		2.0	0.37	
LPS 0.25mg/ml		1.9	0.5	

References

[1] P. Christe, F. delope, G. Gonzalez, N. Saino and A. P. Moller, The influence of environmental conditions on immune response ,morphology and recapture probability of nestling house martins (Delechsn Urbica) Occlogia Vol.126 pp.333-338, 2001.

[2] P. Christe, A. P. Moller, N. Saino and F. delopes, Genetic and environmental components of phenotypic variation immune response and body size of colonial bird, Delichon Urbical the house mortin. Heredity 85.pp75-83, 2000.

[3] J. E. Smits and T.D. Williams, Validanin of immunotoxicology Techniques in passerine chicks Exposel to oil sand Tailling water, Ecotoxicol. Envir.Saf. 44, pp,105-112,1999.

[4] J. E. Smits, G. R. Bortolotti J. L. Tella, Simplifying the phytohaem agglutinin skin testing technique in studies of avian immune competence, Funcl. Ecol. Vol. 13, pp.567-572, 1999.

[5] P. Christe, A. P. Moller and F. delope, Immunocomptence and nesting survival in the house martin. the tasty chick hypothesis, Oiks, Vol.83.pp.175-179.

[6] J.J. Solar, A.P. Moller, M. Soler and J.G.Martinez, Interaction between a brood parasite and its host in relation to parasitism and immune defense, Evol. Ecol. Res.Vol. 1, pp. 189-210.

[7] T. Sarv and P. Harak, Phytohaemagyltinin has a log-lasting effect on immune cells, J. Avian Biol, Vol. 40, pp., 559-751, 2009.
[8] I.M.S. Shnawa and L. A. A. Al Bayatee, An invivo Phytolectin induced skin test and T cell mitogenicity, Al Qdisiya J. Vet. Med. Sci. Vol. 8, no, pp. 1-7, 2009.
[9] J.B.G. Kwapinski, Methodology of Immunochenical and Immunological Research, New York, Wiley inter science, 1972, pp. 164-184.
[10] J. V. Dacie and S.M. Lewis 4th ed. London Janna, A. Churchill, 1970, 114-132.
[11] L. J. Bradshaw, Laboratory Immunology, 2nd ed., Washington, Saunder College Publishing, 1996, pp. 127-133.
[12] D.D. Banker, Modern Practice In Immunization, Bombay, Popular Prakashan, 1980, pp.164-184.
[13] S. H. R. Kaufmann, Novel vaccination strategies federal republic of Germany, Wiley .VCH, 2004, pp. 243-245.
[14] J. Horton and N. A. Ratcliffe, Evolution of immunity in immunology, 6th ed. I Roitt, J. Brostoff and D. Male Eds, New York, Mosby, 2001, pp. 211- 229.