Journal of Environmental and Applied Bioresearch
Published online 05 December, 2015 (http://www.scienceresearchlibrary.com)
Vol. 02, No. 02, pp. 49-51
ISSN 2719 8745

Research Article **Open Access**

CELL MEDIATED IMMUNITY INDUCED BY HELMINTH ALLERGEN OF GASTROINTESTINAL NEMATODE(*ASCARIDIA GALLI*) IN *GALLUS DOMESTICUS*

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Received: 01 October, 2015 / Accepted : 08 November, 2015

Abstract

Cell mediated immunity involve study of by antigen specific-T lymphocyte, B lymphocyte, monocytes, neutrophils,macrophages.(Dey N C et.al. 1996). Present study have been done against helminth allergen(*A. galli*-GI nematode) in sensitized and hypersensitized *Gallus domesticus* (after the 10th day of the primary sensitization) with the low dose and high doses of the embryonatd eggs of *A. galli*. T lymphocyte and B lymphocyte were identified according to size and density using standard technique. Monocytes, promotes growth of macrophages were found to be increased and neutrophils(PMN) act as accessory cell for T cell activation, were found to be decreased

Key words-Hypersensitivity reaction IV, *Ascaridia galli*, white leg horn chicks, T lymphocyte, B lymphocyte

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Introduction

Parasitic infection account for hundred of millions of dollars in annual losses and medicated cost sin the livestock and poultry industry throughout the world. The most costly parasite in terms of production losses are the gastrointestinal nematodes in ruminants and poultry. Parasites live in the intestine and stomach walls, secrete antigens that act as allergens. For larger worm –as for gastrointestinal nematode(*Ascaridia galli*), the host (*Gallus domesticus*) will often develop inflammation and hypersensitivity. Hypersensitivity or allergy is an inappropriate immune response that cause in tissue damage with second or subsequent contact with the same antigen (helminth allergen) in already sensitized host. Most allergic reactions involve the allergen binding on to special immune system cells and causing these cells to release compounds that affect surrounding tissue. Hypersensitivity reaction-IV involve study of cell mediated immunity by antigen specific-T lymphocyte, B lymphocyte, monocytes, neutrophils, macrophages. This is the only class of hypersensitive reactions to be triggered by antigen-specific T cells. They were originally termed DTH after the alternative name for this reaction - delayed type hypersensitivity. Delayed type hypersensitivity results when an antigen presenting cell, has picked up antigen, processed it and displayed appropriate peptide fragments bound to class II MHC is contacted by an antigen specific Th1 cell patrolling the tissue. The resulting activation of the T cell produces cytokines such as chemokines for macrophages, other T cells and, to a lesser extent, neutrophils as well as TNFbeta and IFNgamma(Ward et al.1998) The consequences are a cellular infiltrate in which mononuclear cells (T cells and macrophages) tend to predominate.

MATERIAL AND METHOD –

Experimental host- white leghorn chicks
Experimental parasite- *Ascaridia galli*
Dose of infection – low dose- 1000 embryonated eggs of the *A.galli*
High dose-2000 embryonated eggs of the *A.galli*

Maintenance of experimental host –

One day old white leg horn chicks were purchased from the poultry hatcheries and kept in the cages of animal house of the Zoology department under hygienic condition. The treatment was started after 15 days of maintenance.

Procurement of Parasites –

Adult worms were isolated from the intestine of fowl from the local abattoir.Female worms were kept in petridishes separately. These petridishes were incubated at low temperature of 34ºC.

Culturing of the egg-

A .galli eggs were cultured upto infective stage according to Reidel method (1947). The worms were gently squeezed from backward to forward, with uniform pressure until

the entire reproductive organs passed out into the culture medium in the petridish.The uteri containing mature eggs were gently squeezed for fertilized eggs.The eggs were kept in sterile solution at 32°C for embryonation for 2-3 weeks.Two drops of 0.1% fromaline was added to prevent fungal infections. Now thesse embryonated eggs have been used for sensitization and hypersensitization of the chicks.

Preparation of the inocula-

The number of embryonated eggs per dose was estimated by dilution technique.The embryonated and infective eggs were counted with the help of stereoscopic binocular microscope.

Counting of T lymphocyte and B lymphocyte-

The blood of different groups of chicks such as nonsensitized (control), sensitized , and hypersensitized were taken into the small glass vial which contained 10 units/ml/ preservation free heparin. Tand B lymphocyte were separated by ficoll hypaque and identified according to size and density (Deys et,1995).For counting of T lymphocyte and B lymphocyte 10ųl aliquots was kept on the counting slide. A cover slip was kept over the counting slide and slide was studied under light microscope for the counting of the lymphocyte. Counting of lymphocyte was repeated three times and mean was taken.

Y=Mean of number of Tand B lymphocyte in 10ųl aliquots

Y x1OO=X

X= Number of T and B lymphocyte/ml of blood

Percentage of T lymphocyte = Number of T lymphocyte x100

Total number of T and B lymphocyte

Percentage of B lymphocyte = Number of B lymphocyte x100

Total number of T and B lymphocyte

Counting of neutrophil, monocyte-

For monocyte and neutrophil, a thin blood film was prepared and then slide were stained in leishmania stain for 10 min then wash with running tap water to remove excess of stain.

EXPERIMENTAL DESIGN-

During present investigation chicks were divided into 3 groups- Group-A having nonsensitized chicks, group-B having chicks sensitized and hypersensitized with the low dose of the egg antigen Group-C having chicks sensitized and hypersensitized with the high dose of the egg antigen, each group comprise of 6 chicks .Primary infection of low dose and high dose were given to the 15 days old white leg horn chicks. Now both the group-B and Group-C is further divided into two group-Ba &group-Bb and group-Ca & group-Cb for the hyper sensitization of the chicks. 2 Chicks from each group (Aa, Ba, Ca) were autopcised after 10 days of the primary sensitization and rest chicks were hyper sensitized with the secondary infection of the low dose (Bb) and high dose (Cb) subsequently. The chicks were autopcised at 35th (Bb_1&Cb_1) and 45 days (Bb_2&Cb_2) day of the experimental design.

OBSERVATION AND STATISTICAL ANALYSIS-

Data on different cell values were statistical calculated by using T test and analysis of varience. Observation have been taken during cell mediated immunity were shown in tables-1,2 and 3.

Table-1 Parameters of cell mediated immunity in *Gallus domesticus* sensitized with the low dose and high dose of embryonated eggs of the *A.galli* at 25th of the experimental design

Parameters		Nonsensitized	Sensitized with low dose	Sensitized with high dose
T lymphocyte %	S.D	66	62	58.4
		±4.472	±1.581	±1.14
	S.E	±2	±0.7071	±0.5099
B lymphocyte%	S.D	30.4	34.4	37.4
		±1.14	±1.14	±1.673
	S.E	±0.5099	±0.5099	±0.7483
Monocyte percentage M%	S.D	4	6	6
		±1.581	±1.581	±2.236
	S.E	±0.7071	±0.7071	±1
Neutrophil percentage N%	S.D	40	38	35
		±2.55	±1.581	±1.581
	S.E	±1.14	±0.7071	±0.7071

Table-2 Parameters of cell mediated immunity in *Gallus domesticus*, sensitized and hypersensitized with the low dose and high dose of embryonated eggs of the *A.galli* at 35th of the experimental design

Parameters		Nonsensitized	Sensitized with low dose	Hypersensitized with low dose	Sensitized with high dose	Hypersensitized with high dose
T lymphocyte	S.D	65.2	59.8	58	56.6	57.6
		±0.8367	±0.8367	±1.581	±1.14	±1.14
	S.E	±0.3742	±0.3742	±0.7071	±0.5099	±0.5099
B lymphocyte	S.D	31.6	36.6	40	38	36.8
		±1.14	±1.14	±1.581	±1.581	±1.643
	S.E	±0.5099	±0.5099	±0.7071	±0.7071	±0.7348
Monocyte percentage M%	S.D	6	6.6	7	291	8.2
		±1.581	±1.14	±1.581	±3.4	±0.8367
	S.E	±0.7071	±0.5099	±0.7071	±1.52	±0.3742
Neutrophil percentage N%	S.D	42	35.2	33	13.77	32
		±1.581	±1.304	±2.236	±0.4606	±1.581
	S.E	±0.7071	±0.5831	±1	±0.206	±0.7071
	S.E	±0.7071	±0.3742	±1.14	±0.206	±0.5831

Table-3 Parameters of cell mediated immunity in *Gallus domesticus*, sensitized and hypersensitized with the low dose and high dose of embryonated eggs of the *A.galli* at 45th of the experimental design

Parameters		Nonsensitized	Sensitized with low dose	Hypersensitized with low dose	Sensitized with high dose	Hypersensitized with high dose
T lymphocyte	S.D	66.2	58	53.8	54	57
		±0.8367	±1.581	±1.643	±1.581	±1.581
	S.E	±0.3742	±0.7071	±0.7348	±0.7071	±0.7071
B lymphocyte	S.D	33.4	38	42	40	38.2
		±1.14	±1	±2.55	±2.345	±1.789
	S.E	±0.5099	±0.4472	±1.14	±1.049	±0.8
Monocyte percentage M%	S.D	5	7	8	10	10
		±1.581	±0.8367	±0.7071	±1	±1
	S.E	±0.7071	±0.3742	±0.3162	±0.4472	±0.4472
Neutrophil percentage N%	S.D	45	36	34	32.4	32.4
		±2.236	±1.643	±2.236	±1.517	±1.517
	S.E	±1	±0.7348	±1	±0.6782	±0.6782

RESULT AND DISCUSSION –

The cells responsible for the improved protection are antigenic experienced Tand B lymphocyte that can persist for long period of time and can reactive quickly following recounter with the antigen.T lymphocyte, the main effector cell during hypersensitivity reaction (iv) because the differentiation of T lymphocyte depend on the amount of antigen binding (Dong c et . al 2001). Low level of antigen binding promote differentiation of T cell into Th_2 cell. High level of antigen binding bring differentiation of naive cell to Th_1 cell. These cells produce different interleukins, lymphokines for the proliferation of different cell of immune system (DeVries et al 1999).

During present investigation, the percentage of B-lymphocyte (essential for t cell activation) were increased in sensitized and hypersensitized chicks while the percentage of T-lymphocyte, main effector cell in cell mediated immunity were found to be suppressed during sensitization and hypersensitization of the chicks (shown in table-1, 2 and 3).The reason for the present investigation was that the B cells and antibodies were required for resistance to the gastrointestinal nematode. The present results correlate with the results of Blackwell et al. (2000) and Tew et al. (1992) observed the migration of B cells from the germinal central to bone marrow where they differentiate to long lived plasma cells and also likely contribute to the prolonged elevation in serum antibody levels.

Present author found the reason behind suppressed T lymphocyte was that in suppressed T lymphocyte immunoregulatory function could facilitate B cell proliferation, stimulated by specific or nonspecific lymphocyte activators of helminthes or endogenous origin. The significant decrease of the T lymphocyte reflected a decreased proliferation or an increased apoptosis after long-term antigenic stimulation. For the opposite results of B and T lymphocyte the main cause is that the functional activity of B cells lasted longer than that of T-cells. A reduction in the percentage of T cells and increase in B cells was observed by Canals et al. (1997). He studied the cytokine profile induced by a primary non-protective infection with *Osteriagia ostertagi* in cattle. This infection resulted in decreased level of IL-2 mRNA expression and increase in IL-4 and IL-10 transcription.Levkul et al. (1999) observed the similar results in lambs after infection of *Ascaris suum.* The production of specific IgG antibodies was significantly high in highly infected group while the significant decrease in CD8+ T cells was observed.

In the present experiment the differential leucocyte count revealed a significant rise in monocytes which promotes the growth of antigen presenting cell (macrophages) with a fall in neutrophils during experimental ascaridiasis in sensitized and hypersensitized groups in comparison to nonsensitized groups. The present investigation revealed rise in monocytes was due to effective immune responses against *Ascaridia galli* infection in *Gallus domesticus*. The elevation of monocytes and suppression of neutrophil represent strong inflamating reaction in cell mediated immunity for killing the parasites and protect the host. The fall in neutrophils was obviously due to the suppression of immune response against low and high doses of *Ascaridia galli* infection in WLH chicks. A rise monocytes at the cost of heterophils with no change in basophils were reported by Sharma et al (1984) in chicks experimentally infected with *Toxocara canis.*

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