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IMMUNE RESPONSE OF CHICKEN INFECIED WITH QUARANFIL VIRUS

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INTRODUCTION

Quranfile virus (QRFV) is one of the unclassified group viruses of Arina viruses (Taylor et al., 1966) at the last few years it was believed that this virus is tick borne Arboviruses. It seems quite clear that birds constitute the basic vertebrate hsot, and that the maintenance cycle is from tick to birds and back to tick (Hoogstraal, 1973).

Studies employed in vitro correlates of cellular immunity have revealed further evidence of suppressed immne reactivity durring acute viral infection. The reactivity of lymphoid cells to antigen or phytohaemagglutinin (PHA) was decreased in animals naturally infected with viruses or vaccine induced as measles (Zweiman, 1971 and Zweiman et al., 1971), influenza (Reed et al., 1972) and hepatitis virus (Willems et al., 1969). On the other hand, Gimboro disease suppressed B cell mainly present in Bursa of Fabricous.

The aim of this investigation is to through a light on the role of QRFV on the chicken immune response.

MATERIAL AND METHODS

Sixty chicks (Dokki-4) about 14 days old were used in this investigation. These chicks were obtained through the Anshas Research Station, Ministry of Agriculture. The chicks were divided into 6 gorups, each of 10 The first group was injected intraperotenial (I/P) with 1 ml sheep red blood cells in a dose of 10^8/ml (Hegazi, 1979), the second group was injected I/P with bovine serum albumin (BAS) (Sigma Lab. U.S.A.) in a dose of 50 mg/ml (Hegazi et al., 1985 b), the third group was injected intramascular (I/M) with quaranfil virus (QRFV)(AR-1117 in suckling mous brain, Rocky Mountain Lab. U.S.A.) in a dose of $10^{7.1}$ $ISMBILD_{50}$/ml (Intracerabral suckling mous brain infective lethal dose fifty) (Kouka, 1978). The fourth group was injected I/P with SRBC (sheep red blood oells) 1 ml and I/M with $10^{7.1}$ $ISMBILD_{50}$/ml ORFV. The fifth group was injected I/P with BSA (50 mg.ml) & I/M with $10^{7.1}$ QRFV. While the sixth group was kept as normal control. All groups were kept under observation.

Five chicks from each group were taken after four days post treatment to collect heparnized blood and spleen. The whole blood culture and splenocytes were used to study the dose response curve (Hall and Gorden, 1976 & Hegazi, 1981). The plaque assay was embloyed on the splenocytes (Jerne and Nordin, 1963 and Hegazi, 1979) by using SRBC, SRBC conjugated with BSA and SRBC conjugated with QRFV as indicator cells for detection of $PFC/10^6$ splenocytes.

All groups after 14 days were injected intradermally (I/D) on the right foot pad (Hegazi et al., 1985 a) with its specific antigen, while the left once injected with normal saline and considered as control. The sixth group (control group) was injected in both food pads with normal saline. The foot pads were measured (Goto et al., 1978) and recorded for detection of the foot pad index.

RESULTS

Table, 1 shows the stimulation index (SI) (dose response curve) of whole blood culture and spleen lymphocyte cultures to PHA in the six groups.

a. **Whole blood culture:** The normal stimulation indecies were 8.3, 6.3 and 5.1 at PHA dilutions 1:25, 1.75 and 1:225 respectivelly. It is noticed that a great reduction in the stimualtion index in chicks injected with SRBC, QRFV + SRBC, while an increase in chicks injected with BSA, ORFV + BSA and QRFV . The optimum PHA dilution was 1:225 which gaves the highest SI in groups infected with QRFV.

b. **Spleen lymphocyte culture:** The SI of the normal spleen lymphocyte culture was 0.5, 6.9 and 1.3 at 1:25, 1:75 and 1:225 PHA dilutions respectively . There was slight increase in SI of spleen lymphocyte culture of chicks injected with SRBC, BSA, QRFV + SRBC and QRFV + BSA. While a great increase of SI in chicks infected with QRFV. The optimum PHA dilution was 1:225 specially in groups infected with QRFV.

c. **Foot pad index:** Table, 2 demonstrated the delayed hypersensitivity (intradermal skin test) as a sequential reactivity post injection with each specific antigen as sensitized antigens. From this table it is clear that the highest foot pad index in chicks injected with BSA (2.0 mm), QRFV + BSA (1:1) and QRFV (1.0), while the lowest foot pad index in chicks injected I/D by SRBC (0.3 mm) and there are a moderate reaction in chicks injected with QRFV + SRBC (0.5 mm).

d. **Plaque forming cells:** The PFC/10^6 spleen lymphocytes was shown in the **Table (2)**, it is clear that the highest PFC level was 440 in SRBC group, while a reduction in BSA, SRBC + QRFV, BSA + QRFV and a suppressive effect was observed in chicks infected with QRFV only (7).

Table (1): Stimulation index of chinken whole blood culture and spleen lymphocyte cultures to PHA.

Groups	PHA dilution	In vitro stimulation: Whole blood culture SI[a]	Whole blood culture DPM[aa]	Spleen cell culture SI	Spleen cell culture DPM
Control	1 : 25	8.3	1336	0.5	254
	1 : 75	6.3	1021	6.9	15919
	1 : 225	5.1	758	1.3	886
SRBC	1 : 25	4.0	687	1.02	142
	1 : 75	2.3	292	3.4	6040
	1 : 225	4.5	785	1.07	184
BSA	1 : 25	4.0	587	2.2	845
	1 : 75	6.0	919	1.6	400
	1 : 225	12.0	2069	20.5	6962
QRFV	1 : 25	6.5	634	8.0	324
	1 : 75	4.5	415	7.0	7250
	1 : 225	7.2	219	5.0	303
SRBC + QRFV	1 : 25	1.4	151	[illegible]	[illegible]
	1 : 75	1.5	169	7.1	4138
	1 : 225	2.8	679	2.2	548
BSA + QRFV	1 : 25	3.4	1733	4.0	810
	1 : 75	3.9	1810	1.9	249
	1 : 225	6.2	736	3.4	210

SI : Stimulation index DPM : Disentigr ation per minute

Table (2) Foot pad index and PFC / 10^6 spleen lymphocytes in chickens.

Test	Control	SRBC	BSA	QRFV	SRBC+QRFV	BSA+QRFV
Foot pad index 72 hours	0.0	0.3	2.0	1.0	0.5	1.1
PFC/10^6 spleen cells 96 hours	35	440	100	7	200	54

DISCUSSION

Regarding the results of the stimulation index (mitogenic dose respons curve) of whole blood culture and spleen lymphocyte cultures revealed that the PHA used in the experiment induced a noticeable blastformation in all PHA dilutions. These results proved that the cells (whole blood & spleen lymphocyte cultures)under examination are functioning which concided with observations obtained by Kirohner and Blaese (1973) and Hegazi (1981) who reported that peripheral blood lymphocytes, splenic lymphocytes, bone marrow cells and even thymus cells exposed to PHA will developed blastformation.

The optimum stimulation of lymphocytes to PHA varied depending on the dose of PHA, yet it was the first aim to determine such parameter. It was noticed that optimum PHA dilution was 1:225 in all groups under the examination. Also many authers reached different optimum stimulation using different PHA dilution as Lee (1975); Lee (1978) and Hegazi (1981) who found a significant increase in PHA response up to 270 fold using 20 ug PHA, while Maheswaran and Thies (1975) used Con A at dilution of 0.4 ug and pokweed 80 ug at a cell concentration 2 x 10^6 cells.

Concerning the delayed hypersensitivity skin test as a sequential reactivity post injection with each (sensitized antigen) specific antigen revealed that the highest foot pad index in chicks injected with BSA, QRFV + BSA, while the lowest foot pad index was observed in chicks injected with SRBC and a moderate reaction in QRFV. The rise of foot pad index indicated that there was an increase in foot pad due to stimulation with specific antigen. These findings also observed by many authers used different specific antigens in chickens as purified protein derivative (PPD) of tuberculine (Cheville and Richards, 1971);

Newcastle disease virus (Cheville and Beard, 1972 & Hegazi, 1981); PHA (Goto et al., 1978 and Mac Corkle et al., 1979); BSA (Hegazi et al., 1985 a) and Reo virus (Hegazi et al., 1989).

In order to apply the Jerne test for the enumeration of the antibody producing cells after inoculation with different antigens (QRFV & BSA), it was important to prepare antigen of virus and BSA that can be conjugated to carry the sheep red blood cells which used as an indicator. Conjugation of such antigen to red cells was the bases of Jerne and Nordin, (1963) for enumeration of PFC. The detection of the PFC at the 4th day post injection of different antigens was based of the data obtained by many authors as Eveland (1964); Abramoff and Brien (1968); Martin and Leslie (1974); Romaninkawa (1974); Hegazi (1979) and Hegazi et al., (1985 b) who found that the PFC appeared after the inoculation of antigen. The number of the PFC was usually greater at the fourth day post inoculation. It was clear that the difference in numbers of PFC/10^6 spleen cells in all groups under investigation may be due to the difference in the antigens nature.

From overmentianed data it can be concluded that the QRFV showed an immunosuppressive effect on chicken immune response detected by delayed hypersensitivity and PFC as well as deminished the non specific stimulation with PHA. Also the optimum dilution of PHA used in the stimulation of whole blood and spleen cell cultures was 1:225. in QRFV infected chicks.

SUMMARY

The immune response of chicken infected with Quaranfil (QRFV) virus was investigated. The dose response curve of whole blood culture and spleen lymphocyte cultures were deteated by non specific stimulation with phytohaemagglutinin (PHA) at different dilutions. The optimium stimulation was (1:225) with 0.5 x 10^6 cells in

whole blood culture, also (1:225) in spleen lymphocyte culture. The delayed hypersensitivity skin test as a sequential reactivity post injection with specific antigen revealed moderate reaction against QRFV. The plaque forming cells (PFC) was reduced in case of QRFV if it is compared with the control group (sheep red blood cells).

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