The immune status of rabbits primed with chicken thymic protein

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Abstract

Chicken thymus glands were collected from three weeks old chicken through standard manual dissection technique. The glands were minced, mortared, centrifuged at 5000 RPM for 15 min, and supernatant was saved. Thymic protein were separated by PEG 6%( M.w 6000) solution. This protein is designed as chicken thymic protein (CTP). CTP was used as test immunogen and sensitizer. As an immunogen it was used in concentration ,1.1g/land 0.55 g/l for priming of rabbits using multi site injection protocol with Freunds complete adjuvant. Passive haemagglutination ,NBT ,LIF ,E-rosette and skin test were made on CTP primed rabbits and controls. It was found that CTP could acts as humoral immunogen ,T cell suppressor and LIF cytokine inducer. Thus CTP may contain lapin immunoregulatory protein

Introduction

The avian thymus is an elongated lobulated gland, usually with seven lobes on each side, lying in the neck along side the jugular veins. It arises from third and fourth pharangeal pouches and as in other vertebrates, ectoderm /endoderm interactions play an important part in its induction. The role of the avian thymus in cell mediated immunity of the bird body is very similar to that of mammalian body and many of the function of the thymus mammalian T cells and paralleled in birds. The thymus is rapidly proliferating primary lymphoid organ with the ability to maintain a defined microenvironment and appears to be essential for the production of mature T cells. Thymic function is dependent upon interactions between developing thymocyte neural input, blood borne factors, paracrine effect and feed back mechanism, these interaction are complex including neurotransmitters the products of neurons working in thymus and immunotransmitters working in central nervous system and numerous peptides from the hypothalamus–pituitary axis are immunoregulatory centrally and peripherally. Thymus produces number of peptides which appear to act within the thymus in paracrine fashion and in systemic circulation as a feedback signals to the central nervous system. These peptide are thymosin α1, thymopoietin, thymulin and thymic hormonal factor. Thus chicken thymic protein can contain the four thymic peptide, cytokines and MHC molecules. The objective of the present work was to investigate the immune status of rabbit primed with chicken thymic protein e.i. the function of avian thymic protein in mammalian body (the rabbits)

Materials and Methods

1- Chicken thymic protein (CTP)

The chicken thymus glands were collected from three week old chickens through standard manual dissection technique. They were minced mortared, centrifuged at 5000 rpm, supernatant saved and homogenated the thymic proteins were separated by mixing 1 volume of PEG 6000 6% solutions to 1 volume of thymic homogenate leaved for half hours at room temperature then centrifuged 5000rpm for 30 minutes. supernates were decanted and pellet was washed and reconstituted with ml saline modified from (6). The protein concentration was determined by Biurate method and adjusted to 1.1 g/L. this protein was the immunogen to rabbit. The sensitizer for leuccocyte inhibitory factor (LIF) and the antigen for Passive haemagglutination (PHA).
2- Rabbits:
From a group of native breed rabbits brought from local market nine were proved to be free of ecto and endo parasite and bacterial as well as virus pathogens. These were kept under ad libitum conditions of food and drinks then adapted to housing conditions. These nine rabbits were sub grouped into three groups each of three rabbits, the first test group of 1.1 mg/ml and the second test group 0.55 mg/ml chicken thymic protein and the control.

3- Immunization protocol:
The control group received saline injections through subcutaneous (Sc) and intramuscular (Im) routes. the first test group received 0.55 g/L and the second test group received 1.1 g/L CTP half ml of thymic protein was mixed thoroughly with 1/2 ml of Freund complete adjuvant (FCA). The thymic protein FCA mixture was distributed 1/2 ml Im, ,1/4 ml Sc cervical and 1/4 ml Sc subclavian. Two injections in week a part followed by one week leave.

1- The response of nonprimed rabbits:
1.1: Humoral
The mean titres of anti chicken thymic protein (ACTP) antibody among non primed rabbits were for mucosal antibody and 20 for serum antibody (Table 1).

1.2: Cellular
The neutrophil nitro blue tetrazolium reduction in terms of mean percentages were 25%. The E rosette T lymphocyte count was 58. Mean time the leucocyte inhibitory factor were with means of 0.58, 0.56 for mucosal and systemic leucocyte using chicken thymic protein as sensitized and were 0.98, 0.96. When saline was used as sensitizer. The skin tests were of negative results (Table 1).

2- The immune response of primed rabbits to chicken thymic protein (CTP)
2-1: Humoral:
Whenever the CTP was used in concentration of 0.55 mg/mL /kg body weight rabbits. The A CTP antibody titers were 64,640 for mucosal and systemic compartments while when increased to 1.1 mg/mL/kg. The mucosal and systemic antibody titers were 32 and 320 respectively (Table 2).

4- Blood samplings:
After the one week leave post to last injection in the protocol. Animal were heart punctured for blood. Blood were saved with anticoagulant for cellular test and without anticoagulant for humoral tests.

5- Appendix samplings:
The specific immune primed rabbits were eviscerate and appendix were collected. Using standardized dissection method (7).

6- Appendix mucosal Ig separation:
The mucosal Ig separation was done according to (8).

7- Serology procedure:
Passive haemagglutinin test was done on sera and mucosal immunoglobulin according to (9).

8- Cellular immune procedures:
Leucocyte inhibitory factor was done as in (10). Nitroblue tetrazolium test done according with (11). E rosette T cell count was done as (12). Skin Delayed hypersensitivity test done as in (13).

Results

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CTP initiate LIF cytokines production to a significant limits.

3-2: CTP primed rabbits

CTP is immunogenic inducing antibody responses at both test concentrations for each of mucosal and systemic compartments. It induces increase in NBT % among primed rabbits. It damps the E rosette counts for T lymphocyte to 13 in compare to 58 % in non primed rabbits. LIF % were significant at systemic leucocyte and non significant at mucosal parts for 1.1 and non significant for both compartments at concentration of 0.55 mg/ml and negative DTH skin test among CTP primed rabbits (Table 3).

Table 1: The responses of CTP non CTP primed rabbits:

| Humoral anti CTP | Systemic 20 | mucosal 2 |
|------------------|-------------|-----------|
| Cellular NBT     | 25%         |           |
| E rosette       | 58          |           |
| LIF              | 0.62        |           |
| Skin DTH induration | <5mm     |           |

Table 2: The anti chicken thymic protein (ATP) titers in ATP primed rabbits:

| Treatment | Replicate | Titers |
|-----------|-----------|--------|
|           |           | Mucosal | Systemic |
| Saline control | R1 | 2 | 20 |
|              | R2 | 2 | 20 |
|              | R3 | 2 | 20 |
|              | R’ | 2 | 20 |
| 0.55 mg/ml  | R1 | 64 | 640 |
|              | R2 | 64 | 640 |
|              | R3 | 64 | 640 |
|              | R’ | 64 | 640 |
| 1.1 mg/ml   | R1 | 32 | 320 |
|              | R2 | 32 | 320 |
|              | R3 | 32 | 320 |
|              | R’ | 32 | 320 |

R1-3 = Replicates    R=mean
Table 3: Cellular immune status of CTP primed rabbits

| Concentration mg/ml | Replicate | Functional cellular | Clinical skin DTH | Structural cellular |
|---------------------|-----------|---------------------|-------------------|---------------------|
|                     |           | NBT Mucosal LIF     | Erythema          | Induration (mm)     | Necrosis | E rosette |
| 0.55                | R1 44     | 0.92 0.76           | + 5              | + 6                | 13 12    | 14 |
|                     | R2 44     | 0.92 1              | + 5              | + 6                | 13 12    | 14 |
|                     | R3 57     | 0.92 1.092          | + 5              | + 6                | 13 12    | 14 |
| R’                  | 55 0.97   | 0.89 5.3            | 13 12            | 14 |
| 1.1                 | R1 60     | 0.92 0.77           | ++ 5             | ++ 6               | 13 13    | 13 |
|                     | R2 60     | 0.92 0.58           | ++ 5             | ++ 6               | 13 13    | 13 |
|                     | R3 60     | 0.92 0.62           | ++ 5             | ++ 6               | 13 13    | 13 |
| R’                  | 60 0.92   | 0.62 5.3            | 13 13            | 13 |
| Control             | R1 24     | 0.98 0.97           | - - -            | 60 |
|                     | R2 25     | 0.96 0.96           | - - -            | 56 |
|                     | R3 26     | 0.97 0.95           | - - -            | 58 |
| R’                  | 0.97 0.96 | 58 58              | 58 |

Discussion

Thymic proteins contains peptide cytokines peptide hormones, as well as tissue milieu proteins and MHC antigenic molecules. Chicken thymic proteins reduced leucocyte inhibitory cytokines in nonprimed rabbits (13,14) and T lymphocyte suppressor in primed rabbits. Thus there is a possibility that CTP acting as of acting as cellular immune modulent in the primed rabbits(13,15). CTP increase neutrophil phagocytosis by NBT in concentration dependent fashion(15). CTP at concentration of 1.1 mg /ml induces significant production of LIF cytokines in peripheral blood leucocyte but not mucosal leucocyte while it was non significant for both concentration at both compartments(16). The skin DTH test indicate five mm induration zone with erythema in tuberculin like mild reaction in the prime rabbit. The significant result of LIF at systemic and 1.1mg /ml concentration to skin DHT test indicates dissociate of clinical skin test results from T lymphocyte function(17). CTP was good humoral immunogen as indicated by rise up anti CTP ab titers higher than folds of the base line titers at nonprimed group. The epitope nature deduced in CTP may be T independent B dependent or T dependent inducing Th2 subsets of T lymphocyte(18). Thus the immunologic features of CTP in the prime rabbit can be stated as follows:
1. Humoral immunogenic.
2. T cell suppressors.
3. Inducer to leucocyte inhibitory cytokines and its concentration dependent.
4. Mild skin DTH responses in primed rabbits

Thus an conclusion one may put forward on opinion of presew of lapin immunoregulatory protein present in chicken thymic proteins.

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الحالة المناعية للارنب المنع ببروتين ثايموس الدجاج

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الخلاصة

جمعت 93 متاممو 10 ارنب تم تقسيمهم بعيار عمر متتابع استاب 22-24 يوماً بعدها. وصلت البروتين الثايموسي باستخدام محلول بولي إيثيلين كلايكلور 6%، 5000 دورة/دقيقة بعدها جمع الرائق ما بعد النفاذ. وفصل البروتينات الثايموسية باستخدام براحت والملف. في حالة وصفه ملعقة مكمل للإراقب، واستخدم هذا البروتين بوصفه ملء ومحس للاجانبية. في حالة وصفه ملعقة مكمل للإراقب.

1.1 غم/لتر و 0.55 ج/ل لتمصنع الإراقب وفق جدول تمثيل بالحقن المتعدد مع مساعد فروند الكل. لتقييم الحالة المناعية استخدم اختبار التلازيم المنع في عدد من الاختبارات، اختزال التييرولو، تثبيت هجرة الخلايا البيض والتكوين الوردي للخلايا المناعية.

استخدم اختبار التلازيم المنع في عدد من الاختبارات، اختزال التييرولو، تثبيت هجرة الخلايا البيض والتكوين الوردي للخلايا المناعية. في حالة وصفه ملعقة مكمل للإراقب، واستخدام البروتينات الثايموسية، وفق جدول تمثيل بالحقن المتعدد مع مساعد فروند الكل. لتقييم الحالة المناعية استخدم اختبار التلازيم المنع في عدد من الاختبارات، اختزال التييرولو، تثبيت هجرة الخلايا البيض والتكوين الوردي للخلايا المناعية.

نلاحظ خاصية اكستاذ الفحص وتفسير السيطرة فوجد أن بروتين ثايموس الدجاج يصحح لحالة المناعة الخلوية، خاصية اكستاذ الفحص وتفسير السيطرة فوجد أن بروتين ثايموس الدجاج يصحح لحالة المناعة الخلوية. في حالة وصفه ملعقة مكمل للإراقب، واستخدام البروتينات الثايموسية، وفق جدول تمثيل بالحقن المتعدد مع مساعد فروند الكل. لتقييم الحالة المناعية استخدم اختبار التلازيم المنع في عدد من الاختبارات، اختزال التييرولو، تثبيت هجرة الخلايا البيض والتكوين الوردي للخلايا المناعية.