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Proinflammatory TNF Alfa TNF Beta, Interleukin 6, Anti-inflammatory Interleukin 10 Cytokines and the Associated Cytokine Imbalance in the Specific Immune Primed Rabbit Models with Streptococcus Pneumonia Serotype 1 and Serotype 6 Capsules

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ABSTRACT

The capsular polysaccharide Type 1 and type 6 of the human clinical local pneumotropic isolates of Streptococcus pneumoniae were separated, characterized and purified. Then quantified to concentrations of 1 mg/ml. To be ready as immunogens they were admixed with an equal volume of lanline. These were standing as the test immunogens. The test immune system was that of rabbits. The test rabbit groups were assigned as C 1 group, C6 group and saline S control group each of five animals. The test rabbit groups C 1 and C6 were primed once through intramuscular route separately with capsule-lanoline mixtures and left for 21 days, then bleed. Sera were saved from the primed rabbits blood samples in 0. 5 ml aliqoutes till use. Trachial mucosal immunoglobulin were separated, partially characterized and kept in alqoutes of 0. 5 amounts in forml normal saline till use. One step sandawich eliza technique for TNF alfa, TNF beta, IL6 and IL10 were performed for both sera and mucosal globuilin of the primed rabbits and controls. The immune features of the capsule-lanline primed rabbits were found as; capsules were weak immunogen needs adjuvant in order to become good immunogen, cytokine responses were serotype dependent, mucosal cytokines have shown to be of higher levels than systemic responses, C1 was higher immune convertor than C 6, and both associated with cytokine imbalance towards pro-inflammatory cytokines.

Keywords: Anti-inflammatory, Capsule, cytokine, immune, interleukins, primed, proinflammatory.

INTRODUCTION

Cytokines were a group of cellular secreted small molecular weight homone like proteins. They were generally secreted from nucleated cells. Cytokines have regulatory effects on both of the hemopoietic and non-hemopoietic cells. Cytokine as a term encopasses number of families as; interleukine, tumor necrosis factors, interferons and growth factors. Cytokines are; soluble,

host specific and non-specific cell mediators that have critical important role in immune effector mechanisms both in infectious and non-infectious disease conditions [1-7]. In the present investigation tempts were made to uncover the pro and anti-inflammotory cytokine responses and the associated cytokine imbalance in rabbits primed with capsule 1 and 6 of S. penumonae and in controls.

MATERIALS AND METHODS

Capsule Antigens

The capsular polysaccharide was; separated, characterized, and purified. Then, quantified to a concentration of 1mg/ml. from the human clinical neurotropic isolates of S. pneumoniae serotype 1 and serotype 6. [7, 8, 9, 10, 11].

Adjuvant

Lanolin solution.

Immunogen

One volume of lanolin was admixed with one volume of the capsular antigen. Mixtures were the test immunogen.

Rabbits

Newzeland white rabbits were brought to the animal house, kept add libitum condition of food and drink for two weeks for acclimatization to house environment. Then assiged into three groups each of five rabbits as in the followings;

- Group I Capsule serotype 1-Lanolin . . . . . five rabbits
- Group II capsule serotype 6-lanolin. . . . . five rabbits
- Group III saline . . . . . five rabbits

Immunization Protocols

One ml of capsular antigen- one lanolin mixture was injected once through the intramuscular route and left for 21 days then test bleed and blood collection. [12].

Trachial Mucosal Globulin

Part of rabbit trachia from the three test groups were incised and open up in sterile petri-plate, mucosa scrapped into the petriplate then five ml formal normal saline were added to the scraps and mixed thorughly and tubbed into centrfuge tubes and centrifuged for minutes. Supernates were kept for furthor proccessing to separation of mucosal globulin as in[Shnawa and Thewiani 2002[13]

Cytokine Mappings

The collecte blood samples by cardiac puncture of immunized rabbits were left to clot at room temperature for 45 minutes then centrifuged and sera saved at -18C till use TNF alpha, TNFbeta IL6 and IL10 cytokine kets for the sandwich one step eliza determination following the method of the Immunotech A beecham Coulter CO., France. These assays were done both for sera and trachial globulins.

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Biometery

Mean value, standard error, standard deviation and t test were done as in Graph and Prison Version 4 computer soft ware. P value less than0. 05 level of significance were considered as significant. Cytokine balance was measured as number of folds as related to sham saline controls.

RESULTS

Cytokine Profils

The TNF alpha, TNF beta, IL6 and IL10 cytokine concentration means were higher than that of controls. Mucosal cytokine mean concentrations were higher than systemic concentration means. Serotpe 1 capsule primed rabbits were shownig higher concentration means than that of Serotype 6 primed rabbits. Both, for group I and group II primed rabbits., Table 1.

Cytokine Imbalance

The associations of proinflamatory and anti-inflammatory cytokine; TNFalpha/IL10, TNFB/IL10, andIL6/IL10 were showing cytokine imbalance in capsule sertype 1 primed rabbit reaching four to six folds than that of controls. While up to twolds for IL10. Hence there were shifts towards pro-inflammatory TNF alpha, TNF beta and IL6 as compared to that of IL10., Table-2.

Table 1: Cytokine profiles for rabbits specific immune primed with serotypeS. 1 and 6 of S. pneumoniae.

Capsule Serotype	TNF alpha con. means in pg/ml.	TNFbata con. means in pg/ml. IL6 pg/ml.	IL6 concent Means in pg/ml.	IL10 concen. means in pg/ml.
Sertype 1 Mucosal Systemic	 650. 13 -+34. 5 345. 3-+4. 1	 645. 10-+ 9. 7 380. 531-+10. 8	 450. 82-+14. 8 332. 20-+27. 8	 438. 2-+4. 300. 51-+7. 1
Sertype 6 Mucosal Systemic	 390. 21-+4. 5 241. 51- +10. 6	 238. 72-+22. 6 330. 34- +3. 5	 260-+5-+12. 6 240. 12-+5. 4	 249. 12 - +8. 5 228. 72- +10. 5
Control Mucosal Systemic	 125. 12+-0. 55 115. 29+-11. 7	 110. 35+-11. 5 101+_11. 5	 93. 54+-2. 5 100. 62+_0. 75	 110. 25 +-11. 5 101. 15+-11. 5

Table 2: Cytokine imbalance in rabbits primed with S pneumoniae serotypes 1 and 6

Cytokine Balance associations	Serotype 1 capsule	Serotype 6 capsule	Conclusions
TNF alpha/IL10 Mucosal Ststemic	 6. 9/4. 3 3. 5/2, 72	 3. 1/2. 26 2. 1/2. 26	Shift towads proinflammatory
TNF beta/IL10 Mucosal Systemic	 5. 9/4. 3 3. 27/2. 7	 3. 1/2. 26 2. 1/2. 26	Shift toward pro inflammatory
IL6/IL10 Mucosal Systemic	 4. 5/4. 3 3. 31/2. 7	 2. 8/2. 26 2. 4/2. 26	Shifts towards proinflammatory

DISCUSSION

Cytokine responses have been observed in human infectious and non-infectious disease conditions [1-6], and inline with these observations they were obseved in this work, Tables – i and 2. Mucosal cytokine responses were higher than the systemic in C1 and C6 primed rabbits and the primed rabbits were higher thanthat of controls [24]. Different S. pneumoniae serotypes have shown different responses., Table – 1. [24]. The most evident cytokine imbalance was observed in serotype 1 in mucosal and to lesser extent in systemic responses [14, 19-24]. Serotype 6 was found in mucosal for TNF alpha and systemic for TNF beta, Table-2. The noted imbalance in, Table 2 were inline with with workers on Covi-19[13], vaccination [16], influenza infection [16] Features of C1 and C2 serotypes immunogenicity were as in the followings;

1. S. pneumoniae capsules were weak immunogens needs adjuvant to be good immunogens in a rabbit's model.
2. Cytokine responses to S. pneumoniae capsules were serotype dependent.
3. Serotype 1 capsule have shown to be higher immune convertor than sertype 6.
4. Serotype 1 induces cytokine imbalance and to lesser extent serotype 6.
5. Mucosal cytokine responses for serotype 1 and 6 were higher than that of systemic responses.

CONCLUSIONS

Streptococcus pneumoniae serotype 1 and serotype 6 capsule specific immune responses in primed rabbits have shown pro and anti-inflammatory cytokine profiles with characteristic increase of the immune primed than in controles. Mucosal was higher than systemic responses. Serotype 1 cytokine imbalance was towards pro-inflammatory responses.

Competing Interest

The authors have no competing interest

Eithical Issue

Care, housing, handling and interventions done on rabbits were following the international acts regulating care, housing, handling and interventions of laboratory animals.

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