Brucella Blastongenesis of Rabbits Lymphocytes Sensitized with Outer Membrane Proteins, Lipopolyccharide and Lipid-A. Associated Protein Extracted from melitensis.

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Abstract

Blastogenesis assay wsa performed on rabbit lymphocytes sensitized with outer membrane proteins (OMP), lipopoly saccharine (LPS) and Lipid. A. Associated protein (LPA) extracted from brucella melitensis (filed strain). Results showed that there was an increase in the mitotic index (M.I) induced by LPA (19.48)and OMP (11.7) with wihle LPS induced lower value of MI (7.61).

Introduction

The immune response to Brucella has been measured in a variety of ways including serological test,(1) and delayed type hypersensitivity skin test(2). The lymphocyte blastogensis test using different extracts also been employed (3). They found that various OMPs extracted from *Brucella abourtus* were enhancer for lymphocytes blastogensis whereas, Baldwinet al(4) tested LPS extracted from *B. abortus* and it was that Lpa is amitogenic for slenic lymphocytes. Furthermore LPA preparation extracted from *E. coli* exhibited a potent mitognic activity for mice lymphocytes. (5) In this study we investihated different extracts of *B. melitensis* for mitognic activities.

Materilals and Methods

Animals: Local breed, 6 months old rabbits were used.

Bacteria: Local isolate of *Brucella melitensis*. Isiolated from an abortive lamb and was chararterized according to Alton et al(6).

Antigens: OMPs, LPS and LPA were extracted according to (Vestreate, and Wintr, (1984) (7), Westphal. Etal 1952(8). Wober, and Alaupovic, (1971)(9).

Immunization: Rabbits were immunized s.c with the different extracts (three rabbits for each). The extracts were emulsified with Freund's adjuvant. A further dose was given after two weeks. Blood was aspirated after another two weeks for each antigen.

Blastogenesis assay: for each animal tubes of RBMI 1640 (Gibo, Grand Island, NY) containing. 10mM HEPES buffer (pH 7.3), 2mM glutamine, $5x10^{-5}$M2 mercapto ethanol (2ME) and 10% Fetal calf serum, were used. To the first tube the following compotes were added; 0.5ml heparinzed blood, 0.2ml PHA (provided from the Iraqi atomic energy commission). While the following extracts were added to the rest of the tubes. 5µg/ml OMP,10 µg/ml lps and 10 µg/ml lAP). In sequence together with 0.5 ml of bolld. Tubes were indcubated at 37°C 72 hours. One hours prior to cell harvest 0.lml of colchicine was added (final concentration, 10 µg/ml) to each culture tube. Cell harvest and assay was conducted according to kelsy et al. (10).

Results

The results shown in table (1) indicated that Lpa was more effective in induction of blsatogensis for peripheral blood lymphocytes and gave mean mitotic index equal to (19.48), while OMP was less efficient in induction of blsatogensis than LPA (11.7) the least effective extract was LPS with mean mitotic index (7.61).

Discussion

Of the most sensitive tests for the quantification effect of potentially mitogentic agents is quantifying of cyogentic parameters including index (MI)(11). In this study we used different extracts taken from *B. melitensis* to check their mitogenticity for sensitized lympocytes, and as shown in the results the most mitogenic was extracts was LPA. Although LPA is isolated in association with LPS and shares certain of its biolgic properties but the two B. cell mitogens may be distinguished by several criteria; of primary importance, is the ability of LPA to simulate LPS non responder lymphocytes (5), and

this finding agreed with our findings which showed that LPA preparations exhibited potent mitogenic activity.

Bruclla abourtu LPS was tested by (4), for blastogensis and they fond that it stimulated nylonwool- adhernt cells tken from immunized animals with a stimulation index equal to 1.99+ 0.17.

The antigens injected into animals induce an immune responsiveness against them characterized by an increase in lymphocyte reactivity to these products in vitro; and this may be due to the cytokines produced to bacterial antigens which enhance the anti bacterial activities of different lympocytes subsets (12). So the results herein showed a great validity to use subcellular fraction from the bacteria in order to enhance an immune respons.

Reference

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Table (1) Mitogenic index of different antigens on blood lymphocytes

Antigens	LAP	LPS	OMP	PHA	NO ANTIGEN
Mitotic Index (MI)**		1.4	20.0	9.4	7.9
	19.17	7.5	6.1	48.2	6.4
	12.9	10.3	25.1	11.7	9.8
	38.1	15.9	21.2		
	15.4	5.1	6.0		
	6.5	6.5	6.9		
Mean (MI) ± SE	19.48 ±5.47	7.81 ±1.71	11.7 ±3.66	23.1 ±12.58	8.03 0.98

* Numbers represent repetitive readings

** The M.I was determined as ar atio of the mitotic cells to interphase nuclei 1000 cells.

التحول البلاستي لخلايا الأرنب اللمفاوية المحسسة ببروتينات الغشاء الخارجي، متعدد السكري الشحمي والبروتين المرتبط بالدهن A المستخلصة من جراثيم المالطية

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الخلاصة

درس التحول البلاستي لخلايا الأرنب اللمفاوية المحسسة ببروتينات الغشاء الخارجي (OMP)، متعدد السكر الشحمي (LPS) والبروتين المقترن بالدهنA (LPS) المستخلصة من جراثيم البروسيلا المالطية (عزلية حقلية). أوضحت النتائج بأن هناك زيادة بمؤشر الإنقسام الخيطي (MI) المحفز بمستضدLPA (19.48) يليه التحفيز مستضد OMP حيث MI (11.7) بينما كان MI لمستضد (7.61).