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**Short communication**

# Density of Con A receptors on T cell of bat and its implication on delayed activation

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**Abstract**

The number of Con A receptor sites on T cell surface of bat lymphocyte was measured by using tritium-labelled Concanavalin A ($^3$H-Con A). It was found that the average number of Con A receptor sites on a bat T cell is $3{\cdot}6 \times 10^6 \pm 0{\cdot}45 \times 10^6$. This value is significantly low compared to $10^7$ Con A receptors on a murine lymphocyte and on a rat lymph node cells. Implication of this low density of Con A receptors on bat T cells in delayed activation of the lymphocytes in terms of blastogenesis and DNA synthesis is discussed.

**Key words:** Con A receptors, T cell, activation.

## 1. Introduction

Concanavalin A (Con A), a polyclonal stimulator for murine T cells[1–4], is also known to activate lymphocytes of *Pteropus giganteus*[5], a frugivorous bat. On studying the activation kinetics of bat lymphocyte stimulated with Con A, we[5] have found that the activation phase is delayed where the peak of blastogenesis and DNA synthesis were attained at 120 h *in vitro* while the same peaks were found at 48 h in murine system. In view of this delayed activation phase we decided to study the number of Con A receptor sites on T cell surface as it appears to be the crucial factor for initiating the sequence of events leading to blastogenesis and DNA synthesis. This study will possibly reveal whether the cause for delayed nature of activation phase in bat lymphocyte is at this step.

## 2. Materials and methods

### 2.1. *Animals*

Wild male and female big brown frugivorous bat, *P. giganteus*, were used throughout the experiment. They were housed in wire cages and were fed with fruits and water *ad libitum*. The weight of these animals ranged from 440 to 550 g.

*2.2 Isolation of T lymphocytes*

Spleen and mesenteric lymph nodes were collected asceptically and dissociated separately in phosphate buffered saline (PBS) with the help of stainless steel wire mesh. RBCs from spleen cell suspension were removed by treating with 0·83% ammonium chloride buffered in tris. The cells were then washed with PBS and subsequently layered on Ficoll-Hypaque gradient and spun at 3000 rpm for 10 min. The lymphocytes were collected from the interface and then washed twice with PBS and finally resuspended in Earle's balanced salt solution (EBSS) supplemented with 15% autologous serum. After adjusting the cell concentration to $10^7$ cells in 5 ml, it was gently poured on a prewarmed, EBSS soaked nylon wool column (600 mg of scrubbed nylon fibre, Fenwal Code 4C 2906, Fenwal Lab., Illinois, USA) packed up to 5 ml mark in 10-ml syringe and incubated at 37°C for 45 min. Finally the non-adherent cells (enriched T-cell population) were eluted with 20 ml of prewarmed EBSS supplemented with 15% autologous serum. The T-cell nature of the eluted cells was tested with anti-thymocyte serum raised by injecting brain cell in rabbits following procedure described earlier[6].

*2.3 $^3$H-Con A binding reaction*

Enriched T-cell population thus obtained, was washed with PBS twice and then binding reactions were carried out in culture tubes containing 0·5 ml of PBS. $^3$H-Con A (Sp. act. $13{\cdot}5 \times 10^4$ disintegration $sec^{-1}$. $\mu g^{-1}$; procured from the Bhabha Atomic Research Centre, Trombay, India) was added in different concentrations ranging from 20 to 0·625 $\mu$g per 0·5 ml PBS. After 30 min of incubation at 37°C, 6 ml of PBS was added, the cells sedimented, washed again with another 6 ml of PBS and finally the cells were collected on small filter paper discs (Whatman filter No. 3) under suction pressure. The filter paper discs were dried and placed in scintillation vial containing scintillation cocktail (6 g PPO, 0·05 g POPOP and 1 l toluene) to count for radioactivity in CPM mode in scintillation counter (Beckman LS-1800, Beckman Instrument Co., USA). The data was plotted as described by Steck and Wallach[6].

*2.4 Experiment to show that binding of $^3$H-Con A is Con-A receptor specific*

In one set of experiments, 0·05 M methyl-$\alpha$-D-mannopyranoside ($\alpha$-MM), a competitor for Con-A receptors, was added before the start of binding reaction to show that binding of $^3$H-Con A is receptor-specific.

*2·5 Viability and blastogenic response of lymphocytes cultured with $^3$H-Con A*

In another set of experiment, viability and blastogenic transformation was assessed by stimulating the cells *in vitro* with $^3$H-Con A at different hours of incubation. This was necessary to ascertain whether or not, tritium-labelled Con A retains its full stimulating property on lymphocytes of *P. giganteus*.

## 3. Results and discussion

When the ability of normal bat lymphocytes to find $^3$H-Con A was measured as a function of $^3$H-Con A concentration, it was found that the apparent dissociation constant (K) for Con A binding as obtained from 1/Con A intercept is 6·6 $\mu$g. The mean ± S.D. of three different experiments, the apparent K value stands as 7·1 ± 1·1 $\mu$g Con A. The specificity of $^3$H-Con A to bind at the Con A-receptor site was evident from the increased $K_i$ (dissociation constant in presence of a competitive inhibitor) value when $\alpha$-MM, a competitor for Con A receptor, was introduced during the binding reaction (fig.1). Furthermore, the reasonable viability index and the attainment of blastogenic peak at 120 h (Table I) reflects the persistence of lymphocyte-stimulating property of tritium-labelled Con A which has been used for binding reaction.

Figure 1 also reveals that bat lymphocytes (T cells) on an average bends only 1·23 ± 0·157 $\mu$g Con A per $2 \times 10^6$ cells as calculated from c (Con A bound) intercept. If the molecular weight of Con A tetramer is assumed to be 102000[7] then the average number of Con A receptor sites on a bat T cell is $3{\cdot}6 \times 10^6 \pm 0{\cdot}46 \times 10^6$. Thus Con A binding sites per bat T lymphocytes seems very low compared to $10^7$ molecules of Con A that are capable of binding to the surface of a murine thymocyte, lymphocytes, spleen cell and bone marrow cell and also rat lymph node cell[8,9]. This decreased number of Con A receptor sites is possibly one reflection of the altered cell surface which is responsible for the prolonged activation phase in the lymphocytes of bats. This is in consistence with the earlier finding that lymphocytes from patients with chronic lymphocytic leukemia showing both a delayed and an impaired blastogenic response to phytohaemagglutinin[10] also exhibit decrease in phytohaemagglutinin-receptor sites[11].

Furthermore, Stobe *et al*[12] have calculated that the number of Con A molecules required for murine lymphocyte activation lies within 0·8 to $3 \times 10^7$ range. Interestingly, our calculated value of Con A molecules that can bind on T cell surface of bat is even lesser than the lowest value. However, in bat lymphocytes we do not know the minimum number of Con A receptors, in terms of Con A molecules, which is optimal for blastogenesis and DNA synthesis. But it transpires from the present investigation that Con A receptors on bat T cells are less closely packed than murine lymphocytes. As the ability of an immuno-competent cell to respond to an external stimulus depends mostly on

**Table I**

**Percentages of viability and blast transformation of bat lymphocytes activated *in vitro* with $^3$H-Con* A at different hours of incubation**

| | Mean ± S.D. | | | | | |
|---|---|---|---|---|---|---|
| | 24 h | 48 h | 72 h | 96 h | 120 h | 144 h |
| Viability percentage | 44·6 ± 20·2 | 37·3 ± 13·0 | 32·6 ± 9·8 | 32·0 ± 13·1 | 29·3 ± 10·0 | 24·0 ± 12·1 |
| Blastogenesis percentage | 24·2 ± 7·18 | 38·9 ± 11·35 | 55·13 ± 1·7 | 59·9 ± 6·1 | 76·0 ± 5·2 | 43·06 ± 5·0 |

* dose 10$\mu$g ml$^{-1}$.

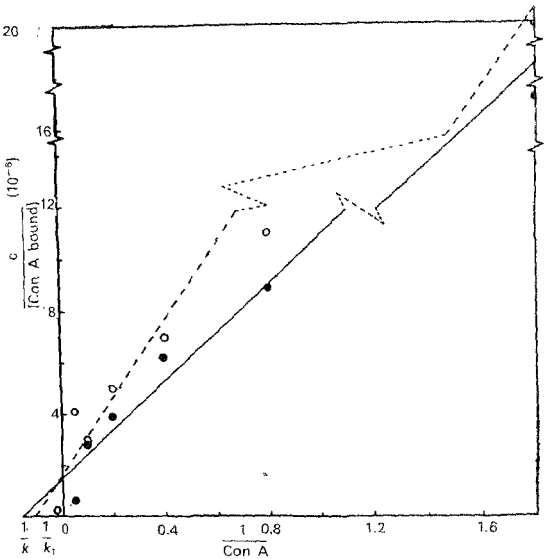


FIG 1 The binding of Con A to bat lymphocytes. Normal lymphocytes treated or not-treated with $\alpha$-MM were incubated with $^3$H-Con A as described in Materials & Methods. The data have been plotted by the method of Steck and Wallach[6] according to the equation:

$$\frac{C}{\text{Con A bound}} = \frac{1}{k\,n} \cdot \frac{1}{\text{Con A}} + \frac{1}{n}$$

where [Con A] = concentration of free Con A ($\mu$g), $n$ = number of Con A-binding sites per cell expressed as $\mu$g Con A bound per lymphocytes, and $c$ = number of cells. The dissociation constant $k$ for maximal Con A binding to lymphocytes was determined from 1/Con A intercept and the $\mu$g Con A bound per lymphocyte, $n$ from the $C$/Con A bound intercept. The 'line of best fit' drawn is obtained by the method of 'least squares' and it is a representative experiment from a triplicate set of data. The experiment was repeated three times. ●——●, binding reaction in normal bat lymphocytes and ○·····○ binding reaction in the presence of $\alpha$-MM, a competitor for Con A binding site.

optimal concentration of the stimulating agent and the concentration of the corresponding receptors on the cell surface, therefore, possibly lesser number of receptors does not allow sufficient binding of Con A molecules at the receptors which is a crucial factor for initiation of activation[11]. Furthermore, the less closely spaced receptors possibly answer why Con A-mediated activation of bat lymphocytes requires a higher dose[5] of the order of

10 $\mu$g. ml$^{-1}$ as against 2–5$\mu$g. ml$^{-1}$ in mouse[1,13,14] for optimal response. This type of critical analysis of receptors for alloantigen of $\beta$ lymphocytes of bat is necessary to understand the mechanism of delayed humoral response in this species as reported earlier[15,16].

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**References**

1. Heininger, D., Touton, M., Chakravarty, A.K. and Clark, W.R. — Activation of cytotoxic function in T lymphocytes, *J. Immun.*, 1976, **117**, 2175.
2. Chakravarty, A.K. and Clark, W.R. — Lectin driven maturation of cytotoxic effector cells: The nature of effector memory, *J. Expl Med.*, 1977, **146**, 230.
3. Falkoff, M.R. and Dutton, R.W. — Evidence that Con A induces cytotoxicity in the same subclass of T cells as does alloimmunisation, *J. Immun.*, 1977, **118**, 1600.
4. Chakravarty, A.K. — Blastogenesis and DNA synthesis in Con A driven regeneration of cytotoxic response in memory T cells, *Indian J. Expl Biol.*, 1978, **16**, 148.
5. Paul, B.N. and Chakravarty, A.K. — *In vitro* analysis of delayed immune response in a bat, *Pteropus giganteus*: Process of Con A mediated activation, *Dev. Comp. Immunol.*, 1986, **10**, 55.
6. Steck, T.L. and Wallach, D.F.H. — The binding of kidney bean phytohaemagglutinin by Ehrlich Ascites carcinoma, *Biochim. Biophys. Acta*, 1965, **97**, 510.
7. Agrawal, B.B.L. and Goldstein, I.J. — Protein-carbohydrate interaction. VI. Isolation of Con A by specific adsorption on cross linked dextran gels, *Biochim. Biophys. Acta*, 1967, **147**, 262.
8. Edelman, G.M. and Millette, C.F. — Molecular probes of spermatozoan structures, *Proc. Natn. Acad. Sci. USA*, 1971, **68**, 2436.
9. Betel, I. and Vandenberg, K.J. — Interaction of Con A with rat lymphocytes, *J. Biochim.*, 1972, **30**, 571.
10. Robbins, J.H. — Tissue culture studies of the human lymphocyte, *Science*, 1964, **146**, 1648.
11. Kornfeld, S. — Decreased phytohemagglutinin receptor sites in chronic lymphocytic leukemia, *Biochim. Biophys. Acta*, 1969, **192**, 542.
12. Stobo, J.D. Rosenthal, A.S. and Paul, W.E. — Functional heterogeneity of murine lymphoid cells. I. Responsiveness to and surface binding of Con A and PHA, *J. Immun* , 1972, **108**, 1.
13. Williams, R.M. and Benacerraf, B. — Genetic control of thymus derived cell function I. *In vitro* DNA synthetic response of normal mouse spleen cells stimulated by the mitogens Con A and PHA, *J. Expl Med.*, 1972, **135**, 1279.

14. BEVAN, M.J AND COHN, M. Cytotoxic effects of antigen and mitogen induced T cells on various targets, *J. Immun.*, 1975, **114**, 559.

15. CHAKRABORTY, A.K. AND CHAKRAVARTY, A.K. Antibody-mediated immune response in the bat, *Pteropus giganteus*, *Dev. Comp. Immun.*, 1984, **8**, 415.

16. CHAKRAVARTY, A.K. AND PAUL, B.N. Analysis of suppressor factor in delayed immune responses of a bat, *Pteropus giganteus*, *Dev. Comp. Immun.*, 1987, **11**, 649.