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Detection of Antigen-Specific Lymphocytes in *Bacillus Calmette Guerin*-Infected Mice by a Direct Rosette Assay

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=국문초록=

BCG 감염마우스의 비장으로부터 BCG항원에 대한 수용체를 가진 세포를 BCG항원으로 감작시킨 면양적혈구(SRBC)를 이용한 rosette 방법으로 분리하였다.

SRBC에 흡착되는 적정 BCG항원 농도를 알아보기 위하여 tannic acid(1:20,000)으로 전처리한 SRBC에 여러 농도의 BCG항원으로 작용시킨 결과 50μg/ml의 BCG항원으로 반응시킨 경우 가장 높은 rosette 형성을 관찰할 수 있었다. 비장세포와 항원처리 SRBC와의 작용시간에 따른 rosette 형성율은 1시간 후에 최고에 달하였고 시간의 경과한다 하여 증가하지 않았다. BCG 감염후 1주에서 8주까지 비장에서의 rosette 형성세포를 관찰한 결과 감염 3~4주 후부터 가장 높은 rosette 형성율을 보였으며 이때 rosette 형성세포는 전 비장세포중 5~7%를 차지하였다.

Key Words: Lymphocyte receptor, Rosette assay, BCG.

INTRODUCTION

The interaction of immune system with antigens in the environment occurs via the variable domains of receptor molecules on lymphocytes. In addition to a binding site for antigen, each variable domain carries a particular set of antigenic determinants, the combination of which is now termed idiotype which is recognized by a particular set of anti-idiotypic antibodies.

Jerne[6] proposed that interactions between idiotype and anti-idiotype constitute a key element in specific regulation of antibody responses, leading to the concept of immuno-regulatory networks. Subsequently, numerous examples of idiotypic regulation have been described. The production of anti-idiotypic antibody has been demonstrated during normal immune responses[4,7,8]. In addition, exogeneously administered anti-idiotypic antibody has been found to regulate relevant idiotype-specific antibody responses[9~11].

For the understanding of the cellular and molecular components involved in idiotypic regulatory interactions, isolation of cells bearing antigen-specific receptors is of fundamental importance. Although several methods have been reported for this (for review, see reference[12]) there is, however, no published work dealing with isolation of antigen-specific cells in the conditions of microbial infections.

This paper decribes a simple rosette assay for detection of antigen-specific lymphocytes in *Bacillus Calmette-Guerin* (BCG)- infected mice using sheep erythrocytes(SRBC) coated with BCG antigen.

MATERIALS AND METHODS

Infection of mice

ICR mice, bred locally, were injected intravenously with 5×10^7 viable BCG cells (French strain 1173).

Preparation of BCG antigen

BCG as 3 weeks of cultures on Sauton medium was sonically disrupted for 30 min using

Fisher sonifier (Model 300) at an output effect of 120 W under cooling on ice. The sonic extract was centrifuged at 20,000 G for 20 min at 4℃. Protein content of the supernatant fluid was measured by the Lowry method and stored at −20℃. This supernatant fluid was used as BCG antigen for the sensitization of tannic acid-treated SRBC.

Preparation of indicator cells

Tanned cell technique was used[1,8]. Briefly, 1 ml of 2.5% SRBC suspension was mixed with 1 ml of 1:20,000 tannic acid solution. The red cells were then incubated at 37℃ for 10 min and washed 3 times with 2 ml of phosphate-buffered saline(PBS, pH 7.2) and resuspended in 0.9 ml of PBS. The tannic acid treated red cells were mixed with 0.1 ml (500 μg/ml) of BCG antigen and incubated at 37 ℃ for 10 min. The cells were than washed 3 times with 2 ml of PBS containing 1% heat-inactivated and SRBC-absorbed(1 ml serum plus 0.25 ml of packed SRBC, 45 min at 4℃) normal rabbit serum(PBS-NRS). The indicator cells were adjusted to a concentration of 1% in PBS-NRS.

Preparation of spleen cells

Spleens from 4 mice which were infected 3 weeks before with 5×10^7 BCG were pooled and cell suspension was prepared in RPMI 1640 supplimented with 5% heat-inactivated fetal calf serum(RPMI-FCS). Contaminating red blood cells were lysed by treating with 0.83% Tris-ammonium chloride buffer followed by washing 3 times with RPMI-FCS.

Rosette assay

One volume(1 ml) of spleen cell suspension (2×10^6/ml) was mixed with an equal volume of 0.5% indicator cell suspension and centrifuged at 200 G for 2 min at room temperature. After incubation for 1 h at 4℃ the pellet was carefully resuspended by gentle pipetting and the percentage of rosette was determined by microscopic examination of about 200 cells in a hemocytometer chamber. Cells with 4 or more SRBC were counted as rosette-forming cells(RFC).

RESULTS

Optimal concentration of BCG antigen in the preparation of indicator cells

The indicator cells were prepared with varying doses of BCG antigen. As shown in Fig. 1, the maximum number of RFC was observed at concentration of 50μg/ml. The num-

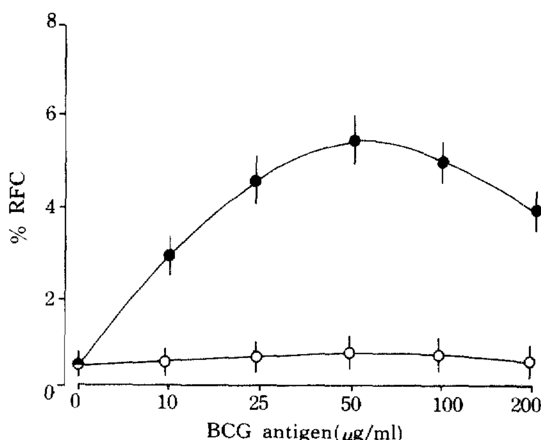

Fig. 1. Effect of varing concentrations of BCG antigen for preparation of indicator cells on rosette formation. Symbols: (●) Spleen cells from BCG-infected mize; (○) Spleen cells from normal mice. Results (mean ± S.E.) from 3 experiments are shown. RFC, rosette forming cells.

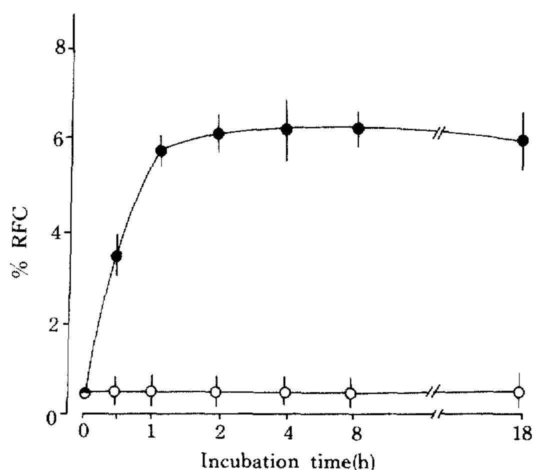

Fig. 2. Effect of incubation time on rosette formation. Symbols: (●) Spleen cells from BCG-infected mics; (○) Spleen cells from normal mice. Results (mean ± S.E) from 3 experiments are shown. RFC, rosette forming cells.

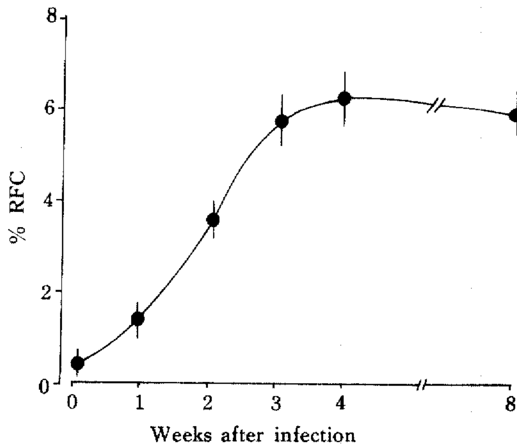

Fig. 3. Change of the number of rosette-forming cells (RFC) during the course of BCG infection. Triplicate determinations were made on 4 spleens for each test.

ber of RFC tended to decrease at concentration higher than 100μg/ml. About 0.5% of normal spleen cells(control rosette) were found to form spontaneous rosette. A concentration of 50μg/ml was chosen for the subsequent test.

Optimal incubation time for rosette formation

Rosette formation was assayed at various incubation time at 4℃. After 30 min incubation, the number of RFC did not reached a maximum value: it was nacessary to incubate for at least 1 h to reach a maximum plateau (Fig. 2). There was no significant difference in rosette formation even after 18 h incubation. One hour of incubation time was chosen for the subsequent test.

Change of the number of RFC during the course of BCG infection

By 2 months after infection, the number of RFC was relatively low at 1 week after infection but increased during the following weeks and reached maximum at 3 or 4 weeks after infection; the number of RFC was account for 5—7% of total spleen cells at this time(Fig. 3).

DISCUSSION

The following three general methods are used for the isolation of antigen binding cells: (1) Rosette assay using red blood cells coated with antigen. (2) Adherence to plastic surfaces like polystyrene coated with antigen. (3) Affinity column chromatography where antigen is chemically bound to polyacrylamide bead matrix. Among these, rosette assay is the most widely used method since it is simple, sensi tive and gives a clear-cut answer.

In this work, tannic acid was used for the treatment of SRBC instead of other coupling agents such as chromic chloride or benzidine compound because tannic acid is routinely used in hemagglutination test for the determination and titration of antibodies against bacterial proteins[2,3]. It was found that the optimal concentration of tannic acid for the preparation of indicator cells was 1:20,000 (data not shown).

The tanned red cells have a tendency of spontaneous agglutination. To avoid this, heat-inactivated and SRBC-absorbed normal rabbit serum was used as a stabilizer and antigen-coated SRBC resuspended in PBS was stand for 1 h at 4℃ and settled erythrocytes at the bottom of the tube was discarded.

The new method for rosette assay described here will be possible to isolate antigen-specific cells by density centrifugation and provide a methodological approach to investigate the direct role of antigen-specific cells in immune regulation in the condition of mycobacterial infections.

SUMMARY

A new method for rosette assay is described for the detection of antigen-specific lymphocytes from BCG-infected mice using sheep erythrocytes coated with BCG antigen. The optimal concentration of BCG antigen for preparation of indicator cells and the incubation time of antigen coated erythrocytes-lymphocytes mixture were 50μg/ml and 1 h, respectively. The number of rosette-forming cells (RFC) during the course of BCG infection sh-

owed gradual increase as infection progressed and RFC was reached maximum (about 5–7 % of splenic lymphocytes formed rosette) at 3 or 4 weeks after infection.

REFERENCES

1) Boyden SV: The adsorption of proteins on erythrocytes treated with tannic acid, and subsequent hemagglutination with antiprotein sera. *J. Exp. Med.* **93**: 107, 1951.
2) Boyden SV and Sorkin E: A study of antigens active in the tannic acid hemagglutination test present in filtrates of cultures of *Mycobacterium tuberculosis*. *J. Immunol.* **75**: 15, 1955.
3) Bhatnagar R, Malaviya AN, Narayanan S, Rajgopalan P, Kumar R and Bharadwaj OP: Spectrum of immune response abnormalities in different clinical forms of tuberculosis. *Ann. Rev. Resp. Dis.* **115**: 207, 1977.
4) Cosenza H: Detection of anti-idiotype reactive cells in response to phosphorylcholine. *Eur. J. Immunol.* **6**: 114, 1976.
5) Frankel S, Reitman S and Sonnenwirth AC: Tannic acid hemagglutination tests. p. 1558, Gradwohl's Clinical Laboratory Methods and Dignosis, 7th ed. The C.V. Mosby Co., Saint Louis, 1970.
6) Jerne NK: Network theory of the immune system. *Ann. Immunology. Paris.* **25C**: 373,1974.
7) Kennedy RC, Ionescu-Matia I, Sanchez V and Dressman GR: Detection of inter-species idiotypic cross-reactions associated with antibodies to hepatitis B surface antigen. *Eur. J. Immunol.* **13**: 232, 1983.
8) Kluskens L and Kohler H: Regulation of immune response by autogenous antibody against receptor. *Proc. Natl. Acad. Sci. USA.* **71**: 5083, 1974.
9) Lee W, Cosenza H and Kohler H: Clonal restriction of the immune response to phosphorylcholine. *Nature (London).* **247**: 55, 1974.
10) Sachs DH, El-Gamil M and Miller G: Genetic control of the immune response to Staphylococcal nuclease. XI. Effect of in vivo administration of anti-idiotypic antibodies. *Eur. J. Immunol.* **11**: 509, 1981.
11) Sy MS, Brown AR, Benacerraf B and Greene MI: Antigen and rereptor driven regulatory mechanisms. III. Induction of delayed-type hypersensitivity to azobenzenearsonate with anti-cross-reactive idiotypic antibodies. *J. Exp. Med.* **151**: 896, 1980.
12) Wolf H, Gattringer C and Wick G: Avian antigen binding cells: Enrichment methods. *J. Immunol. Methods.* **25**: 77, 1979.