วารสารเทคนิคการแพทย์ เชียงใหม่
BULLETIN OF CHIANG MAI MEDICAL TECHNOLOGY
Vol. 6 No. 3
September 1973

STUDIES ON THE COMPETENCE OF SINGLE CELL TO PRODUCE ANTIBODIES OF TWO SPECIFICITY BY ROSETTE-FORMATION TECHNIQUE

Pranee Leechanachai, B.Sc. (Med. Tech.) *
Sanit Makonkawkeyoon, Ph. D. *

Abstract

The study of the competence of single cell to produce antibodies of two specificities by rosette formation technique was carried out. The required antigens should be different in size and shape and should have no cross reaction for each other for example, sheep and chicken red blood cells. After both antigens have been injected together, on the tenth day we get the peak of rosette formation. In any one cell rosette formation against only the sheep or chicken erythrocytes have been observed. Very few cells of the rosette formation against the two different antigens were observed. The amount of both rosette formations against each type of antigens is almost equal. Therefore, we are prefer to agree with the hypothesis that one antibody forming cell is able to produce one antibody against one antigen at one time.

Introduction

One of the prime problems of immunology is to deal with the potential of the immune competent cell to produce antibody of a given specificity. The majority of antibody-forming cells from animals immunized with two or more antigens form detectable amounts of only one antibody at one time. Evidently, many experiments show that after immunization with arbitrary chosen antigens, each immune competent cell can form antibodies of more than one specificity.

A number of methods for studying antibody formation by single cell have been applied to test the predictions of the

* Department of Microbiology, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand.

various theories of antibody production. These include assays for bacterial immobilization and agglutination in microdroplets, phage neutralization in microdroplets, fluorescent antibody and the hemolysin plaque technique. In these studies 0-45% of the cells have been reported to produce antibodies more than one specificity.

In this experiment, a rosette formation technique was used for studying the competent of antibody forming cell. Rosette formation technique is a very sensitive assay system for detecting antibody forming call. Rosette-forming cell is the immunocytoadherence lymphoid cell which attached by sheep or chicken erythrocytes. The attachment is complement independent reaction.

Materials and Methods

Immunization of mice. Albino mice aged 2-3 months were used for immunization with erythrocytes. Sheep red blood cells (SRBC) and chicken red blood cells (CRBC) were collected in Alsever solution. They were washed three times with normal saline solution before injection.

Mice were dlvided into four groups, and each group composed of six mice. The first one is a control group, the second group received intraperitoneally injection of 2% SRBC for 1 ml. The third group was injected with 1 ml. of 2% CRBC and the last one received 1 ml. of 2% mix SRBC acd CRBC.

Assay for rosette formation. Different groups of immunized mice were killed at the 5, 10 and 20th day after immunization. Their spleens were removed and teased in the presence of Engles's medium and the final concentration of cells were 6×10^6 cells / ml. Rosette were prepared by mixing 1.0 ml. of spleen cells with 1 ml. of 2% SRBC or 2%CRBC or the mixture of 2% SRBC and CRBC in a test tube. The mixtures were incubated at 37 C. for one hour and the cells suspension was mounted on the slide and observed for number of rosette per 100 of WBC. Only completely surrounded by SRBC or CRBC or mixed SRBC and CRBC with a berry appearance were counted as rosette. Fig. 1

Results

The results of this series of experiment are summarized in Table I, II, and III. One can noticed that among rosette appears is rosette only against SRBC or CRBC. The peak of rosette fomation was at the tenth day of the immunization and the amount of rosette formation against SRBC and CRBC in the mice that received mixed antigens were nearly equal. Fig 2

Incidencely a few of rosette against both SRBC and CRBC in one lymphoid cell have been observed, but it is very few when compare with the spleen cells.

Therefore it is not obvious to say that one antibody forming cell is able to produce antibodies of two specificity.

Discussion

In this experiment shows that after immunization of an animal with two different antigens, each cell produces antibody of only one specificity. Similar observations have been made in previous studies. However, other reports claimed to have demonstrated the production of antibodies of two specifity by single cell.

The possibility that one cell may simultaneously respond to two antigenic determinants but they may detect by this technique because of the size of both antigens. Chicken red blood cell was larger than Sheep red blood cell, so they may prevent the other to expose the lymphoid cell.

In considering the possibility that one cell may respond to two antigenic determinants, it would appear that the chances of observing such "double" antibody producers, if they exist, might be enhance if the two determinants were so linked that they traveled together as a single molecule. Thus, any cell stimulated by one of the antigenic determinants would have a much greater chance for contact and potential stimulation with the second determinant than the antigenic determinant which carried by separate antigens.

Figure I Various Rosette-forming cells

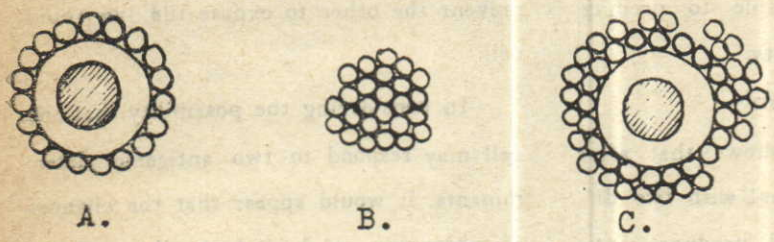

Figure II Per cent of Rosette-forming cells in different period of time.

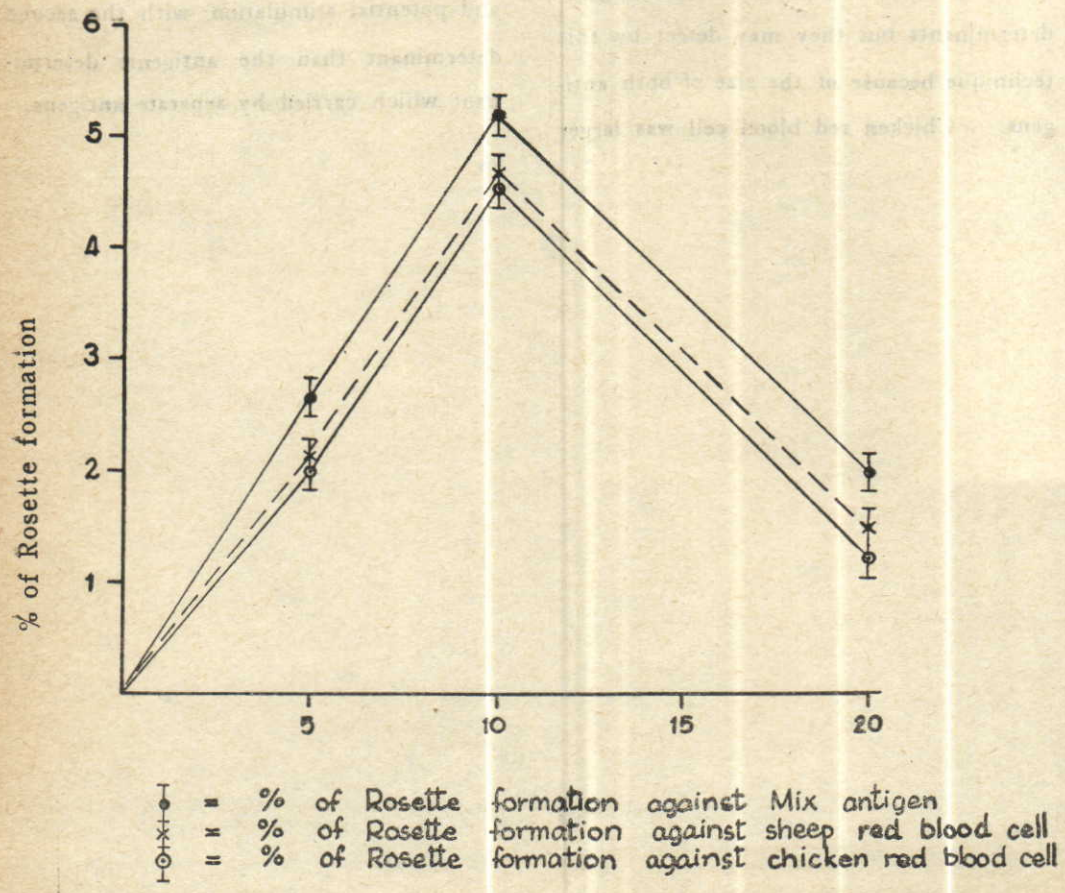

Table I. Number of rosette forming cells on the fifth day after immunization.

Mice	No.	% of total Rosette		% of Rosette against SRBC*		% of Rosette against CRBC**	
			เฉลี่ย		เฉลี่ย		เฉลี่ย
Control	1	0	0	0	0	0	0
	2	0		0		0	
Injected with SRBC	1	2	2	2	2	–	–
	2	2		2		–	
Injected with CRBC	1	2	2	–	–	2	2
	2	2		–		2	
Injected with Mix SRBC & CRBC	1	3	2.7	1	0.7	2	2
	2	2		0		2	
	3	3		1		2	

* SRBC = Sheep Red Blood Cells

** CRBC = Chicken Red Blood Cells

Table II. Number of rosette-forming cells on the tenth day after immunization

Mice	No	% of total Rosette	เฉลี่ย	% of Rosette against SRBC*	เฉลี่ย	% of Rosette against CRBC**	เฉลี่ย
Control	1	0	0	0	0	0	0
	2	0		0		0	
Injected with SRBC	1	4	4.5	4	4.5	–	–
	2	5		5		–	
Injected with CRBC	1	5	4.5	–	–	5	4.5
	2	4		–		4	
Injected with Mix. CRBC & SRBC	1	5	5.3	3	3.0	2	2 3
	2	6		3		3	
	3	5		3		2	

* SRBC = Sheep Red Blood cells

** CRBC = Chicken red blood cells

Table III. Number of rosette – forming cells on the twentieth day after immunization.

Mice	No.	% of total average Rosette	เฉลี่ย	% of Rosette against SRBC*	เฉลี่ย	% of Rosette against CRCB**	เฉลี่ย
Control	1	0	0	0	0	0	0
	2	0		0		0	
Injected with SRBC	1	2	1.5	2	1.5	–	–
	2	1		1		–	
Injected with CRBC	1	2	1.5	–	–	2	1.5
	2	1		–		1	
Injected with Mix. CRBC & SRBC	1	3	2.0	2	1.3	1	0.7
	2	1		1		0	
	3	2		1		1	

* SRBC = Sheep red blood cell

** CRBC = Chicken red blood cell

References

1. Makinodan, T:, Ruth, R.F. and Wolfe, H.R.: Precipitin production in chicken, XI. Site of antibody production, J. Immunol., 72:45-51, 1954.
2. Nossal, G.J.V. and Makela; O.: Kinetic studies on the incidence of cells appearing to form two antibodies, J. Immunol., 89:604-612, 1962.
3. Gudat, F.G., Harris, T.N., Harris, S. and Hummeler, K.: Studies on antibody-producing cell. J. Exptl. Med, 133:305-320, 1971.
4. Wilson, J. D.: The relationship of antibody-forming cells to rosette-forming cells, Immunology, 21: 233-245, 1971.
5. Klinman, N.R.: The mechanism of antigenic stimulation of primary and secondary clonal precausor cells, J. Exptl. Med., 136:241-260, 1972.
6. Vazquez, J. J.: Antibody-or gamma globulin forming cell as observed by the fluorescent antibody technic, Lab. Invest., 10:1110-1125, 1961.
7. Nossal, G.J.V. and Makela, O.: Genetic aspects of antibody formation, Lab. Invest. 10:1094-1109, 1961.
8. Lederberg, J.: Genes and antibodies, Science, 129:1649-1653, 1959.
9. Schwartzman, J.S.: Effect of number of antigens on the quantity of cells producing several kinds of antibody, Nature, 213:925-926, 1967.