

Acta Medica Okayama

Volume 28, Issue 5

1974

Article 7

OCTOBER 1974

Studies on the population of human peripheral lymphocytes forming rosette with dog erythrocytes

Koji Nishiya*

Seizo Yamana[†]

Keisuke Yano[‡]

Katuhide Ensako**

Tadaaki Fujiwara^{††}

Tadashi Ofuji^{‡‡}

*Okayama University,

[†]Okayama University,

[‡]Okayama University,

**Okayama University,

^{††}Okayama University,

^{‡‡}Okayama University,

— BRIEF NOTE —

**STUDIES ON THE POPULATION OF HUMAN PERIPHERAL
LYMPHOCYTES FORMING ROSETTE WITH
DOG ERYTHROCYTES**

Koji NISHIYA, Seizo YAMANA, Keisuke YANO, Katuhide ENSAKO,
Tadaaki FUJIWARA and Tadashi OFUJI

*Department of Internal Medicine, Okayama University Medical School,
Okayama, Japan. (Director: Prof. T. Ofuji)*

Received for publication, June 25, 1974

In 1969, Dr. BACH (1) demonstrated that the lymphocytes in non-immunized mice formed rosette with sheep red blood cells (SRBC). It is well known that a large population of human peripheral lymphocytes that form spontaneous rosettes with SRBC belongs to the thymus derived lymphocytes (T cell). The evidence for this depends on that almost all the human thymocytes form rosettes, and it is blocked by a specific anti-thymocyte serum (2, 3). The mechanisms and the causes of rosette formation, however, are still obscure. Recent reports have demonstrated that a low but significant percentage of human lymphocytes can bind the red blood cells of pig (4).

We examined the spontaneous rosette formation between peripheral lymphocytes and red blood cells in various animals such as dogs, guinea pigs, rats, mice and human beings.

Heparinized peripheral blood was obtained by heart puncture or venopuncture, and the lymphocytes were separated using Conray-Ficoll gradient sedimentation method, and the 5×10^6 /ml lymphocytes were suspended in Hanks' balanced salt solution (Hanks' BSS) including 10% (v/v) fetal calf serum (FCS). Various kinds of red blood cells were also suspended in the identical solution to make the final concentration to 0.5% (v/v) erythrocyte suspension. Equal volumes (0.5 ml) of lymphocyte and red blood cell suspension were mixed in a test tube (10 × 150 mm), centrifuged at 180 g. av. for 5 min and incubated for 30 min at room temperature. After incubation, the suspension was gently mixed at 33 r. p. m. for 5 min. One drop of the mixed suspension was put on a slide glass, mounted by a cover slip, and erythrocyte rosette-forming lymphocytes were counted under the light microscope. Lymphocytes possessing three or more adherent erythrocytes were evaluated as positive cells.

As a result it was found that a low percentage rosette formation was between guinea pig lymphocytes and rat erythrocytes (3.0%), dog lymphocytes

374 K. NISHIYA, S. YAMANA, K. YANO, K. ENSAKO, T. FUJIWARA and T. OFUJI

and guinea pig erythrocytes (2.4%), human lymphocytes and mouse erythrocytes (1.7%). However, an increased number of rosette formation between human lymphocytes and dog erythrocytes (DRBC, 35.1%) was observed. The intensity of this reaction seemed to be temperature-dependent, giving an optimal binding at room temperature *e. g.* appropriately at 15°C.

In addition, the population of DRBC rosette forming lymphocytes (DRBC-RFL) was compared with that of SRBC rosette forming lymphocytes (SRBC-RFL). Namely, peripheral lymphocytes challenged with SRBC in advance were divided equally into two test tubes. To one test tube, an equal volume of SRBC suspension was added again and to the other, the DRBC suspension. The subsequent procedures were as previously described. The former (SRBC) experiment revealed the mean $\pm$ SD = $53.6 \pm 9.0\%$ and the latter (DRBC), $68.3 \pm 9.6\%$ on the average of five preparations ($P < 0.05$). Similar experiments using DRBC rosette forming lymphocytes were performed, which yielded nearly the same results as with SRBC rosette forming lymphocytes. We observed in the present experiments that both sheep and dog erythrocytes would attach to the same lymphocyte. Therefore, we conclude that one group of DRBC-RFL belongs to the same population as that of SRBC-RFL and other group is of a different population. As already mentioned, SRBC-RFL belongs to T cell, so that a major part of DRBC-RFL would belong to the T cell population, but it may be said that at least one group of the DRBC-RFL population might be different from SRBC-RFL population.

TABLE 1 SPONTANEOUS "SRBC AND DRBC" ROSETTE FORMATION OF HUMAN PERIPHERAL LYMPHOCYTES

Human lymphocyte	SRBC	SRBC* + SRBC	SRBC** + DRBC	DRBC	DRBC + DRBC	DRBC + SRBC
1	49.0 (%)	51.5	77.6	2.9	32.8	50.0
2	38.0	37.0	77.7	8.9	25.5	58.0
3	48.8	57.5	66.5	7.2	34.3	51.7
4	51.5	62.4	68.2	12.8	35.0	58.7
5	44.6	59.5	51.6	9.5	34.2	48.4
Mean	46.4	53.6	68.3	8.3	32.4	53.4
$\pm$ SD	$\pm$ 4.7	$\pm$ 9.0	$\pm$ 9.6	$\pm$ 3.2	$\pm$ 3.5	$\pm$ 4.2

(P < 0.05)

* incubated first with SRBC, then repeated, indicating an increase of the rosette forming cells

** incubated first with SRBC, then repeated using DRBC instead of SRBC, indicating an increase of the rosette forming cells as well

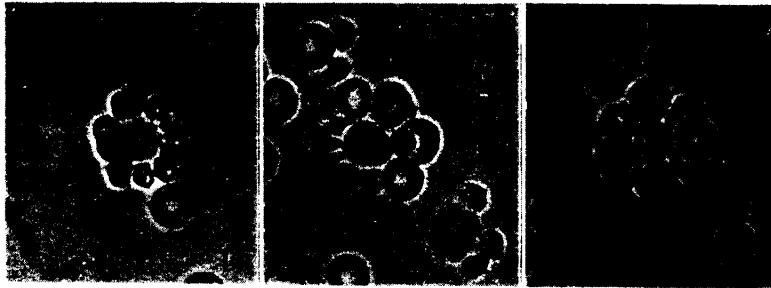


Fig. 1 Rosette forming lymphocytes

1. Human peripheral lymphocyte forming rosette with sheep red blood cells. ($\times 1600$)
2. Human peripheral lymphocyte forming rosette with both sheep (small one) and dog (large one) red blood cells. ($\times 1600$)
3. Human peripheral lymphocyte forming rosette only with dog red blood cells. ($\times 1600$)

REFERENCES

1. BACH, J.F., MULLER, J.Y. and DARDENNE, M.: *In vivo* specific antigen recognition by rosette forming cells. *Nature* **227**, 1251, 1970
2. BACH, J.F., DORMONT, J., DARDENNE, M. and BALNER, H.: *In vitro* rosette inhibition by antihuman antilymphocyte serum. *Transplantation* **8**, 265, 1969
3. BRAIN, P., GORDON, J. and WILLETTS, W.A.: Rosette formation by peripheral lymphocytes. *Clin. exp. Immunol.* **6**, 681, 1970
4. LAY, W.H., MENDES, N.F., BIANCO, C. and NUSSENZWEIG, V.: Binding of sheep red blood cells to a large population of human lymphocytes. *Nature* **230**, 531, 1969