Kawasaki Med. J. Vol. 6: No. 1·2, 65-69, 1980

THE SPONTANEOUS CYTOTOXICITY OF RAJI CELLS TO SHEEP RED CELLS DETECTED WITH THE PLAQUE ASSAY

Ayako UEKI and Tetsuo KIMOTO

Department of Pathology, Kawasaki Medical School, Kurashiki 701-01, Japan

Accepted for Publication on May 27, 1980

Abstract

The authors observed that RAJi cells, a lymphocyte cell line of B cell type derived from Burkitt's lymphoma, show cytotoxic effector potential to sheep red blood cells (SRBC) using the plaque forming method. We have studied three Burkitt's lymphoma cell lines, RAJi, EB-3 and P3HRI, and only RAJi cells showed such a cytotoxic effector potential. This cytotoxicity might be considered as the same quality as observed in rat or mouse fibroblasts or P815Y mastocytoma cells which are capable of killing red blood cells but incapable of killing metabolically active nucleated cells.

The moieties in cell membranes of RAJi cells, which exist only in RAJi cells but not in P3HRI and EB-3 cells and are capable of killing target SRBC, remain to be clarified.

The existence of natural cell-mediated cytotoxicity of normal individuals against a variety of tumor targets has been well documented[1]. Ortaldo et al.[2] studied the generation of NK and K cells in cultures, and characterized the precursors of the Fc receptor positive NK and K cells as Fc receptor negative null cells. Muchmore et al.[3] reported that B cells, T cells and macrophages could act as effectors in a PHA-induced cytotoxicity model. According to Muchmore et al.[4] the type of target cell employed is of paramount importance in such experiments, and there is a fundamental difference between metabolically active nucleated cells compared with more quiescent targets like red blood cells. The ability to kill nucleated tumor cells after stimulation with PHA is a T dependent function, and RBC cytolysis is T independent. Another report by Muchmore et al.[5] shows the evidence that the capacity to lyse RBC targets in the presence or absence of lectin is not limited to lymphoid cells, but cultured rat or mouse fibroblasts or P815Y mastocytoma cells are capable of killing RBC targets.

The authors have observed by chance the cytotoxic effect of RAJi cells

植木絢子，木本哲夫

to SRBC, and tried to compare the effect with those of other lymphocyte cell lines derived from Burkitt's lymphoma.

MATERIALS AND METHODS

Cell lines : RAJi, P3HRI and EB-3 lymphoblastic cells derived from Burkitt's lymphoma were grown in suspension in RPMI 1640 medium (Grand Island Biological CO., Grand Island, N. Y.) supplemented with 30% fetal calf serum (FCS) in the air atmosphere of 5% CO_2 in CO_2 incubator. *Cytotoxic effector potential assay* : Sheep red blood cells were washed three times with phosphate buffered saline (PBS) and resuspended into PBS at the concentration of 1×10^8 cells/ml. Five μl of the suspension was added to each well of Falcon 3034 microtest plate (Falcon, USA) pretreated with poly-L-lysine solution, and a single monolayer of SRBC on the plate was obtained after the centrifugation of 700 rpm for 10 min, and 2-4μl of suspension of cells (1×10^6 cells/ml of RPMI 1640 medium without serum) was added to each well and incubated for 4 hr in a CO_2 incubator. After the incubation the warmed mounting agar (Japn Immunores. Lab. CO., Japn) containing fixatives and staining solution were poured into each well and left in room temperature for 30 min. The plaque forming ratio was counted under a usual light microscope.

RESULTS

The mode of cytolytic plaque varies in different cell groups. Among plaques formed by human peripheral blood monocytes, type 4 can be often observed, additioned to type 1, as shown in Fig. 1. On the contrary K cells

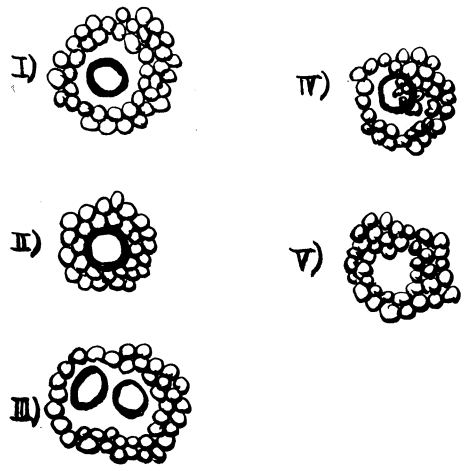

Fig. 1. Schematic classification of types of plaque observed in the cell mediated cytotoxicity.

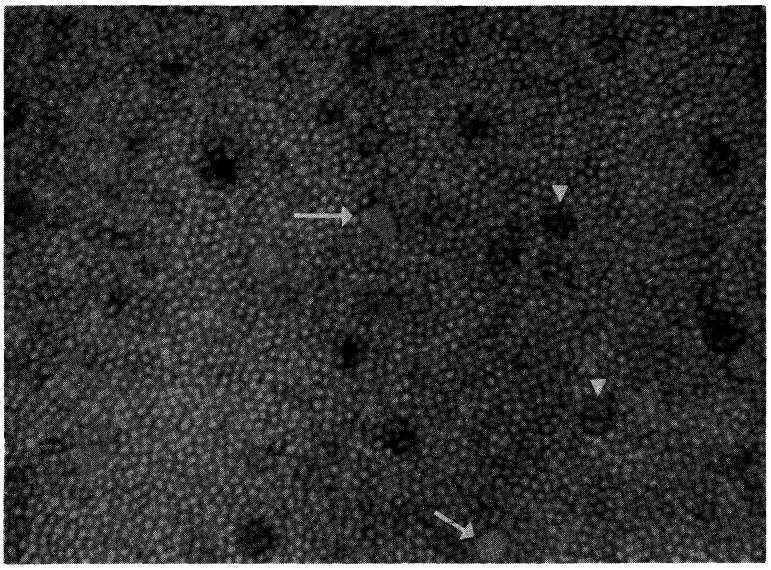

Fig. 2. Lytic plaque formed by RAJi cells. → positive cell
▶ negative cell × 500

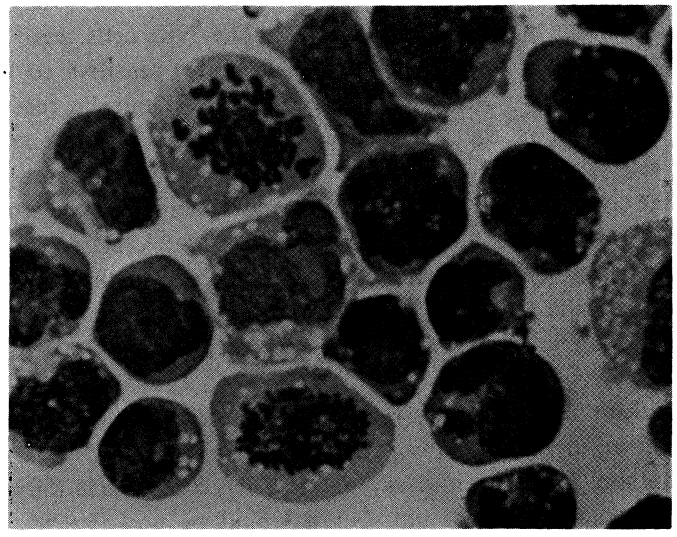

Fig. 3. Smear of RAJi cells. Wright stain. ×1500

and NK cells in human peripheral blood lymphocytes form plaques type 1-3. RAJi cells form plaques with SRBC almost always that of type 2, when they were incubated for 4 hrs at 37°C. As shown in Table 1, the percent of

TABLE 1. The percentage of plaque forming ratio in three cell lines of Burkitt's lymphoma.

Cell line	anti-SRBC antibody(−)	anti-SRBC antibody(+)
RAJi	44.6±1.5(%)	42.8±1.8(%)
EB-3	0.7±0.4	0.5±0.2
P3HRI	1.1±0.5	1.7±0.4

spontaneous plaque formation in RAJi cells was 44.6±1.5%. On the other hand those of EB-3 and P3HRI cells were 0.7±0.4% and 1.1±0.5 respectively. The authors repeated the experiments 4 times, and the results was the same as those obtained previously.

The effect of IgG fraction of anti-sheep red cell antiserum on the plaque formation was also examined. The rosette formation in RAJi cells was 42.8 ±1.8%, and 0.5±0.2% in EB-3 and 1.7±0.4% in P3HRI cells. As known from these results the IgG fraction of antiserum had no effect on the percentage of the spontaneous plaque formation of RAJi cells with sheep red blood cells.

DISCUSSION

Ortaldo et al.[2] reported details about the nature of NK cells, which are capable of killing active nucleated cells. The NK cells were Fc receptor positive, surface membrane immunoglobulin negative, sensitive to trypsin, and boostered by interferon. Most cultured NK cells without Fc receptor form E rosette, and the effect of the treatment of cultured Fc receptor negative cells with anti-T cell serum plus complement was consistent with those of fresh NK cells. On the other hand, Muchmore et al.[5] demonstrated that cultured mouse L cells, rat embryo fibroblasts, and P815Y DBA mastocytoma cells are capable of mediating PHA-induced cytotoxicity when chicken red blood cells are used as targets, and that these cells are usually cytotoxic even in the absence of PHA, although a markedly enhanced cytotoxicity could be seen when PHA was added.

The authors have previously reported about the Concanavalin A induced cytotoxicity of cultured lymphoblastoid cells to tumor cells.[6]

The authors have never tried to test if RAJi cells have the ability to kill active nucleated tumor cells or not, but it would be probable to consider that the cytolytic effector potential of RAJi cells to SRBC is similar to those of mouse or rat fibroblasts and P815Y mastocytoma cells, since RAJi cells are not T cell origin, but derived from B cells of Burkitt's lymphoma. The reason why RAJi cells are cytotoxic to SRBC, and why EB-3 or P3HRI cells are not cytotoxic, remains to be clarified.

REFERENCES

1) Herberman, R. B. and Holden, H. T.: Natural cell-mediated immunity. Adv. Cancer Res. **27** : 305-377, 1978
2) Ortald, J. R., Bonnard, G. D., Kind, P. D. and Herberman, R. B. : Cytotoxicity by cultured human lymphocytes : characteristics of effector cells and specificity of cytotoxicity. J. Immunol. **122** : 1489-1494, 1979
3) Asherson, G. L., Ferluga, J. and Janossy, G. : Non-specific cytotoxicity by T cells activated with plant mitogens in vitro and the requirement for plant agents during the killing reaction. Clin. Exp. Immunol. **15** : 573-589, 1973
4) Möller, G., Sjöberg, and Anderson, J. : Mitogen-induced lymphocyte-mediated cytotoxicity in vitro : Effect of mitogens selectively activating T or B cells. Eur. J. Immunol. **2** : 586-592, 1972
5) Muchmore, A. V., Nelson, D. L. and Blaese, R. M.: The cytotoxic effector potential of some common non-lymphoid tumors and cultured cell lines with phytohemagglutinin and heterologous anti-erythrocyte antisera. J. Immunol. **114** : 1001-1003, 1975
6) Kimoto, T., Ueki, A. and Nishitani, K.: Phagocytosis of lymphoblastoid cells and cell destruction of human malignant tumor cells. Acta Path. Jap. **25** : 99-114, 1975