RAPID COMMUNICATION Jpn. J. Cancer Res. 82, 621–623, June 1991

A New Form of Specific Targeting Cancer Immunotherapy Using Anti-tumor Monoclonal Antibody-conjugated Lymphokine-activated Killer Cells

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Cross-linking of effector T cells to target cancer cells augments their tumor lytic activities. Here we describe a new method of conjugating lymphokine-activated killer (LAK) cells with cancer-specific monoclonal antibody. The LAK cells were biotinylated, treated with avidin, and conjugated with biotinylated monoclonal antibody. These monoclonal antibody-conjugated LAK cells showed specifically enhanced killing activities against anti-tumor antibody-reactive cancer cells, and cold target cells specifically inhibited their activities.

Key words: Anti-tumor monoclonal antibody — Lymphokine-activated killer cells — Specific targeting cancer immunotherapy

Bispecific hybrid antibody composed of anti-CD3 monoclonal antibody and anti-target monoclonal antibody greatly enhances T cell killer activity against cancer cells.[1–4] This finding shows that bispecific antibody induces cross-linking of effector T cells to target cancer cells. In fact, bispecific hybrid antibody was effective in malignant glioma therapy.[5] However, the preparation of bispecific antibody is complicated, and the separation of many kinds of anti-target monoclonal antibodies is time-consuming. To overcome these problems, in this work we developed a new method for conjugation of activated lymphocytes with monoclonal antibody.

We used lymphokine-activated killer (LAK)[5] cells generated from peripheral blood mononuclear cells of healthy volunteers by culture with 100 Jurkat units per ml of interleukin-2 for 5 days. After activation, 10^7–10^8 LAK cells were washed with phosphate-buffered saline (PBS), resuspended in 5 ml of PBS and incubated with N-hydroxysuccinimide (NHS) linked to biotin (5×10^{-7} mol dissolved in 50 μl of dimethyl formamide) for 1 h at 4°C, according to the method reported by Lo *et al.*[6] The biotinylated LAK cells were extensively washed with cold PBS to remove unbound biotin. The cells were then resuspended in 1 ml of cold PBS and mixed with 200 μg of avidin for 30 min at 4°C to allow its conjugation with biotin on the LAK cell surface. The avidin-biotin-conjugated LAK cells were washed with cold PBS to remove unbound avidin, resuspended in 1 ml of cold PBS and incubated with 100 μg of biotinylated monoclonal antibody for 30 min at 4°C (Fig. 1). The yield on generation of monoclonal antibody-conjugated LAK (MAC-LAK) cells was 86.9±9.2%. All these steps were conducted at 4°C to prevent capping and internalization of the conjugate and to preserve the viability of the LAK cells. More than 95% of the cells were conjugated with monoclonal antibody in these steps. The viability of LAK cells was consistently more than 98% and their growth rate after their conjugation with monoclonal antibody was similar to that of unbiotinylated LAK cells within three days.

In initial trials we used 2-70 monoclonal antibody,[7] which reacts with various gastric cancer cell lines, including MKN28[8] derived from differentiated gastric carcinoma and MKN45[8] derived from undifferentiated gastric carcinoma. This 2-70 antibody is a mouse IgM type antibody and has no antibody-dependent cell-mediated cytotoxicity (ADCC) against LAK cells (Table I). The cytotoxic effects of LAK cells were not augmented in the presence of 2-70 monoclonal antibody. The 2-70 antigen(s) that reacted with this monoclonal antibody was not liberated into the culture supernatant.

Eighteen-hour chromium release assays were performed to detect augmentation of cytotoxicity. MAC-LAK cells showed significantly enhanced cytolysis of 2-70 antibody-reactive MKN28 and MKN45 cells ($P<0.01$), but not the Daudi cell line, which does not react with 2-70 antibody (Fig. 2). Cold target inhibition assay

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[5] The abbreviations used are: LAK, lymphokine-activated killer; PBS, phosphate-buffered saline; NHS, N-hydroxysuccinimide; ADCC, antibody-dependent, cell-mediated cytotoxicity; MAC-LAK, monoclonal antibody-conjugated, lymphokine-activated killer.

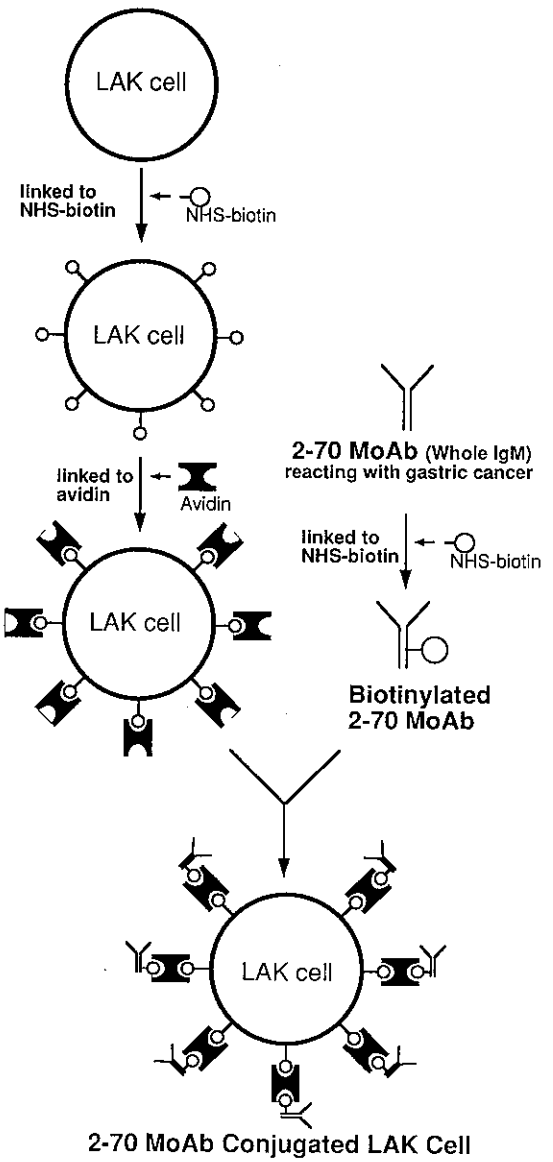

Fig. 1. Scheme for conjugation of monoclonal antibody with LAK cells.

Table I. Cytotoxic Effects with 2-70 Monoclonal Antibody

2-70 Monoclonal antibody concentration (μg/ml)	% Lysis[a)]
0	9.6±1.09
3	9.1±0.11
10	10.1±0.44
30	9.5±1.11

a) The 18-h chromium release assay was performed with various concentrations of 2-70 monoclonal antibody against MKN-45 cells. The effector (LAK):target (MKN45) ratio was 20:1.

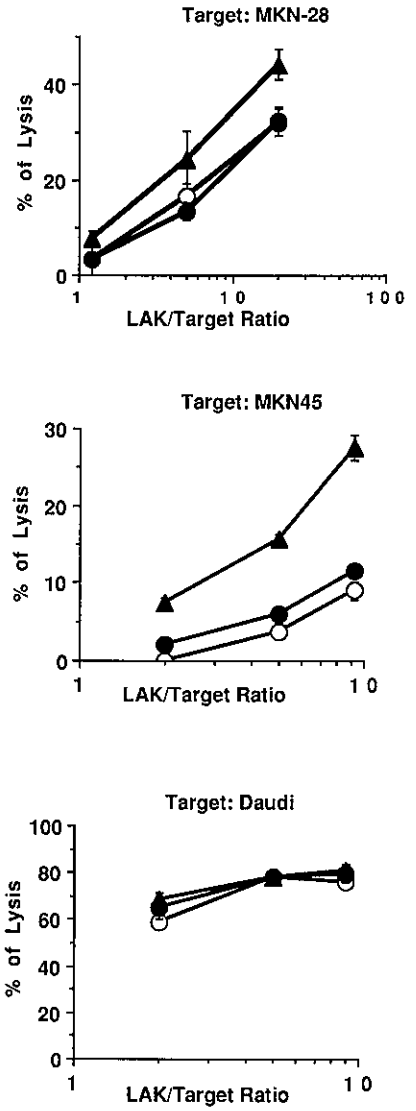

Fig. 2. Cytotoxic effects of MAC-LAK cells against 2-70 monoclonal antibody-reactive MKN28 and MKN45 cells and non-reactive Daudi cells. Results of an 18-h chromium release assay are shown. The effector cells were 2-70 MAC-LAK cells (▲), biotinylated LAK cells (●) and unbiotinylated LAK cells (○). The cytotoxic activities of LAK cells against MKN28 and MKN45 cells but not against Daudi cells were significantly increased by their conjugation with 2-70 monoclonal antibody ($P<0.01$).

was performed. Cold MKN45 cells inhibited MAC-LAK cell activities significantly ($P<0.01$) (Fig. 3). Free 2-70 antibody inhibited MAC-LAK cell activities; 100 μg/ml of 2-70 antibody reduced their activities against MKN45 cells by 22%. The present results suggest that conjuga-

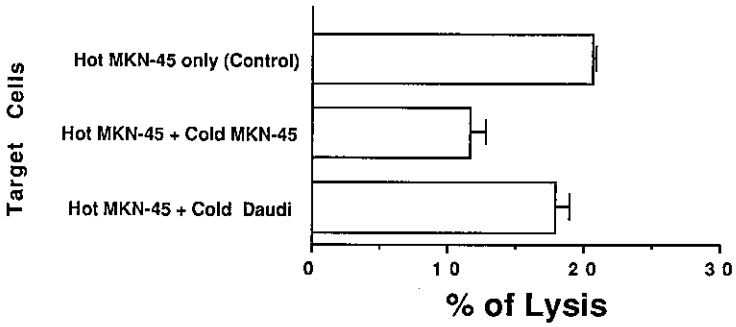

Fig. 3. Cold target inhibition assay of MAC-LAK cells. The hot target was MKN-45 cells and the cold targets were MKN-45 and Daudi cells. The cold:hot ratio was 1:1. The control included no cold cells. The effector (MAC-LAK cells):hot target ratio was 10:1. Values are for 18-h chromium release. MKN-45 cells, but not Daudi cells inhibited hot target lysis significantly ($P<0.01$).

tion of monoclonal antibodies with LAK cells resulted in cross-linking of LAK cells to target cancer cells.

The preparation of bispecific antibodies is time-consuming, and preparation of IgM class antibodies is difficult. However, the MAC method can be used for any class of biotinylated immunoglobulin. This monoclonal antibody conjugation method appears to be an easier and useful way to achieve specific targeting cancer immunotherapy.

We thank S. Shimizu for technical assistance, and T. Yamazaki, National Cancer Center Research Institute, for discussions. Interleukin-2 (S-6820) was kindly provided by Shionogi Pharmaceutical Co. Ltd. (Osaka). This work was supported by a Grant-in-Aid for the Comprehensive 10-Year Strategy for Cancer Control from the Ministry of Health and Welfare of Japan.

(Received January 31, 1991/Accepted April 5, 1991)

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