Studies on Monoclonal Anti-isotypic and Anti-idiotypic Antibodies against Leukemia and Myeloma: V. The Effects of Monoclonal Antibodies and Interferon on the Levels of Cyclic Nucleotides in Leukemic Cell Lines*

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Summary, After the leukemic cell lines were treated with monoclonal antibodies (McAbs) and interferon (IFN-α), the changes of cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) levels in the corresponding leukemic cell lines were measured by radioimmunoassay. The results showed that when the ratio of antigen to antibody was 80 to 1, the cAMP levels in the leukemic cell lines were obviously higher than those in the controls while the cGMP levels were obviously lower after being treated with the corresponding McAbs for 16-24 h (P<0.001). The average level of intracellular cAMP was remarkably increased and that of cGMP underwent no significant changes in the leukemic cell lines after treatment with IFN-α.

Key Words: cyclic nucleotide, leukemic cell lines, monoclonal antibodies, interferon

In recent years, it has been proved that the changes in the cAMP and cGMP levels were observed in malignant cells, namely, a decrease in cAMP level and an increase in cGMP level[11]. These changes could be corrected by some immunofactors. The modulations of IFN, thymosin, transfer factor (TF) on intracellular cyclic nucleotide level have been reported in the literature at home[21]. However, little was reported regarding the effect of McAb on intracellular cyclic nucleotides. In our experiment, we studied the effects of McAbs and IFN-α on metabolism of intracellular cyclic nucleotides and presence or absence of the modulation by McAb and IFN-α, thereby providing a scientific basis for their application.

MATERIALS AND METHODS

1. Tumor cell lines cultured in vitro
B lymphoma cell line Daudi, T
lymphocytic leukemic cell line Molt-4, plasmacytoma cell lines LB-84-4, LB-84-5, common acute lymphocytic leukemic cell line KM3 were all kindly presented by Dr. He Yue (Heidelberg University, Germany). Granulocytic leukemic cell lines HL-60, U937 and erythroleukemic cell line K562 were kindly provided by the Institute of Tumor, Academy of Military Medical Sciences and Department of Microbiology, Tongji Medical University respectively.

2. Reagents
SM6 monoclonal anti-Id antibody to IgM from a patient with chronic lymphocytic leukemia was prepared in our Department. OKT10, McAb against B lymphocyte and IFN-α were purchased from Wuhan Institute of Biological Products. The kits for the detection of \(^{3}\text{H}-\text{cAMP}\) and \(^{3}\text{H}-\text{cGMP}\) were purchased from the Isotype Laboratory of Shanghai Second Medical University.

3. Method
Peripheral blood mononuclear cells (PBMC) were isolated from peripheral blood of healthy volunteers by Ficoll-Hypaque density gradient centrifugation. The concentration of PBMC was adjusted to \(1 \times 10^7\) cells/ml. The tumor cell lines were cultured with 20% FCS-RPMI-1640 medium at 37°C, 5% CO\(_2\) for 24-48 h. The staining with Trypan-blue showed that the activity of living cells was over 95% in every kind of cells as described above. Tumor cells, which were adjusted to appropriate concentration and mixed with different dilution of McAb and IFN-α respectively, were cultured for different periods of time and then washed 2 times with Hank's solution. Finally, the concentration of cells was adjusted to \(1 \times 10^7\) cells/ml for use.

Intracellular cAMP and cGMP were extracted from the cells by way of heating at 100°C. The levels of cAMP and cGMP were detected with the kit of \(^{3}\text{H}-\text{cAMP}\) and \(^{3}\text{H}-\text{cGMP}\), and a standard curve was established at every test. The concentrations of intracellular cAMP and cGMP were calculated according to regression equation.\(^{[4]}\)

RESULTS
1. The levels of cAMP and cGMP in PBMC from healthy subjects were at \(5.82 \pm 0.76\) and \(0.81 \pm 0.21\) (n=15) respectively.

2. Selection of time and concentration of McAb producing effects on tumor cells.

The checkerboard titration demonstrated that the levels of intracellular cAMP and cGMP underwent remarkable changes after the cells were incubated for 16-24 h with McAb diluted at 1:80 (fig. 1, 2). Therefore, 16 to 24 h were selected as the optimal active time and 1:80 as the optimal active McAb concentration.

![Fig. 1. The change of cAMP and cGMP levels of Daudi cells after treatment with McAb (SM6) in different times.](image)

![Fig. 2. The effects of cAMP and cGMP levels of Daudi cells by McAb in different concentration.](image)

3. The effects of McAb on intracellular cAMP and cGMP
The levels of cAMP and cGMP of