(4) Chemoimmunotherapy of Acute Leukemia with Cell Wall Skeleton of BCG

Kazumasa YAMADA, MD

The First Department of Internal Medicine, Nagoya University School of Medicine

There have been an increasing number of clinical trials in patients with a variety of neoplastic diseases which demonstrate that immunotherapy using living cells of *Mycobacterium bovis* strain of BCG can favorably improve prognosis at least temporarily and tilt the balance in favor of the patient. The difficulties inherent in the use of living bacteria, in terms of safety, stability and reproducibility, however, have stimulated interest in non-viable materials.

The present clinical trial was designed to evaluate toxicity, immunological response and possible therapeutic benefits of immunotherapy of BCG-CWS in adult patients with acute leukemia.

1) Studies of Tumor Specific Antigens on Leukemia Cells by Mixed Lymphocyte-Leukemia Cell Cultures.

Prior to immunotherapy, mixed cultures of lymphocyte and autochthonous leukemia cells were performed, attempting to detect-tumor specific antigens on acute leukemia cells. The lymphocytes used were obtained from the patients in chemotherapy-induced complete remission. The leukemia cells had been taken from the peripheral blood or the bone marrow of patients before chemotherapy and been preserved in liquid nitrogen in the medium supplemented with 10% DMSO and 10% FCS until use. Mixing ratios of lymphocytes and leukemia cells (untreated or 4,000R irradiated) were 1:1 or 1:2, and the mixtures were cultured for 7 days in RPMI 1640 medium supplemented with 20% fresh human AB serum at 37°C in CO₂ in air.

Among 28 cases of acute leukemia in remission, lymphocytes of 16 cases showed significant blastoid response to the pre-

Table 1. Blastogenic response of remission lymphocytes against autochthonous leukemic cells

Type	No. of cases	Blastogenic	Response	% positive
		positive	negative	
AML	12	9 (2)	3	75 %
AMoL	5	4	1	80 %
ALL	11	3 (2)	8	27 %
Total	28	16 (4)	12	57 %

() positive only against non-irradiated leukemic cells

served autochthonous leukemia cells. Nine out of 12 AML, 4 out of 5 AMoL showed positive response, whereas only 3 out of 11 ALL exhibited positive. Normal bone marrow cells which had been preserved in the same process as leukemia cells did not stimulate autochthonous lymphocytes in the mixed cultures (Table 1).

2) Chemoimmunotherapy of Acute Leukemia with BCG-CWS

After remission was induced and consolidated with a quadruple combination chemotherapy (DNR/ARA-C/6MP-R/Pred; DCMP) BCG-CWS was added to the bi-monthly schedule of maintenance DCMP chemotherapy. A mixture containing 200µg of oil-attached BCG-CWS and 10⁷ autologous leukemia cells (irradiated) was injected intradermally once a week until relapse (Fig. 2).

Twenty nine patients (13 AML, 3 AMoL, 13 ALL) who achieved complete remission entered the trial.

Toxicity: No serious side effect attributable to BCG-CWS was noted. The most important side effect was local skin reaction. Majority of patients developed

Fig. 1. Chemoimmunotherapy of acute leukemia

Fig. 2. Chemoimmunotherapy of acute leukemia with BCG-CWS—Survival time for A.M.L. in adults

ulcerations which were troublesome, and the dose and interval were modified in some cases.

Immunological Response: Most of patients exhibited conversion of originally negative skin reaction to a battery of recall antigens and progressive increase in positive response during BCG-CWS therapy, reaching plateau after 8 to 10 vaccinations. The greatest increase was noted in skin test to PPD.

***In vitro* lymphocyte blastogenic response to PPD, Con A and PWM** showed substantial increase in comparison to the pretreatment level after 6 to 8 vaccinations. There found no significant change of immunoglobulin level in the serum during immunotherapy. **Remission Length and Survival Time:** Therapeutic effectiveness of BCG-CWS was evaluated in 16 adult patients with acute non-lymphatic leukemia receiving immunotherapy as compared to that for historical control group of 40 consecutive patients given chemotherapy

alone. The two groups of patients were thought to be comparable by analysis for those known to affect prognosis in adult acute leukemia. The median duration of remission for the group of chemotherapy alone was 8 months and that for the group of chemoimmunotherapy was 7.8 months. In contrast to the lack of effect of BCG-CWS in prolonging the first remission length, significant improvement of the survival time was noted for immunotherapy group. The median survival time for patients group treated with chemotherapy alone was 13 months, whereas for group treated with chemoimmunotherapy was 20 1/2 months (Fig. 2).

In the present study, no serious systemic side effect attributable to BCG-CWS was noted. The most important side effects observed was local skin reaction. The ulcerations were troublesome and the dose had to be reduced and the interval of injection was prolonged occasionally.

Substantial and consistent increase in immunological states of patients was evoked by BCG-CWS therapy, including delayed type skin reactions and *in vitro* lymphocyte blastogenic response to mitogens. The *in vitro* blastogenic response to autochthonous leukemia cells demonstrated the evidence indicating that leukemic myeloblasts were more antigenic than leukemic lymphoblasts.

Our study demonstrated that 16 BCG-CWS treated patients with ANLL had a significantly longer survival time than 40 patients maintained with chemotherapy alone, yet there was no difference between the duration of first remission in the two groups.

The increase in survival length produced by any therapeutic modality is made up of two components, prolongation of the first remission and length of survival after the first relapse. The latter can be provided by the ease with which patients enter second and third remission. In our series of patients treated with immunotherapy, 50% of them entered second remis-

sion. Moreover, by heightening resistance to microbial infection, and by protecting immunologically reactive tissue from the assault of chemotherapy, BCG-CWS may protect leukemia patients at critical times in the course of the disease against secondary infection and may make them possible to receive more intensive conventional therapy with reduced risk even at late stage of the disease. Such interpretation is supported, in part, by demonstration of substantial increase in immune response by BCG-CWS therapy.

The prolongation of the first remission could not be obtained in this study. The length of first remission is obviously a function of numbers of residual leukemia cells, and therefore, it is imperative to emphasize that in our protocol the intensity

of maintenance chemotherapy in terms of dose and interval in adjunct of immunotherapy might not be sufficient enough to induce minimal leukemia cell load.

Moreover, there is an increasing criticism on validity and justification in conducting historical assessment system for the therapeutic benefit of immunotherapy.

Reflecting on all the matters discussed above, we have set up a new protocol of immunotherapy for ANLL. The main aspects are as follows; 1) Randomized control system has been used. 2) Cell wall skeleton of *Nocardia rubra* has been used. 3) Allogeneic leukemia cells have been used as an immunogen. 4) Maintenance chemotherapy has been more intensified.

Immunotherapy of ANLL using this new protocol has been under investigation.

(5) Experimental and Clinical Evaluation of Chemoimmunotherapy for Solid Tumors

Akira WAKUI, MD

Department of Clinical Cancer Chemotherapy, The Research
Institute for Tuberculosis and Cancer, Tohoku University

Since the body burden of tumor cells in patients with advanced cancer may be greater than 10^{12} cells, and since it is known in most model systems that the immune system can not eradicate more than 10^5 cells, immunological approach to therapy would have little or no benefit for patients with advanced disease. Accordingly, immunotherapy has been successfully combined with chemotherapy for patients with advanced cancer. The theoretical benefits in favor of the interspersion summarized from many experimental results may be as follows: (1) Most chemotherapeutic agents work on cells in S phase or M phase, but not on G_0 and G_1 phases, while immunotherapy induces a preferential

killing of tumor cells in G_0 and G_1 phases. (2) As tumor cell kill by anticancer drugs follows "first-order kinetics", chemotherapy fails to kill 100% of tumor cells. While tumor cell kill mediated by immunological mechanisms, follows "zero-order kinetics," immunotherapy can destroy all tumor cells if the number of tumor cells is less than 10^5 cells. (3) Immunomodulators may be able to restore immunodepression of patients induced by some anticancer drugs.

On the basis of these theoretical arguments, in order to enhance the effects of chemoimmunotherapy for solid tumors, experiments were designed to determine the role of time of the administration of immunomodulators in treating sarcoma