Peripheral blood lymphocytes from both normal donors and gastric cancer patients contained suppressor cells which could be activated by concanavalin A (Con A) to suppress the proliferative response of lymphocytes from a normal donor. Con A-activated suppressor cells resided in the E rosette-forming cell fraction. An apparent inverse relationship was found between Con A-activated suppressor cell activity and lymphocyte proliferative response to phytohemagglutinin. Significant increases of suppressor cell activities were found in advanced gastric cancer patients. These activities decreased remarkably after surgical resection of the primary tumors. It is likely that nonspecific suppressor cells represent one of the major factors inducing immunosuppression in gastric cancer patients.

Key words: Suppressor cell — Concanavalin A — Human gastric cancer — Lymphocyte proliferation

Regulatory cells, both enhancing and suppressing, play an important role in the modulation of the immune responses. Recent reports indicated that concanavalin A (Con A)-activated peripheral blood lymphocytes suppressed the mitogen or alloantigen stimulation of responder cells and cell-mediated cytotoxicity.7,10,21) There are some reports indicating suppressive activity of Con A-activated cells on other parameters such as antibody production18) and leucocyte inhibitory factor production.15) Assessment of the functional integrity of these cells is thus warranted in various clinical situations such as autoimmunity, aging and neoplasm. Broder et al.5) have found that cells from patients with acute lymphocytic T cell leukemia are capable of suppressing Ig production of normal human B lymphocytes. Thus, it seems likely that under certain circumstances, T cells can express the suppressive activity which clinically characterizes certain diseases.

Information on suppressor cell functions in human tumor systems is limited. Bean et al.1) reported the presence of genetically restricted suppressor T cells that could inhibit the lymphocyte proliferative response to alloantigen in bladder cancer patients. Catalona et al.6) also reported that regional tumor-draining lymph nodes of patients with urological cancer contained suppressor cell precursors that could be activated by Con A to suppress the proliferative response of autologous lymphocytes.

The mechanism by which Con A-activated cells exert the suppression is not sufficiently understood, and there are many variations in the characteristics of Con A-activated suppressor cells. In the present studies, we carried out two types of experiments. The effects of Con A-activated cells on the pro-
liferative response were investigated by means of our assay system, and Con A-activated suppressor cell activities in patients with gastric cancer were observed.

**MATERIALS AND METHODS**

**Patients**

Eighty-five patients with gastric cancer were examined. They ranged in age from 27 to 80 years. Stage determination was done according to the general rules adopted by the Japanese Research Society for Gastric Cancer. Seventy-four healthy volunteers of both sexes, between 20 and 56 years old, served as controls. Student's t-test was used to determine the significance of differences.

**Preparation of Con A-activated Suppressor Cells**

Five ml of heparinized venous blood was allowed to sediment at 37°C for 45 min. The leucocyte-rich plasma was removed. The cells were collected by centrifugation at 300g for 5 min and washed twice in RPMI-1640 medium (GIBCO, U.S.A.). The number of cells was adjusted to 5 x 10^5/ml in RPMI-1640 medium containing 20% human AB serum. The cells were incubated for 24 hr with 10 μg/ml of Con A (Boehringer, West Germany), or without Con A. After incubation, the cells were washed twice and resuspended in RPMI-1640 medium containing 50 μg/ml of mitomycin C (Kyowa Hakko, Ltd., Osaka). After incubation at 37°C for 30 min, three further washes were carried out. The Con A-activated cells and non activated cells were adjusted to 4 x 10^5/ml in complete culture medium. Fresh mononuclear cells were prepared from normal donors in a similar manner and adjusted to 4 x 10^5/ml in complete culture medium. Lymphocyte responsiveness to PHA was determined as described previously.

**Preparation of Enriched T Cells**

Preparation of enriched T cells were performed by a slight modification of the method described previously. Lymphocyte suspensions rich in T cells were obtained by rosetting peripheral lymphocytes with sheep red cells (E) for 30 min at 37°C and sedimenting the lymphocyte-red cell mixture over a Conray-Ficoll gradient. The E rosetting cells were collected, and red cells were lysed in 0.83% of NH₄Cl. The resulting cells were washed and suspended in complete medium.

**RESULTS**

Induction of Con A-activated suppressor cells from normal lymphocytes fractionated on the basis of rosette formation with E was examined. As shown in Table I, treatment of E rosette-forming lymphocytes resulted in strong induction of suppressor cell activity, while non E rosette-forming cells failed to induce suppressor activity.

Suppressor cells were induced in vitro by culturing normal donor lymphocytes with Con A and then testing for their ability to suppress the response of fresh lymphocytes to mitogen and alloantigen (Table II). The responses of fresh lymphocytes to PHA were suppressed by the addition of Con A-activated cells in the range from 9.9 to 24.4%. On the other hand, the responses to alloantigen were suppressed by 9.2 to 51.9%. The mean suppression was 16.0 ± 5.2% with the former, as compared to 30.0 ± 16.4% with the latter, and no significant difference was found between these two groups (P>0.1).

In 85 gastric cancer patients at various clinical stages, peripheral lymphocytes were assayed for Con A-activated suppressor cell activity. As shown in Fig. 1, significant suppressor cell activities were observed in Stage III and IV patients, although suppression was not effective in Stage I and II patients. Mean suppressions were 21.2 ± 5.6% in Stage III patients and 18.0 ± 2.4% in
Stage IV patients, respectively. These results were significantly different from the normal control value ($P<0.05$).

Peripheral lymphocytes from these cancer patients were assayed for responsiveness to PHA in parallel with suppressor cell activity. As shown in Fig. 2, an apparent inverse correlation between these two lymphocyte functions was observed ($P<0.005$).

The effect of surgery on Con A-activated suppressor cell activities was also checked (Fig. 3). An apparent reduction of suppressor cell activities was observed after the surgical resection of the primary tumors. Mean suppressions were $25.5\pm 6.4\%$ in patients of pre-surgical status and $7.7\pm 2.8\%$ in those of post surgical status, and the results were statistically significant ($P<0.05$).
**CON A-ACTIVATED SUPPRESSOR CELLS**

Fig. 1. Con A-activated suppressor cell activities in relation to the clinical stage of 85 gastric cancer patients

Suppression of normal controls ± SE. Significant difference from normal controls, * P<0.05. Horizontal bars indicate the mean suppression and vertical bars indicate the standard error.

Fig. 2. Correlation between lymphocyte responsiveness to PHA and Con A-activated suppressor cell activity in gastric cancer patients

$r = -0.44 (P < 0.005)$

$n = 63$

Fig. 3. Effects of surgery on Con A-activated suppressor cell activities
Horizontal bars indicate the mean suppressions.

Fig. 4. Spontaneous suppression of responder lymphocyte responsiveness to PHA by lymphocytes from 59 normal donors and 59 gastric cancer patients

Significant differences from responder cell proliferation:
* $P<0.05$, ** $P<0.01$. 

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To determine whether spontaneous suppressor cells were present in peripheral lymphocytes from both normal donors and 59 gastric cancer patients, we examined the effect of non activated suppressor cell activity on the lymphocyte responsiveness to PHA. As shown in Fig. 4, non activated spontaneous suppressor cells were found in cancer patients, and these cell activities became greater when activated by Con A in vitro. However, spontaneous suppressor cell activity was absent in normal donors.

**DISCUSSION**

The data presented here indicate that Con A-activated peripheral lymphocytes from normal donors suppressed the in vitro function of homologous T cells. The Con A-activated cells were found to reside in cell fractions enriched in T cells. E rosette-forming cells which were activated by Con A showed remarkable suppressor cell activities, whereas non E rosette-forming cells did not. A number of studies confirmed that the putative effector cells consist largely of T cells, and that human peripheral blood T cells contain a particular subpopulation of T lymphocytes which have already been programmed to express suppressor activities even before their exposure to Con A. Jandinski et al. provided further evidence that such suppressor T cells have already been committed to exhibit suppressor activity before they encountered specific or non specific stimulation.

In our assay system, alloantigen-induced proliferation was as sensitive as PHA-induced proliferation to the suppression by Con A-activated cells, and Con A-activated cells could also inhibit Con A-induced proliferation of fresh lymphocytes (unpublished data). These results are in agreement with others, which demonstrated that lymphocyte proliferative response was inhibited by suppressor cells generated with the same antigen as well as other antigens. Thus, Con A-activated suppressor cells were found to correspond to T cells, and showed remarkable suppressor activities in our assay system.

Previous studies in both animal and human tumor systems have suggested that the impairment of immune responses in cancer may be caused in part by suppressor cells that have been activated by tumor associated antigens. Glaser et al. reported that tumor-bearing animals had splenic suppressor cells that inhibited the proliferative response to tumor associated antigen as well as to mitogens. Jerrells et al. reported that in some cancer patients with immunodepression, suppressor cells were detectable and may mediate the observed immunodepression. Our present studies indicate the presence of suppressor cells in peripheral lymphocytes from gastric cancer patients that could be activated by Con A to suppress the proliferative responses of homologous lymphocytes. In patients with advanced gastric cancer, significantly increased activities of Con A-activated suppressor cells were found as compared with normal controls. Of particular interest was the observation that a significant decrease of suppressor cells was found after resection of the primary tumors. While we found no evidence that the suppressor cell precursors had been activated in vivo by tumor antigen to become nonspecifically suppressive, it is likely that these cells are affected by the resection of tumors.

Our present results indicated an apparent inverse correlation between Con A-activated suppressor cell activity and lymphocyte responsiveness to PHA. Our previous reports demonstrated that low lymphocyte responsiveness to mitogen was found in gastric cancer patients. These results may indicate that, in gastric cancer patients, there is a subpopulation of cells which can develop suppressor function after Con A activation, and that low lymphocyte responses are partly due to the effect of suppressor cells.

It has been reported that tumor-bearing hosts exhibit various effector mechanisms...
against tumor antigen, and more than one suppressive mechanism could be activated in such a host.\textsuperscript{14} Berczi and Sehon\textsuperscript{2} reported that antibody or immune complex might serve as a signal for the activation of suppressor T cells \textit{in vivo}. We demonstrated that lymphocytes from cancer patients which had not been activated by Con A suppressed the proliferative response of lymphocytes from normal donors, and this activity became more apparent after activation by Con A, while spontaneous suppression was not found in lymphocytes from normal donors. Catalona \textit{et al.}\textsuperscript{6} reported the absence of spontaneous suppressor cells in lymph node cells of cancer patients, and indicated that the absence of Con A-activated suppression is not due to \textit{in vivo} activation of suppressor cells. Our results suggest that suppressor cells which are activated specifically or non specifically \textit{in vivo} are present in cancer patients.

The candidacy of a number of immuno-suppressive factors in lymphocytes of cancer patients can be considered. In peripheral blood, a part of the suppressor cell population appears to consist of monocytes.\textsuperscript{3,23} Quan and Burton\textsuperscript{19} reported that lymphocyte reactivity was increased by treatment with carageenan, a macrophage toxic agent, and that the depressed lymphocyte reactivity was due to the presence of suppressor cells that might have been monocytes. In our experiments, adherent cell populations were not depleted, so the effects of suppressive monocytes are unclear; further work is in progress.

In conclusion, the data presented here indicate that peripheral lymphocytes from cancer patients contain suppressor cells which can be activated by Con A to suppress further the proliferative response of lymphocytes from normal donors. These mechanisms may be of major importance in governing the outcome of antitumor responses.

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\textbf{REFERENCES}