

EFFECTS OF ANTINEOPLASTIC AGENTS, CORTICOSTEROID AND ANTIBIOTICS ON MURINE T- AND B-LYMPHOCYTES

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With increasing clinical use of antineoplastic agents and corticosteroid for cancer therapy, the property of the immunosuppressive effects of these agents have become important. Immunosuppressive effect of antineoplastic agent with or without glucocorticoid treatment causes secondary immunodeficiency status, resulting severe infection.

The influence of cyclophosphamide (CY) on immunocompetent cells of experimental animals has already been reported. CY can lead to augmented responses of T-cells as measured by increased levels of delayed type hypersensitivity (DTH) reaction^{1)~5)}. In these studies, the CY-induced decrease in antibody level was thought to be directly responsible for the increased DTH reaction. ASKENASE *et al.*⁶⁾, however, demonstrated CY-mediated augmentation of DTH reaction at the drug doses which did not affect antibody responses, and thus suggested that CY-sensitive suppressor T-cells might exist. On the other hand SCHWARTZ *et al.*⁷⁾ reported that CY was toxic for the population of mouse T-cells which is active in graft-versus-host reactions.

The iatrogenic immunosuppression might be consisted of several points on host defense mechanism. One of the reasons of immunosuppression of host can be explained by retardation of immunocompetent cells. This study was attempted to evaluate the effects of the following agents upon immunocompetent cells of mice. Agents tested were as follows; three antineoplastic agents (cyclophosphamide, mitomycin-C and N-(tetrahydrofuryl)-5-fluorouracil), hydrocortisone and three antibiotics (Ampicillin, Cephalothin and Kanamycin). T- and B-cells in the spleen of mice and in the lymphnodes were measured after treatment with these agents. After cyclophosphamide treatment restoration of T- and B-cells in the spleen and the effect on recirculating lymphocytes were also observed.

To clarify which cell population is affected with these agents, we used sheep red blood cells (SRBC) or lipopolysaccharide (LPS) for the stimulation, the former being a T-cell dependent antigen⁸⁾ and the latter a T-cell independent antigen⁹⁾.

Materials and Methods

Mice Inbred female C₃H mice of 6~8 week old, weighing about 20g were obtained from Funabashi Animal Farm, Chiba Prefecture.

Agents Cyclophosphamide (CY, Endoxan®, Shionogi Co., Osaka), mitomycin-C (MMC, Mitomycin-S®, Sankyo Co., Tokyo), N-(tetrahydrofuryl)-5-fluorouracil (FT, Futraful®, Taiho Co., Tokyo), hydrocortisone sodium succinate (CS, Sol-Cortef®, Japan Upjohn Ltd., Tokyo), Ampicillin (ABPC, Viccillin®, Meiji Seika Co., Ltd., Tokyo), sodium cephalothin (CET, Keflin®, Shionogi Co., Osaka) and Kanamycin sulfate (KM, Kanamycin®, Meiji Seika Co., Ltd., Tokyo) were used. The doses of these agents were determined on the basis of their LD₅₀ values with reference to a preliminary finding that CY decreased the number of spleen cells to about 20% of the control value when injected intraperitoneally at a dose of 40 mg/kg per day for 5 consecutive days. Each agent was injected intraperitoneally on 5 consecutive days. The total dose of each agent corresponded to 46.3% of the LD₅₀ in mice. The daily doses of these agents were: CY, 40 mg/kg; MMC, 0.48 mg/kg; FT, 83 mg/kg; CS, 116 mg/kg; ABPC, 185.2 mg/kg; CET, 526.2 mg/kg; and KM, 29.3 mg/kg. Normal saline was injected into the control mice. Sheep red blood cells (SRBC, Japan Biotest Laboratories, Tokyo) were washed three times with HANK's Balanced Salt Solution (BSS, Nakarai Chemical Co., Kyoto) and then 0.25 ml of a 20% SRBC suspension per mouse was injected intravenously. *Escherichia coli* lipopolysaccharide O55:B5 (LPS, Difco Lab., Detroit, Michigan) was dissolved in normal saline and boiled for 1 hour for detoxification and then 30 µg of LPS per mouse

was injected intravenously. SRBC or LPS was given to the mice on the second day of drug administration.

Preparation of cells Mice were killed two days after the final injection of drugs for 5 days. Their thymus, spleen and lymphnodes (inguinal, retroperitoneal, axillary) were removed and single cell suspensions were prepared individually by pressing the organs through a 150-gauge platinum sieve into cold EAGLE's minimal essential medium (MEM, Nakarai Chemical Co., Kyoto) and breaking up clumps of cells in a pipette. Red blood cells in a suspension of cells were lysed in hypotonic solution. Cell viability was tested by trypan blue dye exclusion cytotoxicity test and cells were regarded as available when more than 90% were viable. The numbers of spleen cells were calculated by hemocytometer. Cell suspensions were adjusted to a concentration of 2×10^6 cells/ml in MEM containing 10% fetal calf serum (FCS, Gibco, Grand Island, N.Y.) for assay of θ -carrying cells. A concentration of 5×10^6 cells/ml in MEM was used for the identification of immunoglobulin (Ig) bearing cells.

Preparation of anti θ -C₃H serum Anti θ -C₃H serum was prepared by the method of REIF and ALLEN¹⁰. The specific cytotoxic activity was confirmed by absorption with adult C₃H brain tissue. The titer against C₃H thymocytes was 1:80.

Identification of θ -carrying cells Identification of θ -carrying cells was carried out by the method of microdroplet assay described by TERASAKI *et al*¹¹. The natural toxicity of guinea pig serum on mouse cells was removed by absorption with agar¹² (Difco Noble). The proportion of θ -carrying cells calculated by the formula: $\frac{T-C}{100-C} \times 100$, where T means the percentage of cells killed with complement plus antiserum and C means percentage of cells killed with complement alone respectively. The whole numbers of θ -carrying cells per spleen were calculated by the formula: (whole numbers of spleen cells) $\times$ (proportion of the θ -carrying cells).

Identification of immunoglobulin-bearing cells (Ig-bearing cells) Ig-bearing cells were detected by the direct immunofluorescent method¹³. The cell suspension was incubated with antimouse immunoglobulin rabbit serum labeled with FITC (Boehringerwerke, West Germany) for 30 minutes at room temperature. After incubation, cell sus-

pension was washed twice with EAGLE's MEM at 4°C and resuspended in glycerol-MEM (1:1, v/v). Fluorescent cells were counted as B-cells under a fluorescence microscope (Olympus Optical Co., Tokyo).

Lymphocyte recirculation Inguinal, retroperitoneal, axillary and mesenteric lymphnodes were collected from normal C₃H mice. A suspension of single cells was prepared and labeled with ⁵¹Cr as sodium chromate at a concentration of 30 μ Ci/10⁸ cells per ml in BSS containing 15% FCS for 30 minutes at 37°C. The labeled cells were washed twice with BSS at 4°C. The viability of these cells was more than 90%. Doses of 1.5×10^7 ⁵¹Cr-labeled lymphnode cells were injected intravenously into syngeneic normal mice. Immediately after injecting the cells, CY was injected intraperitoneally at a dose of 40 mg/kg. The CY-injections were continued for 5 consecutive days and the mice were killed 48 hours after the last injection. Their thymus, spleen, lymphnode (axillary, inguinal, retroperitoneal and mesenteric lymphnodes), liver and both femurs were removed. These organs were put into plastic tubes and their radioactivities were counted in a well type gamma-counter (Spectro Scaler RDM-1, Toshiba, Tokyo). The radioactivity per gram in each organ was calculated as a percentage of the total radioactivity in the thymus, spleen, lymphnodes, liver and femurs.

Restoration of θ -carrying cells and Ig-bearing cells after CY-treatments CY was injected intraperitoneally at a dose of 20 mg/kg for 5 consecutive days or at a single dose of 200 mg/kg. Then the numbers of θ -carrying cells and Ig-bearing cells in spleen were measured repeatedly after the last injection of CY.

Results

Effects of antineoplastic agents and CS on thymocytes

The numbers of thymocytes at 2 days after the last injection of antineoplastic agents and CS are shown in Table 1. Their number decreased markedly in mice treated with CY. On treatments with CS, FT and MMC the numbers of thymocytes decreased to 18.6%, 41.1% and 51.6% of the control, respectively.

Effects of antineoplastic agents and CS on spleen cells, the proportion and number of θ -carrying cells and Ig-bearing cells in spleen with or without antigenic stimulation

Table 1 Changes in numbers of thymocytes treated with CY, MMC, FT and CS

Group	Number of thymocyte ($\times 10^7$) (Mean $\pm$ S.E.)			
	CY	MMC	FT	CS
Control	11.5 $\pm$ 1.3 (5)	22.1 $\pm$ 0.8 (5)	11.2 $\pm$ 0.2 (5)	25.3 $\pm$ 1.2 (5)
Treated	0.6 $\pm$ 0.2 ^{a)} (6)	11.4 $\pm$ 2.1 ^{b)} (6)	4.6 $\pm$ 1.5 ^{c)} (6)	4.7 $\pm$ 0.7 ^{a)} (6)

The abbreviations used are ; CY, cyclophosphamide ; MMC, mitomycin-C ; FT, N-(tetrahydrofuryl)-5-fluouracil ; CS, hydrocortisone sodium succinate.

Number of mice are shown in parentheses.

Significance of difference from the saline control by STUDENT's t-test :

a) $P < 0.001$ b) $P < 0.005$ c) $P < 0.01$

Table 2 Changes in numbers and the proportion of θ -carrying cells in the spleen of mice treated with CY, FT, MMC and CS

Experiment 1)	Without stimulation				
	Saline	CY	FT	MMC	CS
No. of mice	10	6	6	6	6
Whole No. of spleen cells ($\times 10^7$)	18.6 $\pm$ 2.9	3.8 $\pm$ 0.9 ^{a)}	16.3 $\pm$ 1.2	11.2 $\pm$ 1.2 ^{a)}	11.9 $\pm$ 1.5 ^{b)}
Proportion of θ -carrying cells (%)	31.6 $\pm$ 2.9	65.9 $\pm$ 2.1 ^{c)}	28.0 $\pm$ 1.6	32.5 $\pm$ 3.9	48.2 $\pm$ 8.0 ^{d)}
Total θ -carrying cells ($\times 10^7$)/spleen	5.9 $\pm$ 0.7	2.0 $\pm$ 0.2 ^{b)}	4.4 $\pm$ 0.2	3.6 $\pm$ 0.4 ^{b)}	3.9 $\pm$ 0.9
Experiment 2)	Stimulated with LPS				
	10	8	5	7	5
No. of mice	10	8	5	7	5
Whole No. of spleen cells ($\times 10^7$)	22.5 $\pm$ 2.0	3.8 $\pm$ 0.6 ^{a)}	23.8 $\pm$ 0.3	16.2 $\pm$ 1.6 ^{d)}	14.7 $\pm$ 0.6 ^{d)}
Proportion of θ -carrying cells (%)	25.9 $\pm$ 2.8	90.1 $\pm$ 6.3 ^{a)}	27.8 $\pm$ 4.9	44.6 $\pm$ 2.9 ^{a)}	47.1 $\pm$ 1.6 ^{a)}
Total θ -carrying cells ($\times 10^7$)/spleen	5.6 $\pm$ 0.8	3.5 $\pm$ 0.2 ^{d)}	6.7 $\pm$ 0.6	6.9 $\pm$ 0.5	6.8 $\pm$ 0.4
Experiment 3)	Stimulated with SRBC				
	10	5	5	5	7
No. of mice	10	5	5	5	7
Whole No. of spleen cells ($\times 10^7$)	24.9 $\pm$ 0.9	5.3 $\pm$ 1.3 ^{a)}	22.7 $\pm$ 2.8	15.2 $\pm$ 1.1 ^{a)}	9.7 $\pm$ 0.6 ^{a)}
Proportion of θ -carrying cells (%)	25.5 $\pm$ 2.3	74.0 $\pm$ 12.4 ^{a)}	27.7 $\pm$ 1.9	30.2 $\pm$ 1.4	39.4 $\pm$ 4.0 ^{d)}
Total θ -carrying cells ($\times 10^7$)/spleen	6.2 $\pm$ 0.3	3.3 $\pm$ 0.4 ^{a)}	6.2 $\pm$ 0.6	4.6 $\pm$ 0.4 ^{d)}	3.7 $\pm$ 0.3 ^{a)}

Results are geometric means $\pm$ S.E.

Significance of difference from the control by STUDENT's t-test :

a) $P < 0.001$, b) $P < 0.005$, c) $p < 0.01$, d) $P < 0.05$

The effects of CY, FT, MMC and CS on the whole number of spleen cells, the total number of θ -carrying cells and the proportion of θ -carrying cells are shown in Table 2. CY-treatment decreased the whole number of spleen cells to 20% of the control value without stimulation. Treatment with MMC and CS decreased the number significantly but FT caused no significant change. Total θ -carrying cells were also decreased significantly on treatment with CY or MMC. It was noted that the proportion of θ -carrying cells in the spleen increased significantly on treatment with CY or CS.

The changes in the proportion of θ -carrying

cells were examined after LPS stimulation (Experiment 2). CY caused a greater increase in the proportion of θ -carrying cells with LPS stimulation than without it. MMC-treatment also increased the proportion of θ -carrying cells significantly with LPS stimulation, although less than CY-treatment. Changes in the proportions of θ -carrying cells and Ig-bearing cells were also examined after antigenic stimulation with SRBC (Experiment 3). SRBC stimulation increased the proportion of θ -carrying cells on CY-treatment, but the changes on treatments with other agents were similar with and without stimulation. Changes in the Ig-bearing

Table 3 Changes in numbers and the proportion of Ig-bearing cells in the spleen of mice treated with CY, FT, MMC and CS

Experiment 1)	Without stimulation				
	Saline	CY	FT	MMC	CS
Proportion of Ig-bearing cells (%)	37.4±1.6	20.9±2.1 ^{a)}	39.4±0.6	36.0±1.2	28.9±2.1 ^{c)}
Total Ig-bearing cells (×10 ⁷)/spleen	7.0±0.6	0.9±0.2 ^{a)}	7.5±0.3	4.9±0.4 ^{d)}	4.1±0.8 ^{c)}
Experiment 2)	Stimulated with LPS				
	Saline	CY	FT	MMC	CS
Proportion of Ig-bearing cells (%)	32.4±1.6	10.2±1.2 ^{a)}	30.8±1.5	33.6±1.2	33.4±1.5
Total Ig-bearing cells (×10 ⁷)/spleen	6.9±0.8	0.5±0.1 ^{a)}	7.1±0.5	5.4±0.3	5.4±0.3
Experiment 3)	Stimulated with SRBC				
	Saline	CY	FT	MMC	CS
Proportion of Ig-bearing cells (%)	39.2±1.8	19.3±2.0 ^{a)}	32.3±1.3	39.3±2.7	32.0±2.2
Total Ig-bearing cells (×10 ⁷)/spleen	9.9±0.7	0.6±0.1 ^{a)}	8.6±0.5	6.0±0.5 ^{b)}	5.1±1.0 ^{b)}

Ig-bearing cells were measured in the same mice in which θ -carrying cells were measured. Results are geometric means±S.E.

Significance of difference from saline control by STUDENT's t-test :

a) $P<0.001$ b) $P<0.005$ c) $P<0.025$ d) $P<0.05$

Table 4 Changes in the proportion of θ -carrying cells in the lymphnodes of mice treated with CY, FT, MMC and CS

	Saline	CY	FT	MMC	CS
No. of mice	38	5	13	13	5
Proportion of θ -carrying cells (%)	72.0±1.1	91.5±2.7 ^{a)}	71.8±2.1	77.2±1.2 ^{b)}	76.5±4.1

Results are geometric means±S.E.

Significance of difference from saline control by STUDENT's t-test : a) $P<0.001$ b) $P<0.025$

Table 5 Effects of cyclophosphamide on lymphocyte recirculation

Group	Number of mice	Sum of radioactivity	Thymus	Spleen	Pooled lymphnodes ^{a)}	Liver	Femurs
			Counts per min./g (Mean±S.E.) ^{b)}				
Control	6	24,332±3,592 (100) ^{c)}	530±50 (2.5±0.3)	7,067±1,100 (29.0±0.3)	5,141±468 (21.3±0.3)	10,983±2,065 (44.2±1.2)	610±61 (2.6±0.3)
Cyclophosphamide ^{d)}	6	28,018±438 (100)	563±19 (2.3±0.1)	8,700±1,141 (30.9±2.0)	5,142±468 (18.0±1.1)	13,003±532 (46.2±1.4)	590±61 (2.5±0.1)

a) The inguinal, axillary, retroperitoneal and mesenteric lymphnodes were pooled.

b) The radioactivity of each organ was counted in a well-type gamma-counter.

c) Values in parentheses show percentages of the total radioactivity.

d) After injection of ⁵¹Cr-labeled lymphnode lymphocytes, cyclophosphamide was injected intraperitoneally at a dose of 40 mg/kg for 5 consecutive days.

Table 6 Changes in numbers and the proportion of θ -carrying cells and Ig-bearing cells in thymocytes and spleen cells treated with CET, ABPC and KM

	Dose	No. of mice	No. of thymocytes (×10 ⁷)	No. of spleen cells (×10 ⁷)	θ -carrying cells in spleen		Ig-bearing cells in spleen	
					Proportion (%)	Total No. (×10 ⁷)	proportion (%)	Total No. (×10 ⁷)
Control		6	15.9±1.8	18.3±1.0	22.4±2.2	3.6±0.4	33.8±1.0	6.1±0.2
CET	526.2mg/kg×5	6	16.8±1.1	20.8±1.2	20.1±3.6	4.2±0.7	32.4±0.4	6.8±0.8
ABPC	185.2mg/kg×5	6	13.7±0.7	18.3±1.3	29.5±3.9	5.5±1.0	36.8±2.2	6.8±0.8
KM	29.3mg/kg×5	6	17.0±1.5	20.0±0.6	25.1±1.3	5.0±0.3	35.6±2.3	7.1±0.5

Results are geometric means±S.E.

cells in the spleen are shown in Table 3. The total number of Ig-bearing cells was decreased on CY-treatment and also moderately on CS- and MMC-treatment. The proportion of Ig-bearing cells also decreased greatly on CY-treatment and the change was greater with LPS stimulation (Experiment 2). These effects were inversely related to those on θ -carrying cells. There was no significant change of Ig-bearing cells in mice treated with FT with or without stimulation. Treatment with CS decreased the proportion of Ig-bearing cells in the absence of antigenic stimulation but the number of Ig-bearing cells was not affected by stimulation with LPS.

Change in the proportion of θ -carrying cells in the lymphnodes of mice treated with antineoplastic agents and CS

Using the similar experimental conditions, we examined the change in the proportion of θ -carrying cells in the lymphnodes. The results in Table 4 show that the proportion increased significantly only with treatment with CY or MMC.

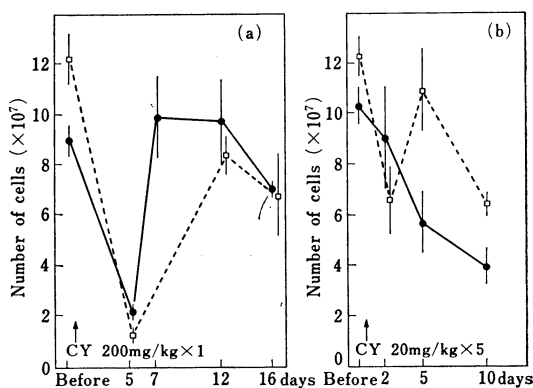
Effects of CY on lymphocyte recirculation

FORNIER *et al.*¹⁴⁾, using ^{51}Cr -labeled mouse lymphnode cells, observed that azathioprine caused selective action on recirculating lymphocytes. Therefore, in order to understand the meaning of the above results, we next examined the effect of recirculation of lymphocyte on CY-treatment. Our experiment was designed to test whether non- θ -carrying cells are selectively suppressed by the cytotoxicity of CY or whether recirculating lymphocytes, which are reported to be of thymic origin¹⁵⁾, tend to accumulate in the lymphnodes on CY-treatment or not. As described in the methods, we injected ^{51}Cr -labeled lymphnode cells intravenously into mice on day 1, injected CY intraperitoneally on day 1 to 5 and killed the mice on day 7. Then the radioactivity in each organ was measured. No significant difference was found in the radioactivities of the control and CY-treated groups (Table 5). Thus there was no increase in accumulation of recirculating lymphocytes in the lymphnodes on CY-treatment.

Restoration of T- and B-cells after treatment with CY

Restoration of T- and B-cells in the spleen of mice was observed after two different schedules of CY-treatments. After treatment with a single dose of 200 mg/kg (Fig. 1 a) T- and B-cells were

Fig.1 Time course of T- and B-cells in the spleen of mice after cyclophosphamide treatment



Cyclophosphamide was intraperitoneally injected (a) at a single dose of 200 mg/kg or (b) at a dose of 20 mg/kg for 5 consecutive days.

Vertical lines represent standard deviations.

●—● : θ -carrying cell
□---□ : Ig-bearing cell

strongly suppressed on the 5th day, but T-cells were recovered to normal value on the 12th day although the restoration of B-cells was retarded. On the other hand, treatment with 20 mg/kg for the 5 consecutive days retarded T-cell restoration more strongly than B-cell (Fig. 1 b).

Effects of antibiotics on murine lymphoid organs

Under the experimental conditions, CET, ABPC and KM did not affect the number of thymocytes and spleen cells and proportion of T- and B-cells in the spleen as shown in Table 6.

Discussion

It is important to know the mechanisms of the antineoplastic agents for cancer chemotherapy or immunochemotherapy. The immune system is consisted of various kinds of cells and factors, but the number of immunocompetent cell may be the parameter to evaluate the immunological status of the host. In this work we studied the effects of antineoplastic agents and corticosteroid on the number of thymocytes and spleen cells, and particularly on the T- and B-cell subpopulation of lymphocytes in the spleen of mice treated with these agents.

We compared the suppressive effects on murine lymphoid cells of the representative antineoplastic

agents such as CY, as an alkylating agent, FT, as an antimetabolite and MMC, as an antibiotic. In our experiment, designed on the basis of preliminary studies, mice were treated with about half the LD₅₀ of each agent in 5 divided injections on the consecutive days. Under these conditions, the different agent had different suppressive effects on murine lymphoid cells. Of the 4 agents tested, CY had the greatest over-all suppressive effect. It caused decrease in the absolute number of both θ -carrying (T) cells and Ig-bearing (B) cells, but it suppressed B-cells more than T-cells on the second day after the last injection of CY, it caused increase in the proportion of T-cells. These results were similar to those obtained by POULTER *et al.*²¹. The changes in the proportion of lymphocyte-subpopulation in the spleen caused by CY-treatment were greater when the mice were stimulated with LPS, or less markedly with SRBC. This might be the result from that B-cells were stimulated by LPS and became more sensitive to CY.

Another possible explanation for the reason why the proportion of T-cells increased in the spleen and lymphnodes is accumulation of θ -carrying cells in the lymphoid organs. But the results of experiments using ⁵¹Cr-labeled lymphnode cells revealed that CY did not increase the accumulation of recirculating lymphocytes to lymphoid organs. Thus the increase in the proportion of T-cells seems to be due to their greater resistance to the cytotoxic effect of CY than B-cells. On the other hand, a single treatment of large dose of CY preferentially retarded the B-cell restoration, but continuous treatments with relatively small dose of CY retarded the T-cell restoration. STOCKMAN *et al.*¹⁶ reported that the effect of CY on the B-cell compartment was more severe and long-lasting than the effect on the T-cell compartment in mice treated with a single sublethal dose of CY. These results revealed that selective effects of CY on lymphocyte subpopulations could be changed by the state of the host immune system, dose of CY, either a single treatment or continuous treatment and time after CY-treatments.

Increase in the proportion of θ -carrying cells was also observed on CS-treatment without antigenic stimulation. This result suggests that unless antigenic stimulation B-cells may be more sensitive to CS than T-cells. Similar results were obtained

by VISHER¹⁷. The number of Ig-bearing cells did not decrease on LPS stimulation. This result would be explained by the report that activated B-cells are resistant to CS (COHEN *et al.*¹⁸). On LPS stimulation, MMC and CS did not decrease the number of θ -carrying cells. These results do not rule out the possibility that T-cells may be influenced by LPS injection.

The suppressive effects of Rifampicin were reported by several investigators¹⁹⁻²⁰. The immunosuppressive effect of commonly used antimicrobial antibiotics is another problem in chemotherapy. We checked the 3 kinds of antibiotics, namely CET, ABPC and KM on the same schedule of anticancer agents. From our results, these antibiotics did not affect on the murine lymphoid cells.

Our results indicate that each antineoplastic agent revealed individual cytotoxic effect on the lymphocyte subpopulation and these immunosuppressive effects may give suggestion in cancer immunochemotherapy or in the immunosuppressive therapy of autoimmune diseases.

Summary

The effects of cyclophosphamide (CY), FT-207 (FT), mitomycin-C(MMC), hydrocortisone(CS) and antimicrobial antibiotics (cephalothin~CET, ampicillin~ABPC, kanamycin~KM) on thymocytes, T- and B-lymphocytes in the spleen of mice were studied. The total dose of each agent given to mice was about half the LD₅₀. A single large dose of CY preferentially retarded the B-cell restoration, whereas the continuous treatment with relatively small dose of CY retarded the T-cell restoration. On the second day after the CY-treatment, its suppressive effect was noticed mainly on B-cells, resulting in an increased proportion of T-cells. This selective suppression of B-cells by the CY-treatment was most remarkable on stimulation with lipopolysaccharide. The increase in the proportion of T-cells in the lymphoid organs by the CY-treatment was not due to the accumulation of T-cells, judging from results obtained from adoptive transfer experiment with ⁵¹Cr-labeled lymphocytes. CS also caused selective suppression of B-cells without antigenic stimulation. MMC suppressed the number of spleen cells slightly and no selectivity among T- and B-cells was apparent. FT decreased the number of thymocytes but did not suppress the number of spleen cells. Com-

monly used antibiotics, such as CET, ABPC and KM did not suppress the number of the murine lymphoid cells.

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