Cancer Immunol Immunother (1986) 22: 37–42

Cancer Immunology Immunotherapy

© Springer-Verlag 1986

T cell recruitment from the thymus to the spleen in tumor-bearing mice

I. Analysis of recruited cells by surface markers

Kazuo Tanaka, Yasuhiro Koga, Kazuto Taniguchi, Kazufumi Kamikaseda, and Kikuo Nomoto

Department of Immunology, Medical Institute of Bioregulation, Kyushu University, Higashi-ku, Fukuoka 812, Japan

Summary. After inoculation of tumor cells (methylcholanthrene-induced sarcoma), the number of Thy 1^+ cells and PNA (peanut agglutinin) binding cells, which were shown to be different subpopulations were increased in the spleen of thymus-intact mice, in contrast this increase was not observed in adult thymectomized mice. In experiments performed concurrently with splenic cell analysis, we found that the plasma PGE_2 levels declined in parallel with the tumor growth. Prevention of such a decline of plasma PGE_2 level by replenishment with exogenous PGE_2 inhibited the splenic cell increase in tumor bearers. In the tumor-bearing mice, cell traffic systems from the thymus to the periphery was ascertained by injecting fluorescein diacetate (FDA) into the thymus and observing fluorescein positive cells in the periphery. We suggest that increased recruitment of thymic cells to the periphery may be mediated by PGE_2 in the presence of a tumor.

Introduction

The function of splenic lymphocytes seems to be modified following the inoculation of tumor cells [2, 6, 8, 9, 10, 13]. In many cases, the number of splenic lymphocytes is increased after tumor inoculation. However, the mechanism (or factor) participating in such modification, and the increase of splenic cells observed in tumor bearers are poorly understood. Such qualitative and quantitative changes of splenic cells can be explained by cell proliferation in the spleen or by emigration of cells from other lymphoid tissues.

Recently, we demonstrated that a T cell traffic system from the thymus to the peripheral lymphoid tissues is mediated by decreasing levels of PGE_2 in the plasma [15, 16, 17, 18, 25]. Thus, it may be that changes in splenic lymphocytes in tumor bearers occur with an enhanced emigration of T cells from the thymus as the result of decreasing PGE_2 level in the plasma. Other investigators have reported elevated PGE_2 levels in the plasma and increased production of PGE_2 by macrophages, in tumor-bearing states [4, 20, 26]. If these findings can be extrapolated to all cases of a tumor-bearing state, changes in splenic cells in tumor bearers cannot be explained by PGE_2-mediated T cell traffic systems. On the contrary, we detected a decline in PGE_2 levels in the plasma after the inoculation of methylcholanthrene-induced sarcoma into syngeneic mice. We now report data supporting the proposal that PGE_2-mediated T cell traffic relates to changes in splenic cells in tumor bearers.

Offprint requests to: Kazuo Tanaka, Department of Immunology, Medical Institute of Bioregulation, Kyushu University, 3-1-1, Maidashi, Higashi-ku, Fukuoka 812, Japan

Abbreviations: PNA^+ cells, peanut agglutinin binding cells; Ig^+ cells, surface IgM positive cells; Thy 1^+, Thy 1.2 antigen positive cells; PGE_2, prostaglandin E_2

Materials and methods

Animals. Female C57BL/6 mice 7 to 8 weeks old were supplied from Shizuoka Laboratory Animal Center (Shizuoka, Japan). Adult thymectomy was performed 7 days before the experiments.

Tumor cells. MBS-1, a fibrosarcoma induced by methylcholanthrene in a female C57BL/6 mouse, was maintained by in vivo passage in syngeneic female C57BL/6 mice. The tumor mass obtained from in vivo passage was treated with 0.25% trypsin and then washed three times with Hanks' balanced salt solution. Mice were inoculated with 1×10^6 tumor cells s.c. in the left flank. When mice were inoculated with 1×10^4 tumor cells, no palpable masses were detected, and when inoculated with 1×10^7 tumor cells, all mice were dead in a week or so. At the dose of 1×10^6 tumor cells, tumors normally reached 1 cm in diameter 1 week after an inoculation. All mice died about 15 days after an inoculation, when the tumor had reached 2 cm or larger.

Cell identification. Nucleated cells were obtained by squeezing the spleen and were counted using a hemocytometer. Splenic cell populations were enumerated by the direct immunofluorescent method using fluorescein isothiocyanate (FITC)-conjugated monoclonal anti-Thy 1.2 antibody (Becton Dickinson, Calif, USA), FITC-conjugated monoclonal anti-mouse IgM (Becton Dickinson), and FITC-PNA (E. Y. Laboratories, Calif.). Fluorescent positive cells were measured using a FACS 440 (Becton Dickinson). Scatter was used to exclude red and dead cells. Background was as high as $200/10^4$ cells analyzed.

Intrathymic injection. The intrathymic injection technique was performed as described elsewhere [18, 23, 24]. Briefly, fluorescein diacetate (FDA, Sigma, Mo, USA) was dis-

form : methanol : HCl acidified water (pH 2.0) 200 : 100 : 75, just before use. The mouse was anesthetized, the chest opened and 5 µl of FDA solution injected into each thymic lobe with a microsyringe. After 2 h, peripheral blood was collected. After lysing red blood cells with Tris-NH_4Cl, fluorescein positive cells could be identified and quantitated using the FACS 440. Lymphocyte populations were identified using a 0°/90° light scatter, and 10^4 lymphocytes were examined for FACS analysis.

Determination of DNA content. To determine the DNA content of splenic T cells, at first fluorescence staining was performed as above. Then cells were fixed with ethanol, and treated with phosphate buffered saline (PBS) containing 1 mg/ml RNase (RNase A, type I-A, Sigma, Mo., USA) at 37 °C for 30 min. Finally cells were stained with propidium iodide (50 µg/ml, Sigma). The relative number of cells in each phase of the cell cycle (G1,S and G2 + M) was estimated by a cell cycle analysis algorithm by Dean's method.

Double immunofluorescent technique. Biotin-conjugated monoclonal anti-Thy 1.2 and monoclonal anti-mouse IgM antibody (Becton Dickinson) were used for indirect immunofluorescence. The diluted antibody (50 µl) and FITC-PNA (5 µl) were added to the 100 µl of cell suspension adjusted to 1×10^7/ml and the mixture was incubated for 45 min on ice. After two washings for indirect immunofluorescent staining, the cell pellet was resuspended in 50 µl of Texas red-conjugated streptavidin (diluted to 1/10, Amersham International Amersham, UK) for microscopical analysis. In the microscopic analysis, 200 FITC positive cells were detected and the number of Texas red positive cells in those 200 FITC positive cells was counted by changing the filters.

Extraction and radioimmunoassay (RIA) for PGE_2. PGE_2 was quantified using the method described by Inagawa [14]. Briefly, blood specimens from each group were pooled and centrifuged to separate the plasma. Then 1 ml of the plasma was mixed with 20 ml of chloroform : methanol 2 : 1 solution, and the preparation shaken vigorously. After elimination of the crude sediment using a filter, the organic solvents were evaporated and the residue redissolved in LP solution and applied to a Sephadex G-25 column soaked with UP solution. LP solution was the lower phase formed by mixing the following solution, chloroform : methanol : HCl acidified water (pH 2.0) 200 : 100 : 75, by volume, and UP solution was the upper phase. The eluate was dried, redissolved in carbon tetrachloride and added to 10% ($^v/_v$) methanol phosphate buffer. After shaking and centrifigation, the upper layer was collected, acidified with HCl and added to ethyl acetate. After further shaking and centrifigation, the ethyl acetate layer was dried in vacuo. The residue was then applied to thin layer chromatography. The area corresponding to standard PGE_2 was scraped off, extracted with 0,5% ($^v/_v$) acetate-methanol, dried under a stream of N_2 and redissolved in an assay buffer. Recovery of PGE_2 was assessed in separate samples by adding a known quantity of ^3H-PGE_2 before the extraction procedures. The concentration of PGE_2 was then determined by RIA. Standard PGE_2 and anti-PGE_2 antiserum were generous gifts from the Ono Pharmaceutical Co. (Osaka, Japan).

Administration of PGE_2. PGE_2 was dissolved in ethanol (1 mg/ml) and kept at −20 °C. For one injection, the ethanolic solution was diluted in PBS, then 200 µg of PGE_2 was injected in the right flank of the mice. In the preliminary experiments, the levels of PGE_2 in the plasma were 6.6, 12.4, 9.7, 6.9 ng/ml, 0, 3, 12, 24 h after the challenge of 200 µg of PGE_2, respectively.

Statistical analysis. Student's *t*-test was used to determine the statistical significance.

Results

Enumeration of lymphocyte subpopulations in tumor-bearing mice

The number of lymphocytes in the thymus and the spleen was examined after inoculation of the tumor cells (Table 1). The numbers of total spleen cells and splenic T cells were increased about twofold and fivefold, respectively, 14 days after the inoculation though the ratio of T cells was decreased. The number of Ig^+ cells was slightly increased 14 days after tumor inoculation, although the ratio of it was also decreased. PNA^+ cells were increased both in the number and the percentage. The number of non-T non-B cells was increased markedly in the tumor-bearing state. On the other hand, the total number of thymocytes was decreased to about one-fourth less than that in nontumor bearers.

Table 1. Enumeration of thymus and spleen cells in tumor-bearing mice

Days after inoculation[a]	Tymus Cell no ($\times 10^7$)	Spleen Cell no ($\times 10^7$)	Thy 1 %	Thy 1 $\times 10^7$	IgM %	IgM $\times 10^7$	PNA %	PNA $\times 10^6$
0	12.7 ± 1.4[b]	2.7 ± 0.5	34.6 ± 4.2	0.9 ± 0.1	34.1 ± 1.6	1.0 ± 0.1	6.1 ± 1.7	1.6 ± 0.3
7	4.9 ± 0.8	4.5 ± 0.5	29.3 ± 3.5	1.3 ± 0.2	23.7 ± 4.3	1.1 ± 0.2	13.9 ± 0.9	6.4 ± 0.6
14	3.3 ± 0.6[c]	10.1 ± 0.9[c]	20.7 ± 1.9	2.1 ± 0.3[c]	15.9 ± 0.7	1.6 ± 0.3[d]	8.1 ± 0.2	8.3 ± 0.7[c]

[a] Each group consisted of 6 mice
[b] mean ± SD
[c] $P < 0.0001$
[d] $P < 0.001$

Table 2. Relative DNA content of splenic T cells in tumor-bearing mice

Days after inoculation[a]	Percentages of cells in[b]	
	Gl	S+G2/M
Exp.1		
0	85.0±1.4	15.1±1.4
5	86.7±2.4[c]	13.2±2.1[d]
Exp.1		
0	84.2±2.1	15.9±2.2
10	83.0±3.4[e]	17.0±3.4[f]

[a] Each group consisted of 5 mice
[b] Relative DNA contents was estimated by flow cytometry
[c] $P=0.21$
[d] $P=0.13$
[e] $P=0.52$
[f] $P=0.56$

Relative DNA content of spleen cells and thymocytes of tumor-bearing mice

As shown in Table 1, the number of lymphoid cells increased in the tumor bearers. These data suggested the possibility that the lymphocytes in the spleen of tumor bearers were either actively proliferating or had emigrated from other organs. Cell cycle analysis of splenic T cells demonstrated that the percentages of G_1 cells or $S+G_2/M$ cells did not change in the tumor-bearing state, compared with the nontumor-bearing state (Table 2). This result removed the possibility of the enrichment of cycling cells in the splenic T cell population of tumor-bearing mice. In contrast, in the thymus of the tumor-bearing mouse, the percentage of synthesizing cells (cells in S and G_2/M phase) was increased (Fig. 1), in spite of the decrease in the number of thymocytes (Table 1).

The effect of thymectomy on the number of splenic subpopulations

We have reported that thymus cells migrate to the periphery [15–18, 25]. In thymectomized mice, the increase in splenic T cells and PNA binding cells was not observed after tumor inoculation (Table 3). These findings strongly suggest that the increase of Thy 1^+ and PNA^+ cells was due to cell recruitment from the thymus. Even though the total number of spleen cells was increased about fourfold in nonthymectomized tumor bearers, the number in the thymectomized tumor bearers was increased twofold. This increase was mainly due to the increased non-T non-B cells in the spleen of thymectomized tumor bearers.

Intrathymic injection of FDA

To ascertain cell recruitment in the tumor-bearing state, FDA was injected intrathymically. FDA was injected into the thymus of tumor-bearing mice challenged with tumor cells 3 days before. Peripheral blood was collected from the femoral artery 2 h later, and the number of fluorescein positive cells was counted analysing 10^4 peripheral lymphocytes (Table 4). In a preliminary experiment, we confirmed that FDA gives a random labeling pattern within the thymocyte population and stained about 30% of total thymocytes [18]. In tumor-bearing mice, thymus cell migration to the periphery was significant, but not so in nontumor bearers.

Double immunofluorescent study in increased splenic populations.

PNA is a marker of T lineage lymphocytes [19]. Roelands et al. showed that PNA could bind to T or null cells but not to B cells in the spleen [21]. However, Berrih et al. indicated that PNA could bind to both T and B lymphocytes in the spleen, at high PNA concentrations (10 µg/ml) [3]. In the present study, we used 5 µl of FITC-PNA (0.2 mg/ml). Preliminary data showed 80%–90% of thymocytes and 5%–10% of splenocytes were stained with this concentration. Thymus-dependent increases in splenic T cells and PNA^+ cells were observed in the tumor-bearing mice (Tables 1 and 3). Double immunofluorescent techniques revealed that these two subpopulations were not identical. Firstly, 200 of FITC-PNA positive cells were examined by a fluorescent microscope, then the number of Texas red positive cells in 200 FITC positive cells was counted (Table 5). These data suggested PNA^+ cells in tumor-bearing mice were non-T non-B cells.

Decrease of PGE_2 level in tumor-bearing mice

In a previous study [15], we observed enlargement of the splenic T cell population in mice with decreased levels of PGE_2 in the plasma following administration of indomethacin, a synthetic inhibitor of PGE_2. This increase of T cells was not evident in thymectomized mice or in nonthymectomized mice injected with PGE_2, concomitantly. The next experiment was conducted in order to ascertain whether the thymus-dependent increase of splenic T cells in the tumor-bearing state is induced in cases of lowered levels of PGE_2. As shown in Table 6, PGE_2 levels in the plasma of tumor-bearing mice declined with tumor growth and the concentration of PGE_2 in the plasma was depressed even 5 days after the tumor injection.

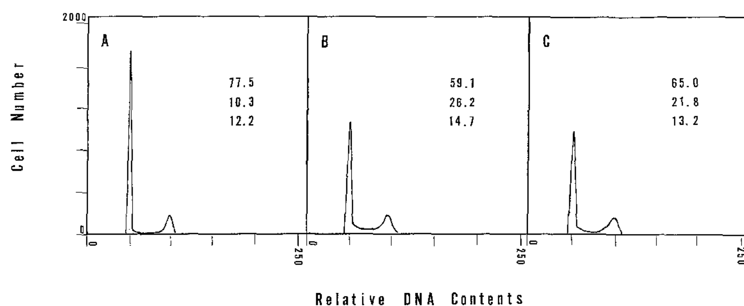

Fig. 1. Flow-cytometric measurements of DNA content in normal thymocytes (A) or thymocytes of tumor-bearing mice (B and C, 5 and 10 days after tumor inoculation, respectively). To measure DNA content, thymocytes were fixed with ethanol and then stained with propidium iodide (PI). Fluorescence intensity of PI was directly proportional to DNA content. The DNA content of G_1 cells corresponded to a fluorescence intensity of 50 arbitary units. The sets of three numbers denote the percentages of cells in G_1, S, and G_2+M phase, from top to bottom, respectively, estimated by Dean's method

Table 3. Enumeration of splenic cells in thymectomized tumor-bearing mice

Days after inoculation[a]	Total cell number	Thy 1		PNA	
	($x10^7$)	%	$x10^6$	%	$x10^6$
0	2.7±0.7[b]	26.9±2.7	7.2±2.0	5.8±2.5	1.5±0.5
7	3.2±0.7	18.0±3.5	5.8±1.6	8.4±1.2	2.6±0.4
14	5.3±0.8[c]	9.0±3.4	4.9±2.5[d]	4.9±1.4	2.6±0.9[d]

[a] Each group consisted of 6 mice
[b] mean ± SD
[c] $P < 0.0005$
[d] not significant

Table 4. Increase in fluorescein positive lymphocytes in the peripheral blood of tumor-bearing mice

Group	Mice	No. of mice	Intrathymic injection of FDA	Fluorescein positive /10000 cells[b]
1.	Normal	4	(−)	255±33[c]
2.	Normal	6	(+)	277±27
3.	Tumor-bearing[a]	6	(+)	355±15

[a] Tumor was inoculated 3 days before
[b] 10000 peripheral lymphocytes were analyzed
[c] mean ± SD
Statistical analysis: Group 1 vs Group 2; not significant
Group 2 vs Group 3; $P < 0.0005$

Table 5. PNA positive cells in the spleen of tumor-bearing mice were Thy 1 negative and IgM negative cells

Surface marker[a]	Positive/total[b]
Thy 1.2	5/200
IgM	3/200

[a] Detected by indirect immunofluoresceint method stained with Texas red
[b] FITC positive cells (200) were examined by fluorescent microscopy

Table 6. PGE_2 level in the plasma of tumor-bearing mice

Days after tumor inoculation	PGE_2 (ng/ml)
0	9.8±3.8[a]
5	1.1±0.3[b]
10	1.8±0.6[b]
14	0.8±0.2[b]

[a] mean ± SD, $n = 3$
[b] $P < 0.05$

Table 7. Inhibition of increase of splenic T cell population in tumor-bearing mice following administration of PGE_2

Tumor[a]	Treatment[b]	Spleen[c]			
		Weight	Total cell no.	No. of T cells	
		(mg)	($\times 10^7$)	(%)	($\times 10^7$)
(−)	PBS	76.0± 5.9[d]	4.1±0.8	34.2±0.3	1.4±0.2
(+)	PBS	146.0±27.6[e]	7.5±2.5[f]	27.9±2.8	2.3±0.6[f]
(+)	PGE_2	103.3± 5.8[e]	4.4±1.3[g]	24.6±3.8	1.0±0.6[g]

[a] Inoculated on day 0
[b] 0.2 ml of PBS or 200 μg of PGE_2 was injected once a day from day 0 to day 5. Each group consisted of 6 mice
[c] obtained on day 5
[d] mean ± SD
[e] $p < 0.001$
[f] $p < 0.01$
[g] not significant

Replenishment with exogenous PGE_2 in tumor-bearing mice

To clarify that cell recruitment from the thymus in the tumor-bearing state was induced by a low level of PGE_2 in the plasma, mice were given 200 µg of PGE_2 s. c. daily for 5 days just after tumor cell inoculation (Table 7). The absolute numbers of whole cells and T cells in the spleen were significantly higher 5 days after the tumor inoculation (7.5 ± 2.5 vs 4.1 ± 0.8; $P < 0.01$). However, administration of exogenous PGE_2 halted such an increase in the spleen (4.4 ± 1.3 vs 4.1 ± 0.8; $P = 0.64$). Exogenous PGE_2 itself had no effect on the number of spleen cells in the normal state.

Discussion

There are some reports on thymus cell migration in normal mice [23, 24]. However, there have not been any studies on the migration of thymus cells in the tumor-bearing state. This is the first report to indicate the massive migration of cells from the thymus in tumor bearers, and also the first to demonstrate suppressed levels of PGE_2, which is related to migration of thymic cells, in the tumor-bearing state.

We have previously reported the role of PGE_2 levels in the regulation T cell population [15–18, 25]. In this study, the level of PGE_2 in the plasma was shown to be low in the tumor-bearing state. We speculated that the increase in T-lineaged spleen cells may be due to a massive emigration from the thymus, as induced by a decrease in levels of PGE_2. The correlation between increased splenic T-lineaged cells and lowered levels of PGE_2 in the plasma may not be fortuitous, as disscussed in the following. The fact that such an increase in the number of spleen cells could be prevented by elevating the level of PGE_2, strongly supported the above correlation. Some investigators have noted an increased level of PGE_2 in the sera of cancer patients [26]. It has also been reported that macrophages, known as the major source of PGE_2 [1], produced increased amounts of PGE_2 in tumor-bearing mice [4, 20]. However, we observed a remarkably depressed level of PGE_2 in the plasma in the tumor-bearing state. Favalli et al. observed low levels of PGE_2 in the spleen of tumor-bearing mice, whereas no significant decrease of levels of PGE_2 was found in the plasma [7]. The different levels of PGE_2 noted in our experiments and others may be ascribed to the difference in tumors used for experiments and also to technical manipulations. The suppressed level of PGE_2 may be due to the inhibition of PGE_2 production from macrophages by some factors from tumor cells or host cells. We have recognized that the depressed level of PGE_2 induced by administration of indomethacin, a PGE_2 synthesis inhibitor, increased the number of T cells in the spleen via mobilization from the thymus [15, 16, 25]. The T cell emigration phenomenon in tumor bearers may relate to the same mechanism. To analyze the increased splenic subpopulations in the tumor-bearing state, it seems to be very important to account for this PGE_2-mediated cell recruitment.

In the tumor-bearing mice which we studied, the number of splenic cells, which were composed of Thy 1^+/PNA$^+$, Thy 1^-/PNA$^+$, and Thy 1^-/PNA$^-$ cells was increased. Thus, increased Thy 1^+/PNA$^-$ and Thy 1^-/PNA$^+$ cells were proved to be not identical. Such increases in Thy 1^+/PNA$^-$ and Thy 1^-/PNA$^+$ cells were not observed in thymectomized mice, even when the tumor-bearing state was advanced. Therefore, the cells in these two populations may belong to T lineage and be derived from the thymus. Moreover, exogenous administration of PGE_2 prevented the increase in splenic T cells of tumor bearers. This result suggested that the source of increased splenic T cells of tumor-bearing mice is the thymus.

The analysis of cell surface markers of increased splenic cell populations provided information on the maturation or differentiation of T-lineaged cells. Firstly, Thy 1^+/PNA$^-$ cells, which are increased most intensely, were commended. The expression of PNA binding receptors is related to a low concentration of sialic acid on the cell surface [5], which is characterized as immature T lineage cells. However, the Thy 1^-/PNA$^+$ cells in this study did not have terminal deoxynucleotidyl transferase (TdT) activity, another marker of prethymocytes and cortical thymocytes (data not shown). As TdT activity is thought to be detected only on the thymocytes or under the influence of thymic hormones [12, 22], it might be suggested that Thy 1^-/PNA$^+$ cells lose TdT activity after leaving the thymic environment. As for another problem, namely that PNA$^+$ cells have not expressed Thy 1 antigens, it may be ascribed to strongly suppressed levels of PGE_2, because the expression of Thy 1 antigen has been shown to be affected by PGE_2 [11]. Therefore, these Thy 1^-/PNA$^+$/TdT$^-$ cells were supposed to be derived from cortical thymocytes, whose behavior is strongly modified by the level of PGE_2.

The other increased splenic cell population in tumor-bearing mice was Thy 1^+/PNA$^-$ cells. These cells were also recognized as recruited cells from the thymus by the PGE_2-mediated system. In the T-lineaged cells, PNA binding receptors are masked by sialic acid during the intrathymic differentiation from cortical thymocytes to mature thymocytes. Therefore, it is likely that Thy 1^+/PNA$^-$ cells are maturated thymic cells and that these cells form a large part of splenic T cells in an ordinary state. The expression of Thy 1 antigen in this population seems to be completed when serum PGE_2 levels are within the normal range.

The third increased splenic population in tumor bearers concerned the Thy 1^-/Ig$^-$ cells. Though PNA$^+$ cells account for approximately 20% of this population, the majority of the remainder cells were nonlymphoid cells, which were considered to be macrophages by morphological analyses. As the increase in nonlymphoid cells in the spleen of tumor bearers was not affected by thymectomy (data not shown), the increase of this population may be controlled by another mechanism.

In this study, we demonstrated the recruitment of T-lineaged cells from the thymus in the tumor-bearing state. Though it is still not clear how the thymus of an adult contributes to or has a role for the host, we exhibited the role of the thymus in the tumor-bearing state from the viewpoint of PGE_2-mediated cell recruitment. We wish to define the functional characteristics of these recruited cells in the next study.

References

1. Allison AC (1978) Mechanism by which activated macrophages inhibit lymphocyte responses. Immunol Rev 40: 3
2. Behforouz NC, Cerny J, Eardly DD (1983) Activation of T cells in tumor bearing mice. Cell Immunol 79: 93
3. Berrih S, London J, Bonavida B (1981) Murine spleen lymphocytes bearing receptors for peanut aggulutinin. VII. Separation and functional characterization. Cell Immunol 63: 249

4. Denbow CJ, Conroy JM, Elgert KD (1984) Macrophage-derived prostaglandin E modulation of the mixed-lymphocyte reaction: An anomaly of increased production and decreased T-cell susceptibility during tumor growth. Cell Immunol 84: 1
5. Despont JP, Abel CA, Grey HM (1975) Sialic acids and sialyltransferases in murine lymphoid cells: Indicator of T cell maturation. Cell Immunol 17: 487
6. Elgert KD, Farrar WL (1978) Suppressor cell activity in tumor bearing mice. I. Dualistic inhibition by suppressor T lymphocytes and macrophages. J Immunol 120: 1345
7. Favalli C, Garaci E, Etheredge E, Santoro MG, Jaffe BM (1980) Influence of PGE on the immune response in melanoma bearing mice. J Immunol 125: 897
8. Fujimoto S, Greene MI, Sehon AH (1976) Regulation of the immune responses to tumor antigens. I. Immunosuppressor cells in tumor bearing hosts. J Immunol 116: 791
9. Fujimoto S, Matsuzawa T, Nakagawa K, Tada T (1978) Cellular interaction between cytotoxic and suppressor T cells against syngeneic tumor in the mouse. Cell Immunol 38: 378
10. Gabison A, Small N, Trainin N (1976) Kinetics of the response of spleen cells from tumor bearing animals in an in vitro tumor neutralization assay. Int J Cancer 18: 813
11. Garaci E, Rinaldi-Garaci C, Del Gobbo V, Favalli C, Santoro M, Jaffe BM (1981) A synthetic analogue of prostaglandin E_2 is able to induce in vivo theta antigen on spleen cells of adult thymectomizerd mice. Cell Immunol 62: 8
12. Gelabert MJ, Transy C, Penit C (1982) Effect of Facteur Thymic Serique (FTS) on TdT expression in murine thymocyte. Cell Immunol 71: 118
13. Hellstrom I, Hellstrom KE, Bernstein ID (1979) Tumor enhancing suppressor activator T cells in spleens and thymuses of tumor immune mice. Proc Natl Acad Sci 76: 5294
14. Inagawa T (1982) Assay of prostaglandins. J Med Technol 26: 135
15. Koga Y, Taniguchi K, Kubo C, Nomoto K (1983) Thymus dependent increase in splenic T cell population by indomethacin. Cell Immunol 75: 43
16. Koga Y, Taniguchi K, Kubo C, Nomoto K (1984) T cell recruitment regulated by prostaglandin-mediated system and its role in immune response. Immunobiology 166: 382
17. Koga Y, Taniguchi K, Nomoto K (1985) Thymus-recruited peritoneal exudate T lymphocytes. Immunobiology 167: 495
18. Koga Y, Tanaka K, Yokoyama Y, Nomoto K (1985) Thymus cell migration with prostaglandin mediated system. Immunobiology in press
19. London J, Berrih S, Bach J-F (1978) Peanut aggulutinin. I. A. new tool for studying T lymphocyte subpopulations. J Immunol 121: 438
20. Pelus LM, Bockman RS (1979) Increased prostaglandin synthesis by macrophages from tumor bearing mice. J Immunol 123: 2118
21. Roelands GE, London J, Mayor-Withey KS, Serrano B (1979) Peanut aggulutinin. II. Characterization of the Thy-1, Tla and Ig phenotype of peanut aggulutinin-positive cells in adult, embryonic and nude mice using double immunofluorescence. Eur J Immunol 9: 139
22. Sasaki R, Bollum FJ, Goldschneider I (1980) Transient populations of terminal transferase positive (TdT^+) cells in juvenile rats and mice. J Immunol 125: 2501
23. Scollay R, Kochen M, Butcher E, Weissman E (1978) Lyt markers on thymus cell migrants. Nature 276: 79
24. Scollay R, Chen W-F, Shortman K (1984) The functional capabilities of cells leaving the thymus. J Immunol 132: 25
25. Taniguchi K, Koga Y, Kato M, Nomoto K (1984) The in vivo regulation of splenic T cell population by the prostaglandin-mediated system. J Clin Lab Immunol 14: 195
26. Williams ED, Karim SMM, Sandler M (1968) Prostaglandin secretion by medullary carcinoma of the thyroid. Lancet I: 22

Received May 8, 1985/Accepted November 6, 1985