Journal of Clinical Immunology, Vol. 10, No. 1, 1990

Decrease in Interferon-γ Production by Peripheral Blood Mononuclear Cells in Patients with Uterine Cervical Cancer

HIDEHIRO MORI,[1] TAKAHIRO HANABAYASHI,[1] YOSHITAKA YAMADA,[2] and TERUHIKO TAMAYA[1]

Accepted: September 21, 1989

The interferon-γ (IFN-γ) productivity of peripheral blood mononuclear cells (PBMCs) was examined in 30 patients with uterine cervical cancer. The patients under 50 years of age had decreased IFN-γ production compared with the age-matched controls. The IFN-γ productivity in the patients over 50 years of age was decreased as well as in the age-matched controls. The proportion of monocytes in PBMCs did not correlate with the IFN-γ productivity. The prostaglandin E_2 (PGE_2) productivity of PBMCs increased with the progress of cancer. PGE_2 inhibited the IFN-γ production by PBMCs, and the sensitivity of PBMCs to PGE_2 was increased in the patients and controls over 60 years of age. The addition of indomethacin resulted in an increase in IFN-γ production by PBMCs. These results suggest that the increased production of PGE_2 and/or increased sensitivity to PGE_2 are responsible for the decreased IFN-γ production in patients with cervical cancer.

KEY WORDS: Peripheral blood mononuclear cells; interferon-γ production; prostaglandin E_2; uterine cervical cancer.

INTRODUCTION

Interferon-γ (IFN-γ), a polypeptide produced by lymphocytes upon stimulation with antigens or nonspecific mitogens (1), plays a crucial role in the modulation of a variety of immune functions. These include immune functions that are of particular importance in the host defense against cancer, such as priming of macrophages for killing of tumor cells (2, 3), induction of specific cytotoxic T cells (4), and enhancement of natural killer-cell activity (5). Patients with uterine cervical cancer have been shown to exhibit decreased cell-mediated immune responses (6, 7). In patients with depressed cellular immune reactivity, it is presumed that there may be some disturbances in the system of IFN-γ production by lymphocytes.

The present study was undertaken to examine the capability of peripheral blood mononuclear cells (PBMCs) from patients with cervical cancer to produce IFN-γ upon stimulation with phytohemagglutinin (PHA), an IFN-γ inducer (8). Prostaglandin E_2 (PGE_2) has been shown to inhibit cellular immune responses such as T-cell proliferation and lymphokine production (9, 10). In human PBMCs, a cell responsible for PGE_2 production is a monocyte (11). Therefore, we also examined the proportion of monocytes in PBMCs, the endogenous production of PGE_2 by PBMCs and the effect of PGE_2 on IFN-γ production by PBMCs.

METHODS

Subjects

The subjects consisted of 30 patients with squamous cell carcinoma of the uterine cervix (aged 35–67 years; mean ± SD, 48.4 ± 9.2 years). None of the patients had received treatment at the time they were investigated. The clinical staging of the patients was based on the criteria of the Federation International of Gynecologists and Obstetricians. The patients were divided into two groups according to the clinical stages. The early disease (ED) group consisted of 6 patients with stage 0 and 13 patients with stage I. The advanced disease (AD) group consisted of five patients with stage II, four

[1]Department of Obstetrics and Gynecology, Gifu University School of Medicine, Gifu, Japan.
[2]Department of Obstetrics and Gynecology, Gifu Prefectural Hospital, Gifu, Japan.

0271-9142/90/0100-0045$0600/0

with stage III, and two with stage IV. The control group consisted of 41 healthy women with no history of recent infection or illness (aged 30–70 years; mean ± SD, 46.2 ± 10.4 years). None of the controls had evidence of any pathologic condition found on physical, pelvic and histological examinations, and ultrasonography. Neither the patients with cervical cancer nor the controls were malnourished.

Reagents

Indomethacin (Sigma Chemical Co., St., Louis, MO) and PGE_2 (Ono Pharmaceutical Co., Ltd., Osaka, Japan) were dissolved in absolute ethanol (10 mg/ml) and diluted to a desired concentration with serum-free RPMI 1640 medium (Gibco, Grand Island, NY) just before use. The final concentrations of ethanol in the cultures did not exceed 0.05%, and this level of ethanol had no effect on IFN-γ production by PBMCs.

Separation of Cells

PBMCs were separated by Ficoll-Hypaque density-gradient centrifugation. PBMCs at the interface were washed three times with Hank's balanced salt solution and were suspended in RPMI 1640 medium containing 10% heat-inactivated fetal calf serum (Gibco) and 25 μg/ml gentamycin (complete medium). The viability of the cells was evaluated by the trypan blue exclusion test. Nonspecific esterase-stained samples of PBMCs were prepared, and the proportion of nonspecific esterase-positive cells (monocytes) in PBMCs was counted.

Cell Cultures

After adjusting viable PBMCs to 1×10^6 cells/ml in complete medium with or without 5 μg/ml PHA-P (Sigma), 500 μl of this suspension was poured into each well of a 24-well culture plate (No. 25820; Corning Glass Works, Corning, NY). The culture plates were incubated for 48 or 72 hr at 37°C in a humidified 5% CO_2 incubator. All cultures were performed in triplicate. After incubation, the supernatant was collected, passed through a 0.45-μm filter, and stored at −70°C until assay.

Assay of INF-γ and PGE_2 in Supernatants

The IFN-γ level was measured in the supernatants from 72-hr culture with a solid phase radioimmunoassay (RIA) (CENTOCOR, Malvern, PA). The IFN-γ level was expressed in reference units (U) based on the National Institutes of Health standard.

The concentration of PGE_2 was measured in the supernatants from 48-hr culture with a competitive binding RIA (New England Nuclear, Boston, MA). Extraction procedures were omitted, because a control with complete medium alone had no significant antibody binding in RIA.

Experiments of PGE_2 and Indomethacin Treatments

To examine the effect of exogenously added PGE_2 on IFN-γ production, the levels of IFN-γ produced by PHA-stimulated PBMCs in the presence of PGE_2 (10^{-11}–10^{-6} *M*) were determined. At the outset of cultures, indomethacin (1 μg/ml) was added to block the endogenous PG production. The PGE_2 dose–effect data were analyzed using the median effect equation (12), and the concentration of PGE_2 required to produce the median effect for IFN-γ production was calculated from a linear regression line.

The percentage change in the level of IFN-γ produced by the addition of indomethacin (1 μg/ml) was calculated by the formula, $(X/Y - 1) \times 100$, where X and Y are the IFN-γ levels with and without indomethacin, respectively.

Statistical Analysis

The statistical analyses were evaluated by Student's t test and by linear regression analysis.

RESULTS

IFN-γ Production by PBMCs

In five controls and six patients, the IFN-γ productions by PHA-stimulated PBMCs formed plateaus at 72 hr, whereas unstimulated PBMCs did not produce detectable levels of IFN-γ (Fig. 1); accordingly, the supernatants after the 72-hr incubation in the presence of PHA were routinely assayed. In the controls, the level of IFN-γ produced by PHA-stimulated PBMCs showed a significant negative correlation with the age of the subjects ($r = -0.525$, $P < 0.01$) (Fig. 2), and the mean IFN-γ levels in the subjects under 50 years of age were significantly higher than those in the subjects

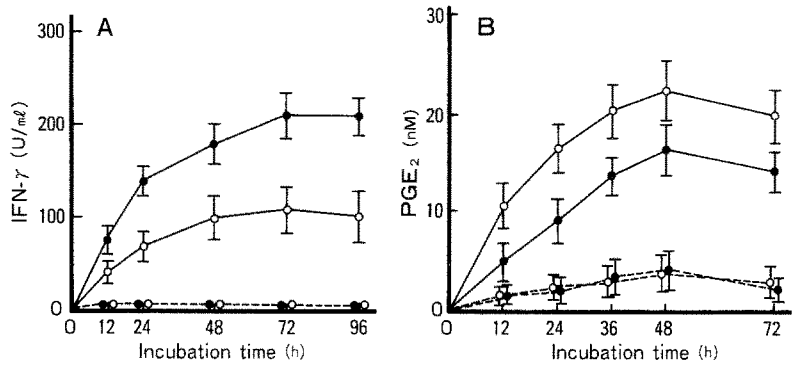

Fig. 1. The time course of productions of IFN-γ (A) and PGE_2 (B) by PHA-stimulated (——) and unstimulated (- - - -) PBMCs. Points represent the mean ± SE in five controls (●) and six patients (○).

over 50 years of age ($P < 0.01$) (Table I). Therefore, the PHA-stimulated IFN-γ production was compared for the two age groups (under 50 and over 50 years of age). In the subjects under 50 years of age, the mean IFN-γ levels in the ED and AD groups were significantly lower than that in the control group ($P < 0.02$ in the ED group, $P < 0.01$ in the AD group), and the mean IFN-γ level in the AD group was significantly lower than that in the ED group ($P < 0.05$) (Table I). In the subjects over 50 years of age, there was no difference in the mean IFN-γ level between the controls and the patients; the mean IFN-γ level in the ED group was somewhat higher, whereas that in the AD group tended to be lower than that in the controls (Table I).

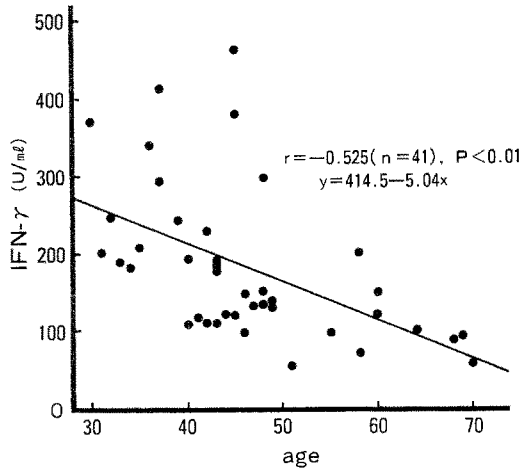

Fig. 2. Correlation between age and IFN-γ production by PHA-stimulated PBMCs in the controls. *r*, correlation coefficient.

PGE_2 Production by PBMCs

In five controls and six patients, the PGE_2 productions by PBMCs in the absence and presence of PHA reached peaks at 48 hr (Fig. 1); accordingly, the supernatants after 48-hr incubation were routinely assayed. In the controls, unlike the PHA-stimulated IFN-γ production, there was no correlation between the age of the subjects and the levels of PGE_2 produced by PBMCs in the absence of PHA ($r = -0.095$, $P > 0.5$) or in the presence of PHA ($r = -0.070$, $P > 0.5$). There was no difference in the mean level of PGE_2 produced by unstimulated PBMCs between the control group, the ED group, and the AD group (Table II). The mean

Table I. IFN-γ Production by PHA-Stimulated PBMCs in the Cervical Cancer and Control Groups[a]

Group	Number of cases	Age	IFN-γ production (U/ml)
Under 50 years of age			
Control	31	41.3 ± 5.6	207.3 ± 99.5*
ED	15	41.9 ± 4.5	139.7 ± 51.4**
AD	3	40.7 ± 5.5	62.8 ± 20.6***
Over 50 years of age			
Control	10	61.3 ± 6.3	102.9 ± 44.7§
ED	4	58.0 ± 3.4	121.8 ± 13.3§§
AD	8	58.5 ± 3.2	68.5 ± 35.5§§§

[a]Mean ± SD.
* vs § $P < 0.01$; * vs **, $P < 0.02$; * vs ***, $P < 0.01$; ** vs ***, $P < 0.05$; § vs §§, $P > 0.1$; § vs §§§, $P > 0.05$; §§ vs §§§, $P < 0.02$.

Table II. Proportion of Monocytes in PBMCs and PGE_2 Production by PBMCs in the Cervical Cancer and Control Groups

Group	Number of cases	Proportion of monocytes (%)	PGE_2 (n*M*) produced by	
			Unstimulated PBMCs	PHA-stimulated PBMCs
Control	41	$12.1 \pm 5.3^{*,a,b}$	$2.30 \pm 1.05^{\S,a}$	$17.41 \pm 4.49^{\#,a}$
ED	19	$11.3 \pm 5.6^{**}$	$2.64 \pm 1.33^{\S\S}$	$19.08 \pm 5.37^{\#\#}$
AD	11	$8.6 \pm 3.3^{***}$	$2.75 \pm 1.56^{\S\S\S}$	$28.83 \pm 6.59^{\#\#\#}$

[a]Mean ± SD.
[b]* vs **, $P > 0.5$; * vs ***, $P > 0.05$; ** vs ***, $P > 0.1$; § vs §§, $P > 0.2$, § vs §§§, $P > 0.1$; §§ vs §§§, $P > 0.2$; # vs ##, $P > 0.2$; # vs ###, $P < 0.01$; ## vs ###, $P < 0.01$.

level of PGE_2 produced by PHA-stimulated PBMCs in the AD group was significantly higher than in the control group ($P < 0.01$) and that in the ED group ($P < 0.01$) (Table II). However, there was no difference in the mean level of PGE_2 produced by PHA-stimulated PBMCs between the control group and the ED group ($P > 0.2$) (Table II). In the patients, the level of IFN-γ produced by PHA-stimulated PBMCs showed a significant negative correlation with the level of PGE_2 produced by PHA-stimulated correlation with the level of PGE_2 produced by PHA-stimulated PBMCs ($r = -0.515$, $P < 0.01$) (Fig. 3). However, there was no such correlation in the controls ($r = 0.063$, $P > 0.5$).

Proportion of Monocytes in PBMCs

In the controls, there was no correlation between the proportion of monocytes in PBMCs and the age of the subjects ($r = 0.075$, $P > 0.5$). There was no difference in the mean proportion of monocytes in PBMCs between the control group, the ED group, and the AD group (Table II). The proportions of monocytes in PBMCs from the controls and the patients showed no correlation with the levels of IFN-γ produced by PHA-stimulated PBMCs ($r = -0.108$, $P > 0.5$, in the controls; $r = 0.153$. $P > 0.2$, in the patients) or with the levels of PGE_2 produced by PHA-stimulated PBMCs ($r = 0.217$, $P > 0.1$, in the controls; ($r = 0.247$, $P > 0.1$, in the patients).

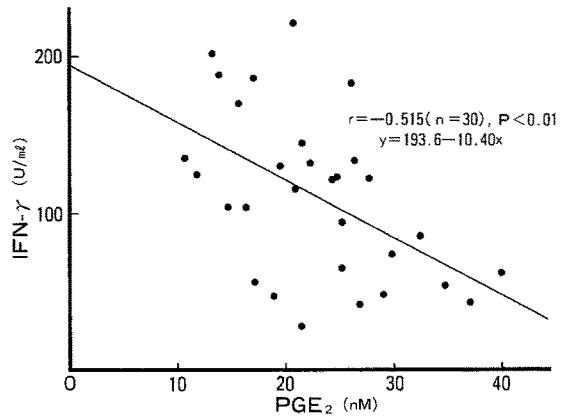

Fig. 3. Correlation between PGE_2 production and IFN-γ production by PHA-stimulated PBMCs in the patients. *r*, correlation coefficient.

Effect of Exogenous PGE_2 on IFN-γ Production by PBMCs

Exogenous PGE_2 suppressed IFN-γ production by PHA-stimulated PBMCs from both the controls and the patients in a dose-dependent manner (Fig. 4). The sensitivity of PBMCs to the suppressive effect of PGE_2 on IFN-γ production tended to increase with age in the controls and the patients (Table III). The sensitivity to PGE_2 of the patients was similar to that of the controls, when compared in similar age groups, and was not influenced by their stage of the disease (Table III). Among the patients (cases 13–23) who showed decreased IFN-γ production by PHA-stimulated PBMCs (lower than the mean −1 SD U/ml of the controls: <107.8 U/ml under 50 years of age, <58.2 U/ml over 50 years of age), seven (cases 13–17, 19, and 20) of the eight patients under 60 years of age showed increased PGE_2 productions by PHA-stimulated PBMCs (higher than the mean + 1 SD n*M* of the controls: >21.90 n*M*), whereas all three patients over 60 years of age (cases 21–23), as well as the controls over 60 years of age, showed increased sensitivities to PGE_2 ($ED_{50} < 10^{-10}$ *M*) (Table III). PBMCs from one patient (case 18) with decreased IFN-γ-producing capability had neither increased production of PGE_2 nor increased sensitivity to PGE_2 (Table III).

Effect of Indomethacin on IFN-γ Production by PBMCs

The effect of indomethacin on PGE_2 production by PHA-stimulated PBMCs was examined in two

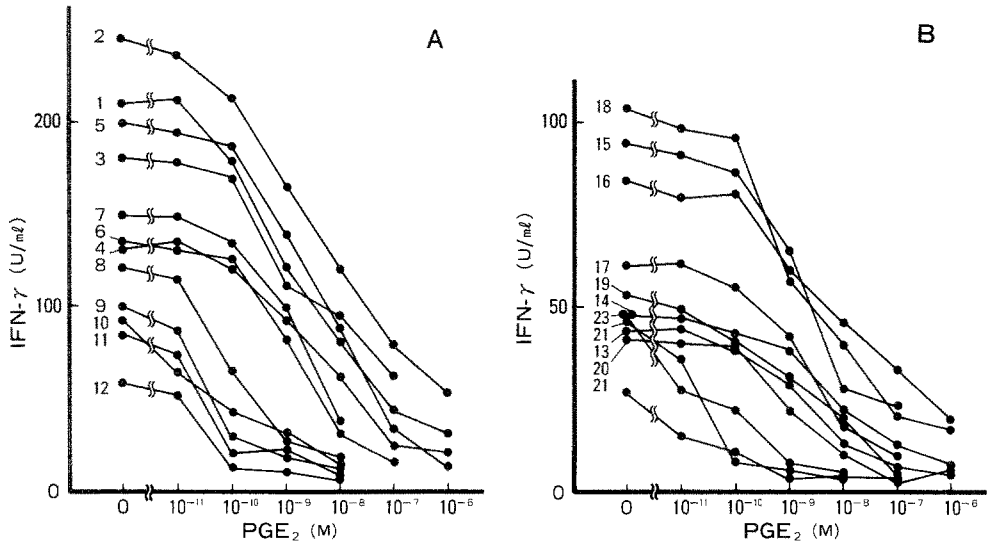

Fig. 4. Effect of PGE_2 on IFN-γ production by PHA-stimulated PBMCs from the controls (A) and patients (B). The numbers of controls (1–12) and patients (13–23) are matched to those shown in Table III.

controls and three patients. Indomethacin (1 μg/ml) blocked virtually all the PGE_2 productions compared to the control cell cultures without indomethacin (<0.30 n*M* for indomethacin-treated cultures vs 20.45 ± 3.12 n*M* for control cultures). In both the controls and the patients, the IFN-γ production was increased by the addition of indomethacin (Table III). However, the mean percentage increase in IFN-γ production by the addition of indomethacin was significantly higher in the controls over 60 years of age (cases 8–12) and the patients with decreased IFN-γ production (cases 13–23) than in the controls under 60 years of age (cases 1–7) (cases 8–12 vs cases 1–7, $P < 0.05$; cases 13–23 vs cases 1–7, $P < 0.01$).

DISCUSSION

The present study showed that the IFN-γ production by PHA-stimulated PBMCs from the controls decreased as a function of age. The patients under 50 years of age had a significantly decreased capability of PBMCs to produce IFN-γ compared with the age-matched controls. The IFN-γ-producing capability was impaired with the progress of cancer. In the subjects over 50 years of age, the IFN-γ-producing capability in the patients with early disease (stages 0 and I) was somewhat increased, whereas that in the patients with advanced disease (stages II, III, and IV) tended to be decreased.

It is known that IFN-γ producers are T cells (13, 14) and NK cells (15) and that the production of IFN-γ is regulated by a complex interaction between monocytes/macrophages and T cells (16, 17). Monocytes or macrophages can release at least two immunoregulators, interleukin 1 (IL-1) and PGE_2. IL-1 stimulates helper T cells to produce interleukin 2 (IL-2), and IL-2 stimulates T-cell growth and promotes IFN-γ production (1). On the other hand, PGE_2 suppresses IFN-γ production through inhibition of IL-2 production by helper T cells (18). We confirmed that PGE_2 at the concentrations detected in the PHA-stimulated 48-hr cultures (about 10^{-8} *M*) suppressed clearly IFN-γ production by PHA-stimulated PBMCs. Although the proportion of monocytes in PBMCs was not associated with aging, the presence of absence of cancer, and the level of IFN-γ produced by PHA-stimulated PBMCs, the PGE_2 production by PHA-stimulated PBMCs was significantly increased with the patients with advanced disease compared to the patients with early disease and the controls. This suggests that the increased PGE_2 production by monocytes is responsible for the decreased IFN-γ production in the patients with advanced disease. The increased PGE_2 production might be the result of either increased synthesis at a cellular level or an increased population of PGE_2-producing monocytes. Since it has been reported that IL-1 and PGE_2 are produced by separate subsets of monocytes (19,

Table III. IFN-γ and PGE_2 Production by PHA-Stimulated PBMCs and Effects of PGE_2 and Indomethacin on IFN-γ Production by PHA-Stimulated PBMCs[a]

Case No.	Age (years)	IFN-γ production (U/ml)	PGE_2 production (nM)	Effect of PGE_2 ($logED_{50}$)[b]	Effect of indomethacin (% increase)[c]
Control					
1	30	210	23.74	−8.36	18.2
2	37	245	19.06	−7.78	31.3
3	37	181	19.28	−7.89	5.9
4	43	131	11.76	−8.04	12.3
5	47	200	18.91	−7.96	21.4
6	49	135	21.92	−8.51	11.0
7	55	149	10.56	−8.62	26.4
Mean					18.1[§,d]
8	61	121	16.22	−9.48	48.2
9	64	100	18.74	−9.97	29.0
10	68	84.2	17.10	−9.98	42.3
11	69	92.1	19.45	−10.02	69.6
12	70	58.3	12.84	−10.05	51.9
Mean					48.2[§§]
Cervical cancer					
13	35 (IV a)[e]	43.2	36.98[f]	−8.30	43.2
14	37 (I b)	47.3	29.00[f]	−8.68	61.8
15	39 (I b)	94.2	24.79[f]	−8.23	52.3
16	41 (III b)	84.2	32.46[f]	−7.48	27.8
17	46 (II a)	61.0	39.76[f]	−8.42	32.5
18	49 (I b)	104	16.33	−8.18	21.7
19	56 (II b)	53.6	34.70[f]	−8.77	34.9
20	58 (IV a)	41.5	26.87[f]	−8.24	59.3
21	61 (III b)	46.1	18.77	−10.49	70.3
22	65 (I b)	27.0	21.39	−11.15	48.4
23	67 (I b)	47.2	21.67	−10.46	52.1
Mean					45.8[§§§]

[a]The case numbers correspond with those shown in Fig. 4.
[b]The dose–effect data shown in Fig. 4 were fitted by linear regression analysis using the median effect equation. ED_{50} was calculated from the linear regression line.
[c]The percentage increase in the level of IFN-γ produced by the addition of indomethacin (1 μg/ml).
[d]§ vs §§, $P < 0.05$; § vs §§§, $P < 0.01$.
[e]Clinical stage of disease.
[f]Higher than the mean + 1 SD n*M* (21.90 n*M*) of the controls.

20), the patients with advanced disease might have a relative increase in PGE_2-producing monocytes. On the other hand, the increased sensitivity of PBMCs to the suppressive effect of PGE_2 seems to be associated with the decrease in IFN-γ production in both the controls over 60 years of age and the patients over 60 years of age, regardless of their stage of the disease. The amount of PGE_2 required to cause 50% inhibition of the IFN-γ production in healthy subjects over 60 years of age was approximately 10^{-9}–10^{-10} *M*. This is obviously lower than the amount required for 50% inhibition in healthy subjects under 60 years of age (10^{-7}–10^{-8} *M*). Walford has theorized that the gradual loss of cellular immunity is the fundamental mechanism of aging (21). This increased sensitivity to PGE_2 appears to contribute to some of the disturbances in cellular immune function found in aging humans.

The IFN-γ production was increased by the addition of indomethacin, a prostaglandin synthetase inhibitor, in both the controls and the patients. This suggests that PGE_2 inhibits the IFN-γ production by PBMCs regardless of the presence or absence of cancer. Furthermore, the increasing rate of IFN-γ production by the addition of indomethacin was significantly higher in the controls over the age of 60 and the patients with decreased IFN-γ production than in the controls under 60 years of age. This finding supports the concept that PGE_2 plays a major role in the decrease of IFN-γ production by PBMCs. However, the early disease patients under 50 years of age, whose PBMCs did not show the increased production of PGE_2, had decreased IFN-γ production by PBMCs; one patient (case 18, Table III) with decreased IFN-γ production had neither increased production of PGE_2 nor enhanced

sensitivity to PGE_2. Furthermore, the early disease patients over 50 years of age had more or less increased IFN-γ production. These findings suggest that other regulatory mechanisms are likely to be involved in the system of IFN-γ production by lymphocytes. Therefore, it is necessary to examine the number of IFN-γ-producing cells, their intrinsic capability to produce IFN-γ, and the activities of helper and suppressor T cells in IFN-γ production.

The interferon system has been implicated in the host's defense against viral and bacterial infections as well as in the defense against tumors (22). Therefore, it may be useful for patients with cervical cancer to restore the IFN-γ-producing capability of lymphocytes for enhancing the resistance to infections and activating the antitumor immunity.

REFERENCES

1. Maeyer ED, Maeyer-Guignard JD: Production of IFN-γ by T cells and modulation of T cell, B cell, and NK cell activity by interferons. *In* Interferons and Other Regulatory Cytokines, ED Maeyer, JD Maeyer-Guignard (eds). New York, John Wiley & Sons, 1988, pp 221–273
2. Le J, Prensky W, Yip YK, Chang Z, Hoffman T, Stevenson HC, Balazs I, Sadlick JR, Vilcek J: Activation of human monocyte cytotoxicity by natural and recombinant immune interferon. J Immunol 131:2821–2826, 1983
3. Schultz RM, Kleinschmidt NJ: Functional identity between murine γ interferon and macrophage activating factor. Nature 305:239–240, 1983
4. Chen L, Tourvieille B, Burns GF, Mathieu-Mahul D, Sasportes M, Bensussan A: Interferon: A cytotoxic T lymphocyte differentiation signal. Eur J Immunol 16:767–770, 1986
5. Platsoucas CD: Regulation of natural killer cytotoxicity by *E. coli*-derived human interferon γ. Scand J Immunol 24:93–108, 1986
6. Levy S, Kopersztych S, Musatti CC, Souen JS, Salvatore CA, Mendes NF: Cellular immunity in squamous cell carcinoma of the uterine cervix. Am J Obstet Gynecol 130:160–164, 1978
7. Shiraki S, Mori H, Kadomoto N, Nakano T, Yamada Y, Noda K: Adjuvant immunotherapy of carcinoma colli with levamisole: Prevention of immunological depression following surgical therapy and radiotherapy. Int J Immunopharm 4:73–80, 1982
8. Epstein LB, Cline MJ, Merigan TC: The interaction of human macrophages and lymphocytes in the phytohemagglutinin-stimulated production of interferon. J Clin Invest 50:744–753, 1971
9. Goodwin JS, Messner RP, Peake GT: Prostaglandin suppression of mitogen-stimulated lymphocytes in vitro. J Clin Invest 62:753–760, 1978
10. Rappaport RS, Dodge GR: Prostaglandin E inhibits the production of human interleukin-2. J Exp Med 155:943–948, 1982
11. Kurland JI, Bockman R: Prostaglandin E production by human blood monocytes and mouse peritoneal macrophages. J Exp Med 147:952–960, 1978
12. Chou TC, Talalay P: Quantitative analysis of dose-effect relationships: The combined effects of multiple drugs or enzyme inhibitors. Adv Enzyme Regul 22:27–55, 1984
13. Archer DL, Smith BG, Ulrich JT, Johnson HM: Immune interferon induction by T-cell mitogens involves different T-cell subpopulations. Cell Immunol 48:420–426, 1979
14. Epstein LB, Gupta S: Human T-lymphocyte subset production of immune (γ) interferon. J Clin Immunol 1:186–194, 1981
15. Handa K, Suzuki R, Matsui H, Shimizu Y, Kumagai K: Natural killer (NK) cells as a responder to interleukin 2 (IL2). II. IL2-induced interferon γ production. J Immunol 130:988–992, 1983
16. Torres BA, Farrar WL, Johnson HM: Interleukin 2 regulated immune interferon (IFNγ) production by normal and suppressor cell cultures. J Immunol 128:2217–2219, 1982
17. Torres BA, Yamamoto JK, Johnson HM: Cellular regulation of immune interferon (IFNγ) product: Lyt phenotype of suppressor cell. Infect Immun 35:770–776, 1982
18. Torres BA, Russell JK, Johnson HM: Eicosanoids and gamma interferon. *In* Prostaglandins in Cancer Research, E Garaci, R Paoletti, MG Santoro (eds). Berlin, Springer-Verlag, 1987, pp 227–234
19. Picker LJ, Raff HV, Goldyne ME, Stobo JD: Metabolic heterogeneity among human monocytes and its modulation by PGE_2. J Immunol 124:2557–2562, 1980
20. Khansari N, Chou YK, Fudenberg HH: Human monocyte heterogeneity: Interleukin 1 and prostaglandin E_2 product by separate subsets. Eur J Immunol 15:48–51, 1985
21. Walford RL: The immunologic theory of aging: current status. Fed Proc 33:2020–2027, 1974
22. Yabrov AA: Interferon and Nonspecific Resistance. New York, Human Sciences Press, 1980