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Monophosphoryl Lipid A (MPL) Upregulates Major Histocompatibility Complex (MHC) Class I Expression by Increasing Interferon-γ (IFN-γ)

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Abstract

Tumor immunity is primarily mediated by cells as $CD8^+$ cytotoxic T lymphocytes (CTL) recognize tumor antigen by MHC class I molecules. But most tumors are associated with a decreased expression of MHC class I to escape the antitumor immunity of the host. Our previous data have demonstrated that MPL has an antitumor effect on metastatic lung cancer of B16 melanoma with enhancing cytotoxicity due to increase of IFN-γ and IL-2, and decrease of IL-4, which indicates the stimulation of type 1 helper T cells (Th1). To determine the effects of MPL, IFN-γ, TNF-α, and IL-1α on MHC class I expression of B16 melanoma cells, we evaluated the expression of MHC class I molecules with treatments of MPL, IFN-γ, TNF-α, and IL-1α by flow cytometry. The supernatant of MPL-treated spleen cells in vitro upregulated the expression of MHC class I molecules of B16 melanoma cells compared to the control supernatant of spleen cells. The MHC class I expression of B16 melanoma cells treated with IFN-γ, but not TNF-α or IL-1α, increased in a time-dependent manner. In conclusion, MPL upregulated MHC class I expression of B16 melanoma cells by activating spleen cells via IFN-γ. These data suggest that increased IFN-γ by MPL is responsible for the upregulation of MHC class I expression to augment cytotoxicity. Therefore, we suggest that MPL could play an important role in immunotherapy.

Key Words: Monophosphoryl lipid A, MHC class I, IFN-γ

INTRODUCTION

Major histocompatibility complex (MHC) molecules are essential for cell-mediated immune function.[1] $CD8^+$ cytotoxic T lymphocytes (CTL) are effector cells that recognize tumor antigens, which are intracellularly processed into small peptides and presented with MHC class I molecules on the cell surface. Also, $CD4^+$ T helper (Th) cells, collaborating with macrophages and other cells, play an important role by amplifying CTL responses.[2,3] Released tumor antigen from tumor cells, which was taken up and processed into peptides by antigen-presenting cells (APC), then displayed on MHC class II molecules, can be recognized by $CD4^+$ T cells. Peptides generated from viral or mutated cellular proteins, but not normal cellular proteins by themselves, can trigger a T-cell response.[4]

The downregulation of human leukocyte antigen (HLA) class I expression is a widespread phenomenon in tumor biology[5-7] and probably reflects selected tumor escape mechanisms owing to the role of HLA molecules in presenting immunogenic peptides to T cells.[8]

MPL has an antitumor effects with increased cytotoxicity due to increase of IFN-γ and IL-2 in our previous study.[9] In this study, we evaluated the expression of MHC class I molecules of B16 melanoma cells treated with MPL to determine whether antitumor effects of MPL is due to the upregulation of MHC class I expression of B16 melanoma cells. The effect of IFN-γ on MHC class I expression of B16 melanoma cells was studied. Furthermore, to observe the effects of TNF-α and IL-1α, which can be induced by MPL as a derivative of bacterial lipopolysaccharide (LPS), MHC class I expression of B16 melanoma cells with the treatments of TNF-α or IL-1α were examined. We found that the expression of MHC class I was increased by the supernatant of MPL-treated spleen cells and also that the treatment of B16 melanoma cells with IFN-γ upregulated MHC class I expression in a time-dependent manner.

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MATERIALS AND METHODS

Cell lines and chemicals

B16 melanoma cells, a highly-malignant melanoma cell line, were derived from a spontaneous tumor occurring in a C57BL/6 mouse (H-2b). The cells were developed by I. Fidler (M. D. Anderson, Houston, Texas, USA), and maintained in a 5% CO_2 incubator at 37°C in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS) (GibcoBRL, Grand Island, N.Y., USA) and antibiotics (100 U/ml penicillin and 100 μg/ml streptomycin) (GibcoBRL, Grand Island, N.Y., USA).

MPL was purchased from Ribi Immuno Chem Research Inc. (San Francisco, CA, USA) and diluted in a 0.15% NaCl solution, and stored at −4°C.

Preparation of single cell suspension of splenocytes

The spleen was isolated from a C57BL/6 mouse and lymphocytes were collected after compressing the spleen with sterile slide glasses, following the lysis of red blood cells with hypotonic solution. Spleen cells (2×10^6/ml) were plated in a 25 cm^2 culture flask and incubated in the presence or absence of 10 μg/ml MPL in 10% FBS-containing DMEM for 24−48 h in a 5% CO_2 incubator at 37°C. Supernatants were collected and added to B16 melanoma cells.

Treatment of B16 melanoma cells with cytokines

B16 melanoma cells were treated with different doses of IFN-γ (50−800 U/ml) (PharMingen, San Diego, CA, USA) for 24−48 h. To evaluate the effect of other cytokines, TNF-α (50 U/ml) (PharMingen, San Diego, CA, USA), or IL-1α (50 U/ml) (PharMingen, San Diego, CA, USA) were added to B16 melanoma cells for 24 h. Also anti-IFN-γ antibody (5−0.2 μg/ml) (PharMingen, San Diego, CA, USA) was added to B16 melanoma cells in the presence of IFN-γ to abrogate the effect of IFN-γ on MHC class I molecules.

Immunofluorescent analysis

The expression of MHC class I molecules was determined by flow cytometry (FACStar, Becton Dickinson, Lincoln Park, N.J., USA). Cultured B16 melanoma cells (2×10^6 cells/ml) in a 25 cm^2 culture flask were incubated with 1 ml of spleen-cell supernatant, which was cultured in the presence or absence of MPL.

Cells were harvested with phosphate-buffered saline with ethylenediamine-tetraacetic acid (PBS/EDTA) and incubated for 45 min at 4°C with either mouse monoclonal antibody to MHC H-2K class I (Biodesign, Kennebunk, ME, USA) or control IgG (Biodesign, Kennebunk, ME, USA). Cells were washed twice with washing buffer (PBS with 1% FBS and 0.01% sodium azide), and incubated for an additional 45 min at 4°C with diluted FITC conjugated monoclonal goat anti-mouse Ig (Biodesign, Kennebunk, ME, USA). The cells were washed twice with washing buffer and analyzed by flow cytometry, using fluorescence-activated cell sorter (FACS) (FACStar, Becton Dickinson, Lincoln Park, N.J., USA)

RESULTS

The effect of MPL on MHC class I expression

Our preliminary study showed no increase of MHC class I expression of B16 melanoma cells with the treatment of MPL, which sugeest that MPL has no direct effect on MHC class I expression. To reveal the mechanism of increased cytotoxicity of spleen cells, we evaluated the MHC class I molecules of B16 melanoma cells in the presence of supernatant of splenocytes pretreated with MPL for 12−24 h.

The control supernatant of spleen cells in the absence of MPL did not increase the expression of MHC class I molecules of B16 melanoma cells (Fig. 1, A, B and C), but the supernatant of MPL-pretreated spleen cells upregulated the MHC class I expression in a time-dependent manner (Fig. 1, D and E). This suggested that the MHC class I expression of B16 melanoma cells is upregulated by the cytokines of MPL-pretreated spleen cells.

The effects of IFN-γ and other cytokines (TNF-α and IL-Iα) on MHC class I expression

Since MPL increased IFN-γ in our previous study and also upregulated the expression of MHC class I molecules of B16 melanoma cells in this study, in order to see the direct correlation between IFN-γ and MHC class I expression, B16 melanoma cells were incubated with recombinant IFN-γ. IFN-γ increased the expression of MHC class I molecules of B16 melanoma cells compared to the control (percentage of MHC class I positive cells: 89.8% vs 19.3%, respectively) (Fig. 2, A and B).

In a sepsis model, injection of LPS increased TNF-α and consequently IL-Iα.[10] Therefore, we treated B16

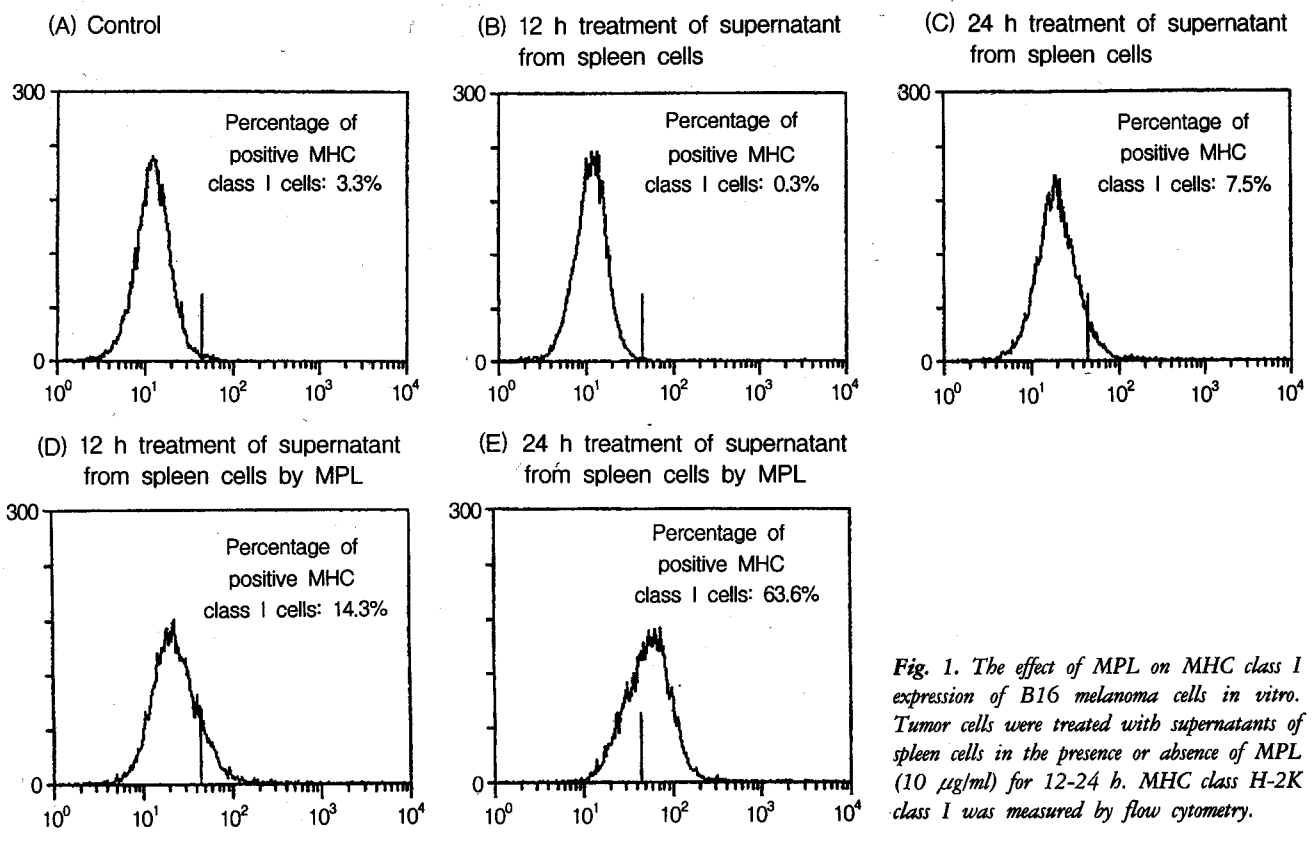

Fig. 1. The effect of MPL on MHC class I expression of B16 melanoma cells in vitro. Tumor cells were treated with supernatants of spleen cells in the presence or absence of MPL (10 μg/ml) for 12-24 h. MHC class H-2K class I was measured by flow cytometry.

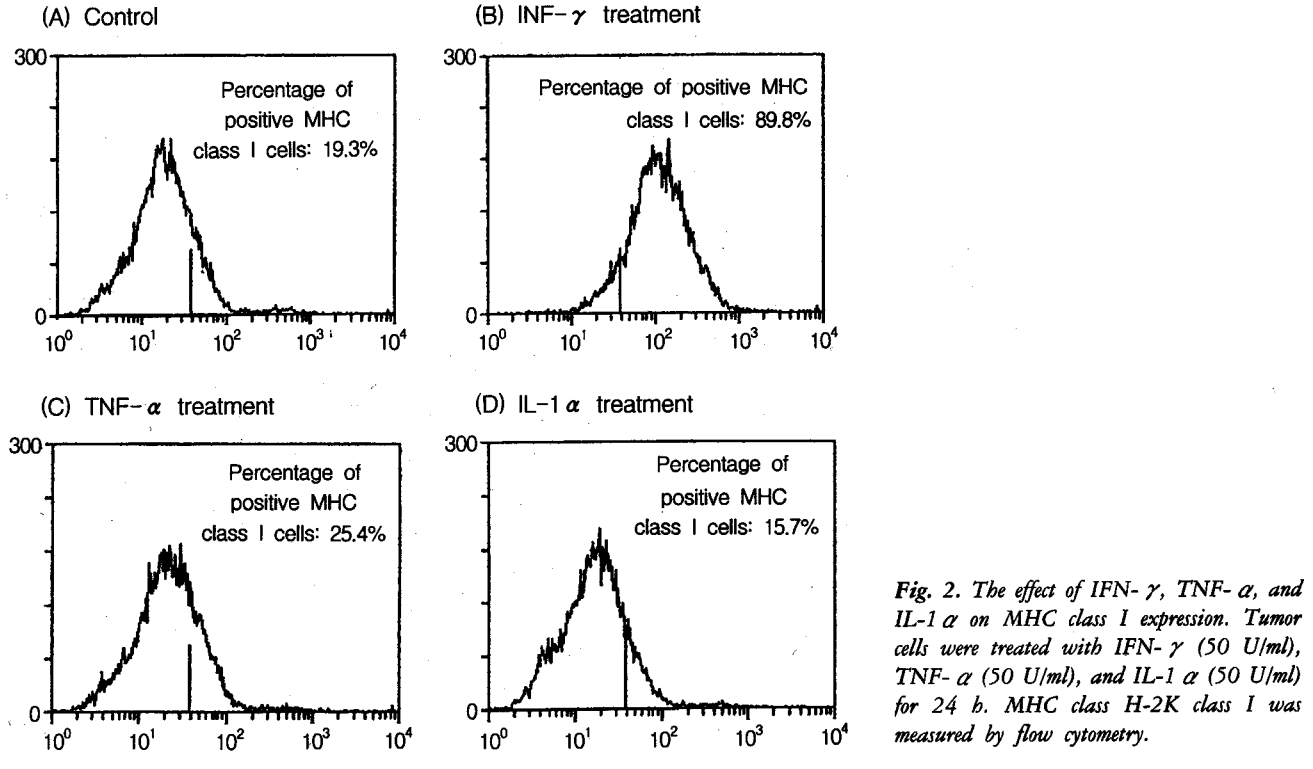

Fig. 2. The effect of IFN-γ, TNF-α, and IL-1α on MHC class I expression. Tumor cells were treated with IFN-γ (50 U/ml), TNF-α (50 U/ml), and IL-1α (50 U/ml) for 24 h. MHC class H-2K class I was measured by flow cytometry.

melanoma cells with TNF-α or IL-Iα to observe the effect of these cytokines on MHC class I expression, since MPL is a detoxified derivative of bacterial lipopolysaccharide (LPS),[11] and TNF-α is a co-factor for the induction of IFN-γ by MPL.[12] In contrast to IFN-γ, the MHC class I expression of B16 melanoma

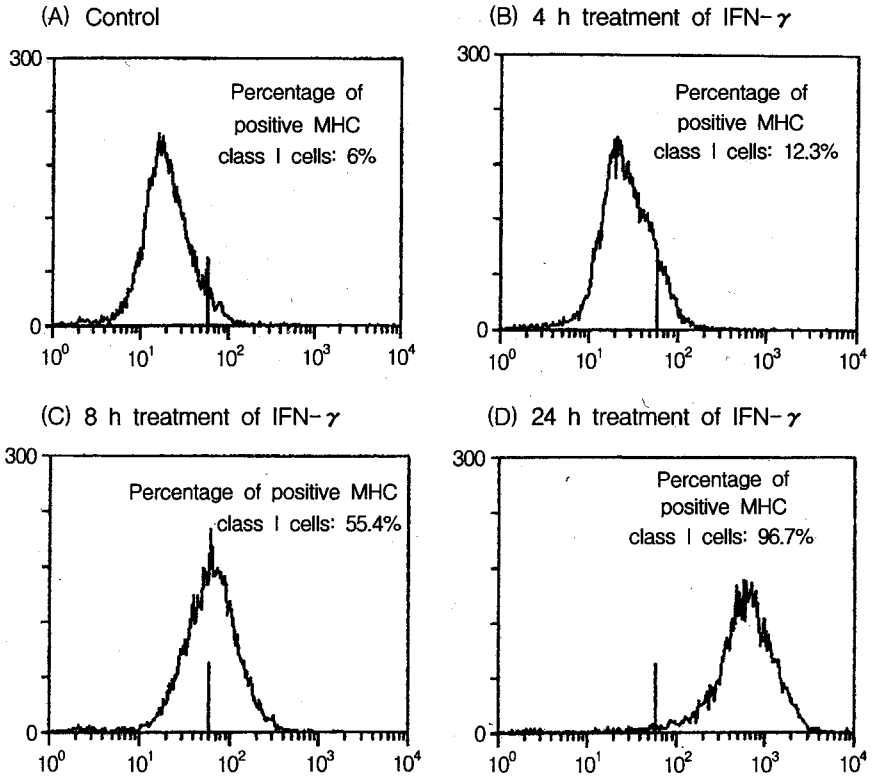

Fig. 3. Time dependent effect of IFN-γ on MHC class I expression. Tumor cells were treated with IFN-γ (50 U/ml) for 4-24 h. MHC class H-2K class I was measured by flow cytometry.

cells treated with TNF-α or IL-Iα did not increase (Fig. 2, C and D). These results suggested that IFN-γ upregulated MHC class I expression whereas both TNF-α and IL-Iα did not.

To observe the effect of IFN-γ on the expression of MHC class I molecules according to incubation time, we added IFN-γ to B16 melanoma cells for different periods (0−24 h). MHC class I expression of IFN-γ-treated B16 melanoma cells increased in a time-dependent manner (percentage of MHC class I positive cells in a 4, 8, and 24 h incubation time; 12.3, 55.4, and 96.7%, respectively) (Fig. 3, B, C and D) compared to the control (percentage of MHC class I positive cells of control; 6%) (Fig. 3A).

MHC class I expressions with high doses of IFN-γ (100−800 U/ml IFN-γ) showed similar increases of MHC class I expressions (All above 96%) (data not shown). Also the addition of anti-IFN-γ antibody to abrogate the function of IFN-γ on MHC class I expression showed a partial decrease of MHC class I expression compared to IFN-γ (data not shown).

DISCUSSION

CTL and helper T (Th) cells are effector cells that kill tumor cells. $CD8^+$ T cells, which recognize MHC class I molecules, can destroy tumor cells directly, while $CD4^+$ T cells, which recognize MHC class II molecules, produce cytokines that amplify CTL responses and activate macrophages or antigen presenting cells (APC). However, the alteration of human MHC (HLA) can occur during tumorigenesis, invasion and metastasis, which are often characterized by the accumulation of multiple genetic events to escape the immune mechanisms. Therefore, to develop an effective anti-tumor effect by CTL, enhancement of MHC class I expression can be a possible strategy to overcome the tumor escape mechanism.[13]

The loss of an MHC antigen associated with H-2K in a mouse lymphoma was first described in 1976.[14] Tumors frequently exhibit a loss of HLA class I expression when derived from HLA^+ mucosa, but may also upregulate HLA expression when derived from HLA^- or weakly-expressing tissues.[15,16] These changes may directly influence an immuno-surveillance mechanism, as MHC molecules play a central role in presenting potentially immunogenic peptides to T cells.

There have been reported that MHC alterations of class I expression are correlated with tumor invasiveness, differentiation and metastatic potential. The decreased expression of HLA class I antigen expression is correlated with poor tumor differentiation in breast, lung and larynx cell carcinomas,[17-20] which indicates that the loss of MHC class I antigen shows poor survival. Furthermore, melanomas with a loss of HLA class I expression are more invasive with a

greater metastatic capacity.[21]

It has been known that an increased expression of MHC class I proteins on tumor cells enhances their ability to be lysed by tumor-specific cytotoxic T lymphocytes in vitro. Studies showed that enhancing expression of MHC class I by IFN-γ[22-24] increases CTL activity against cancer cells.[24] Also increased MHC class I expression by gene transfection resulted in decreased tumorigenic capacity and/or metastatic capacity of murine tumor models.[25,26]

It has already been shown in our previous study that MPL, a derivative of lipopolysaccharide (LPS) from *Salmonella typhi*, increased the cytotoxicity of spleen cells and peritoneal macrophages with an accompanying increase of IFN-γ and IL-2 and a decrease of IL-4, which indicated that the stimulation of the type 1 helper T cells (Th1) had an antitumor effect.[9] In only regarding to IFN-γ level of our previous study, IFN-γ production of spleen cells with the treatment of MPL (346.66 ± 110.04 ng/ml) was more than 10 folds higher than that of control (29.60 ± 24.11 ng/ml). Therefore, we evaluated whether MPL regulates MHC class I expression of tumor cells, and also whether increased IFN-γ is responsible for the upregulation of MHC class I expression. We have used both the MHC class I expressions of control and treated group of each experiment, since MHC class I expressions of each experiment were different. In this study, we found that MPL increased MHC class I expression by inducing cytokines from spleen cells, and also that recombinant IFN-γ upregulated MHC class I expression. But there was a little difference of MHC class I expressions between the treatment of supernatant of MPL-pretreated spleen cells (Fig. 1) and direct addition of IFN-γ (Fig. 3) on B16 melanoma cells according to incubation time, which might be due to different productions of IFN-γ in both group or other cytokines by MPL pre-treated spleen cells, that could modulate the expression of MHC class I. All of these results suggest that IFN-γ, which is induced from host immune cells by MPL, upregulates the MHC class I expression of B16 melanoma tumor cells, resulting in increased lysis of tumor cells by CTL.

MPL is a derivative of lipopolysaccharide, induces TNF-α and IL-Iα in a sepsis model. Thus we used recombinant TNF-α and IL-Iα to observe the effect on MHC class I expression. However the effect of TNF-α or IL-Iα on MHC class I expression was not significant, which results suggested that only IFN-γ may be the main cytokine to increase the expression of MHC class I molecules.

MPL increased IFN-γ and IL-2 (cytokines of Th1-type $CD4^+$ T cells), and decreased IL-4 (cytokine of Th2-type $CD4^+$ T cell), which suggests that MPL stimulates Th1 cells by inhibiting of Th2 cells. These findings are consistent with others showed that MPL stimulates CTL and Th1 cytokines (IFN-γ) in aged mice.[27]

Most spontaneous tumors appear to be non-immunogenic, which suggests that immunomodulators or immunization to increase the tumor antigenicities by inducing MHC class I or II molecules could provide an attractive approach to cancer therapy. Therefore, MPL could be considered as an useful immunotherapeutic agent for the treatment of cancer by upregulation of MHC class I expression.

REFERENCES

1. Hellström KE, Hellström I. Cellular immunity against tumor antigens. Adv Cancer Res 1969;12:167-223.
2. Greenberg PD. Adoptive T cell therapy of tumors: mechanisms operative in the recognition and elimination of tumor cells. Adv Immunol 1991;49: 281-355.
3. Melief CJ. Tumor eradication by adoptive transfer of cytotoxic T lymphocytes. Adv Cancer Res 1992;58:143-75.
4. Yewdell JW, Bennink JR. Cell biology of antigen processing and presentation to major histocompatibility complex class I molecule-restricted T lymphocytes. Adv Immunol 1992;52:1-11.
5. Garrido F, Cabrera T, Concha A, Glew S, Ruiz-Cabello F, Stern PL. Natural history of HLA expression during tumour development. Immunol Today 1993;14:491-9.
6. Garrido F, Cabrera T, Lopez-Botet MA, Ruiz-Cabelco F. HLA class I antigens in human tumors. Adv Cancer Res 1995;67:155-95.
7. Stern PL. Immunity to human papillomavirus-associated cervical neoplasia. Adv Cancer Res 1996;69:175-211.
8. Townsend AR, Rothbard J, Gotch FM, Bahadur G, Wraith D, McMichael AJ. The epitopes of influenza nucleoprotein recognized by cytotoxic T lymphocytes can be defined with short synthetic peptides. Cell 1986;44:956-96.
9. Cho CH, Lee BK, Chu MK, Kim SK, Kim JD. Antitumor effect of monophosphoryl lipid A, polyadenylic-polyuridylic acid and cisplatin on B16 melanoma-induced pulmonary metastasis in mice. J Korean Soc Microbiol 1994;29:231-44.
10. Abbas AK, Lichtman AH, Pober JS. Cytokine. In: Cellular and molecular immunology. 3rd ed. Philadelphia: W.B. Saunders Company; 1997. p.249-77.
11. Ulrich JT, Cantrell JL, Gustafson GL, Meyers KR, Rudbach JA, Hiernaux JR. The adjuvant activity of monophosphoryl lipid A. In: Spriggs DR, Koff WC, editors. Topics in vaccine adjuvant research. Boston: CRC Press; 1991. p.133-43.
12. Gustafson GL, Rhodes MJ. Effects of tumor necrosis factor and dexamethasone on the regulation of IFN-r induction by monophosphoryl lipid A. J Immunotherapy 1994;15: 129-33.
13. Garrido F, Ruiz-Cabello F, Cabrera T, Perez-villar JJ,

Lopez-Botet M, Duggan-Keen M, et al. Implication for immunosurveillance of altered HLA class I phenotypes in human tumors. Immunol Today 1997;18:89-95.
14. Garrido F, Festenstein H, Schirrmacher V. Further evidence for depression of H-2 and Ia-like specificities of foreign haplotypes in mouse tumour cell lines. Nature 1976;261:705-7.
15. Lopez-Nevot MA, Esteban F, Ferron A, Oliva MR, Ruiz-Cabello F, Garrido F. HLA class I gene expression on human primary tumours and autologous metastases: demonstration of selective losses of HLA antigens on colorectal, gastric and laryngeal carcinomas. Br J Cancer 1989; 59:221-6.
16. Fernandez JE, Concha A, Aranega A, Ruiz-Cabello F, Cabrera T, Garrido F. HLA class I and II expression in rhabdomyosarcomas. Immunobiology 1991;182:440-8.
17. Ebseban F, Concha A, Delgado M, Perez-Ayala M, Ruiz-Cabello F, Garrido F. Lack of MHC class I antigens and tumour aggressiveness of the squamous cell carcinoma of the larynx. Br J Cancer 1990;62:1047-51.
18. Concha A, Esteban F, Cabrera T, Ruiz-Cabello F, Garrido F. Tumor aggressiveness and MHC class I and II antigens in laryngeal and breast cancer. Semin Cancer Biology 1991;2:47-54.
19. Redondo M, Concha A, Oldiviela R, Cueto A, Gonzalez A, Garrido F, et al. Expression of HLA class I and II antigens in bronchogenic carcinomas: its relationship to cellular DNA content and clinical-pathological parameters. Cancer Res 1991;51:4948-54.
20. Cabrera T, Garrido V, Concha A, Oliva MR, Ruiz-Cabello F, Garrido F. HLA molecules in basal cell carcinoma of the skin. Immunobiology 1992;185:440-52.
21. Lopez-Nevot MA, Garcia E, Romero C, Oliva MR, Serrano S, Garrido F. Phenotypic and genetic analysis of HLA class I and HLA-DR antigen expression on human melanomas. Exp Clin Immunogenet 1988;5:203-12.
22. Bernhard H, Maeurer MJ, Jager E, Wolfel T, Schneider J, Karbach J, et al. Recognition of human renal cell carcinoma and melanoma by HLA-A2-restricted cytotoxic T lymphocytes is mediated by shared peptide epitopes and up-regulated by interferon-γ. Scandinavian J Immunol 1996;44:285-92.
23. Gastl G, Ebert T, Finstad CL, Sheinfeld J, Gomahr A, Aulitzky W, et al. Major histocompatibility complex class I and class II expression in renal cell carcinoma and modulation by interferon-γ. J Urol 1996;155:361-7.
24. Street D, Kaufmann AM, Vaughan A, Fisher SG, Hunter M, Schreckenberger C, et al. Interferon-γ enhances susceptibility of cervical cancer cells to lysis by tumor-specific cytotoxic T cells. Gynecologic Oncol 1997;65:265-72.
25. Hui K, Grosveld F, Festenstein H. Rejection of transplantable AKR leukaemia cells following MHC DNA-mediated cell transformation. Nature 1984;311:750-2.
26. Wallich R, Bulbuc N, Hammerling GJ, Katzav S, Segal S, Feldman M. Abrogation of metastatic properties of tumour cells by de novo expression of H-2K antigens following H-2 gene transfection. Nature 1985;315:301-5.
27. Mbawuike IN, Acuna C, Caballero D, Pham-Nguyen K, Gilbert B, Petribon P, et al. Reversal of age-related deficient influenza virus-specific CTL responses and IFN-γ production by monophosphoryl lipid A. Cell Immunol 1996;173:64-78.