Chinese Journal of Cancer Research 9(4):290-294, 1997

THE EFFECTOR FUNCTIONS OF MACROPHAGES TRANSFECTED WITH INTERFERON- GAMMA GENE MEDIATED BY RECOMBINANT ADENOVIRUS [1]

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Macrophages(Mφs) are not only a kind of immune effector cells but also a kind of antigen-presenting cells(APC). In order to improve their antitumor effect, we transfected interferon-gamma(IFN-γ) gene into Mφs by recombinant adenovirus because IFN-γ is a kind of potent macrophage-activating factor(MAF). High level of IFN-γ could be detected in the supernatants of Mφs after IFN-γ gene transfection and IFN-γ secretion peaked at 20 hour. The cytotoxicity of IFN-γ gene-transfected Mφs increased significantly. The secretion of TNF, IL-1, nitric oxide(NO) also increased to some extent. The results demonstrated that recombinant adenovirus-mediated IFN-γ gene transfection could improve the effector functions of Mφs efficiently.

Key words: Macrophages, Interferon-gamma, Adenovirus, Gene transfection, Cytotoxicity.

Mφs are not only immune effector cells which can kill tumor cells directly, but also antigen presenting cells which can uptake, process tumor antigens and then present them to T cells to induce T cell-mediated immune responses. Although Mφs have spontaneous cytotoxicity to the tumor cells, they can exert potent antitumor effects only after full activation. IFN-γ is a kind of important macrophage activating factor (MAF). In another experiment, we have found that the antigen presenting capacity of MQS could be augmented by IFN-γ gene transfection. In this study, we transfected IFN-γ gene into Mφs in order that IFN-γ secreted by Mφs could activate themselves through autocrine pathway. It is difficult for retroviral vector to transfect genes into Mφs that divided inactively. Because adenovirus can transfect genes into undividing target cells including Mφs efficiently,[1] so we chose adenovirus vector to transfect IFN-γ gene into Mφs.

MATERIALS AND METHODS

Animals and Cell Lines

Pathogen-free male C57BL/6 (H-2) mice, 6-8 weeks of age, were purchased from SIPPR/BK Animal Co. Ltd(Shanghai, P.R.China). L929 fibroblast cells, L1210 leukemia cells were maintained in RPMI-1640 medium supplemented with 10% (V/V) heat-inactivated fetal bovine serum(FBS). 293 cells were kindly provided by Dr.Thomas Blankenstein(MDC for Molecular Medicine, Berlin, Germany)and grown in DMEM containing 10%FBS.

Adenoviral Vectors

The recombinant adenoviral vector containing

Accepted Aug. 8, 1997

[1] This work was supported by the National High Biotechnology Foundation (Z20-01-03).

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the E. Coli β-galactosidase gene (Adex1-RxZ), the recombinant adenoviral vector containing mouse IFN-γ cDNA (Adex1-IFN-γ)were kindly provided by Dr.Hirofumi Hamada (Department of Molecular Biotherapy Research, Japanese Foundation of Cancer Research, Tokyo, Japan).Their titers were 5.2×10^9 pfu/ml (Ad-exl CA mIFN-γ) and 3.4×10^9 pfu/ml (Ad-exl CALacZ) respectively.

Reagents

Murine IFN-γ ELISA Kit was purchased from Endogen Co.; MTT was from Fluka; RPMI-1640 and DMEM medium was from Gibco; Recombinant IL-1 was from Hoffmann-La Roche Co.; Recombinant TNF was from Genetics Co.; LPS was from Sigma .

Preparation and Gene Transfection of Macrophages

As described previously,[2] peritoneal macrophages were obtained from C57BL/6 mice. The mice were killed and their peritoneal exudate cells were harvested by washing with RPMI-1640. The cells were cultured in 12-well culture plates in RPMI-1640 medium. All the nonadherent cells were completely removed from the cultures by allowing cells to adhere to the plastic for 4 hr. For in vitro adenovirus infection, medium was discarded from the peritoneal macrophages seeded in 12-well culture plates, and viral stock was added to each well at a multiplicity of infection(MOI) of 100 pfu per cell. After incubation for 1 hour at 37°C, the cells were washed twice by RPMI-1640 medium to remove free viral particles, then the fresh growth medium was added and the macrophages were cultured.

Assay for IFN-γ

Supernatants of gene-transfected Mφs were collected at the different time for the detection of IFN-γ by a ELISA assay. All tests were performed in triplicates.

Cytotoxicity of Mφ

Indirect MTT assay was used according to the method described previously. [3]

Detection of IL-1, TNF, Nitric Oxide(NO)

Eithteen hours after gene transfection, Mφs were stimulated with LPS (1μg/ml) for 6h, 36h, 48h and the supernatants of them were collected to detect activity of TNF, IL-1, NO separately. L929 cytotoxicity assay was used for detection of TNF; Griess reaction was used for assay nitric oxide.

Statistics

Statistical analysis was performed using the Student's *t* test.

RESULTS

Level of IFN-γ in the Supernatants of IFN-γ Gene-Transfected Macrophages

Freshly isolated peritoneal Mφs were transfected with IFN-γ gene, and the controls including LacZ gene-transfected Mφs and normal Mφs were established at the same time. As shown in Fig.1, 4 hours after IFN-γ gene transfection, high level of IFN-γ could be detected in the supernatants of IFN-γ gene-transfected Mφs with a peak at 20 h, the secretion persisted for 10 days. Normal Mφs and LacZ gene-transfected Mφ didn't secret any IFN-γ . The results indicated that IFN-γ gene could be transfected into Mφs efficiently by recombinant adenovirus.

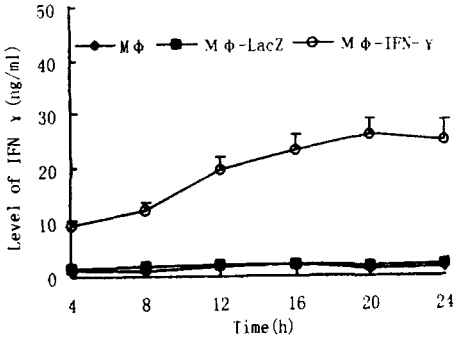

Fig. 1. The level of IFN-γ in the supernatant of mIFN-γ gene-transfected murine macrophages.

Cytotoxicity of IFN-γ Gene-Transfected Macrophages

As shown in Figure 2, cytotoxicity of normal Mφs could be detected but very weak. Cytotoxicity of IFN-γ gene-transfected Mφs was much higher than that of controls($P<0.01$),and were enhanced further markedly after additional stimulation by1μg/ml LPS. These results showed that Mφs could be activated by IFN-γ secreted by themselves after gene transfection and could be activated fully by additional LPS.

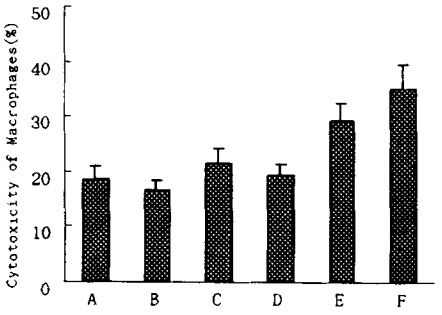

Fig. 2. The cytotoxicity of mIFN-γ gene-transfected peritoneal macrophages.
A: Mφ-RPMI-1640 B:Mφ-LacZ C:Mφ+LPS D:Mφ-LacZ+LPS E: Mφ-IFN-γ F:Mφ-IFN-γ +LPS

Level of TNF, IL-1 Secreted by IFN-γ Gene-Transfected Macrophages

Level of TNF secreted by IFN-γ gene-transfected Mφs hadn't any significant increasement when compared with control, but increased markedly after stimulation with LPS($P<0.01$). High level of IL-1 could be detected after IFN-γ gene transfection and it increased more markedly after stimulation with LPS(Table1).

Level of Nitric Oxide in the Supernatants of IFN-γ Gene-Transfected Macrophages

As shown in Figure 3, normal Mφs or Mφs transfected with LacZ control gene secret little NO even after stimulation with LPS, while high level of NO could be detected in the supernatants of IFN-γ gene-transfected Mφs($P<0.01$). A little increasement could been seen after stimulation with LPS, but there is no marked difference when compared with that of groups without LPS stimulation($P>0.05$).

Table1. The levels of TNF and IL-1 induced from peritoneal macrophages transfected with mIFN-γ gene

Groups	Cytokine Levels ($\bar{\chi} \pm SD$)	
	TNF(U/ml)	IL-1 (ng/ml)
1640	ND*	ND
LacZ	ND	ND
LPS	8.6± 0.9	ND
LacZ+ LPS	9.1± 0.8	ND
IFN-γ	ND	17.4± 2.5
IFN-γ+ LPS	15.3± 2.1	34.0± 5.1

ND* :not detected

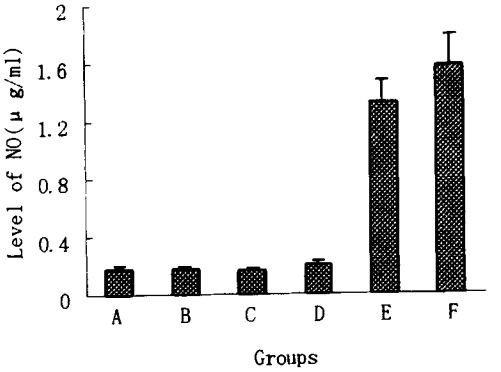

Fig 3. Nitric Oxide production by peritomeal macrophages transfected with mIFN-γgene
A: Mφ-RPMI-1640 B:Mφ-LacZ C:Mφ+LPS D:Mφ-LacZ+LPS E: Mφ-IFN-γ F:Mφ-IFN-γ+LPS

DISCUSSION

IFN-γ plays important roles in the immunological regulation. An optical concentration of IFN-γ can inhibit the growth of tumor cells directly and induce the expression of specific tumor-associated antigens on tumor cells. The most important is that IFN-γ can upregulate the expression of MHC II or costimulatory molecules in the host antigen-presenting cells, leading to effective tumor-antigen presentation to T lymphocytes. Based on these data, animal model experiments as well as clinical trials for cancer therapy using IFN-γ have been developed. To sustain the anti-tumor effects of IFN-γ, repeated administration or continuous perfusion of IFN-γ is required due to their short half-life *in vivo* which

limits its clinical application.

Along with the appearance of concept and methods of cytokine gene therapy, Abe, et al.[4] transfected IFN–γ gene into cytotoxic T lympho-cytes by adenovirus. These gene-transfected CTL could localize in the tumor lesions and regional lymph organs, leading to a high-dose local cytokine delivery to tumor cells as well as host immunocompetent cells. The murine IFN-γ gene was also introduced into tumor cells and the tumorigenicity of IFN-γ-producing tumor cells were abrogated and a long lasting protective immune response against a subsequent tumor were induced.[5-8] A phase I trail using IFN-γ transfected autologous tumor cells are in the progress in patients with disseminated malignant melanoma .[9]

Mφs , as a type of important immune effector cells and antigen presenting cells, play important roles in antitumor effects. A kind of effective APC must have the capacity to internalize and process foreign protein, express major histocompatibility complex (MHC) molecule in order to transport antigenic peptides to the cell surface and it should be able to provide costimulation to T cells. Mφs are phagocytic and they are efficient in internalizing and processing large antigens.[10] Although resting Mφs do not constitutively express MHC class II or B7.[11] IFN-γ has been shown to increase the antigen presenting capacity of Mφs and promote the tumoricidal activity of Mφs. In the present study, we hoped that immune effector and antigen-presenting ability of IFN-γ gene-transfected Mφs could be enhanced at the same time. Early clinical trials had been conducted using recombinant IFN-γ-activated Mφs derived from peripheral blood monocytes for the adoptive immunotherapy of cancer patients. Apart from partial response, no complete reaction was observed. Up to now, there is no report about adoptive immunotherapy of cancer with IFN-γ gene-transfected Mφs. Much research has focused on the Mφs derived from bone marrow precursor or blood monocytes. GM-CSF cDNA was transfected into Mφs derived from bone marrow cells with retroviral vector, the results demonstrated that bone marrow cells could be genetically engineered to secret GM-CSF, resulting in expansion of effective APCs with enhanced stimulatory capacity and improved antigen presenting function.[12] Ramesh et al[1] identified another type of retrovirus containing the adenosine deaminase(ADA) gene under the transcriptional control of the promoters/enhancers of Moloney Murine Leukemia virus and the human cytomegalovirus(CMV) immediated-early gene which could provide optimal gene expression in human hematopoietic cells. Another experiment showed that deficient respiratory burst could be corrected after *ex vivo* infusion of gene-transfected monocyte-derived Mφs of patients with X-linked chronic granulornatous disease.[13]

Activation of Mφs needs priming signal and triggering signal. Fully activated Mφs could be obtained after pulsed with triggering signal. According to the studies of macrophage activation, we observed the changes of immunological effector function of IFN-γ gene-transfected Mφs pulsed with low dose LPS. The results showed that high level of IFN-γ could be detected in the supernants of IFN-γ gene-transfected Mφs, cytotoxicity of them increased markedly. To investigate the mechanisms of the increased cytotoxicity, we studied the secretion of biological mediators from Mφs after gene transfection. The results indicated that high level of IL-1 and NO could be detected in the supernatants of IFN-γ gene-transfected Mφs, it had significant difference against the control, but level of TNF could be increased only after additional stimulation of LPS other than IFN-γ gene transfection. Our results demonstrated: (1) IFN-γ gene had been successfully transfected into Mφs with adenovirus and high level of IFN-γ could be detected in the supernatants of IFN-γ gene transfected Mφs. (2) Mφs would be further activated by LPS. Different biological mediators would be released at the different stages of activation. IL-1 and NO might be released at the beginning of the activation while TNF might be released after completely activation of Mφs. TNF was stored in the Mφs after IFN-γ gene transfection. After pulsed with LPS, Mφs were in the triggering stage and TNF was released at this time. The increased secretion of these mediators might contribute to the enhanced effector functions of Mφs transfected with IFN-γ.

In conclusion, we have demonstrated that IFN-γ gene could be successfully transfected into Mφs with adenovirus vector and these Mφs could be genetically engineered to secret IFN-γ resulting in increasement of their effector function *in vitro*. The results indicated that tumor could be treated with gene-transfected Mφs by direct intratumorally injection or adoptive infusion them after pulsed with tumor antigen.

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