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IMMUNOLOGICAL CHARACTERISTICS OF THE LEUKEMIA CELLS TRANSFECTED WITH ONCOSTATIN M GENE BY RECOMBINANT ADENOVIRUS[1]

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In the present study, FBL-3 murine erythroleukemia cells were transfected with human OSM(hOSM) gene by recombinant adenovirus, then the immunological properties of hOSM gene-transfected FBL-3 cells(FBL-3-OSM$^+$) were investigated. 4 hours after transfection with hOSM gene, hOSM could be detected in the supernatant of FBL-3-OSM$^+$ cells and hOSM secretion peaked at 24 h. The proliferation of FBL-3-OSM$^+$ cells was inhibited markedly. The clonal formation of FBL-3-OSM$^+$ cells was suppressed more obviously in comparison with wild-type FBL-3 cells when analysed in clonal argar culture. Flow cytometry analysis showed that FBL-3-OSM$^+$ cells expressed higher levels of Fas protein, B7 and ICAM-1 molecules.FBL-3-OSM$^+$ cells also expressed higher level of MHC class I molecules(H-2K^b) but remained unchanged in expression of MHC class II molecules (Ia). CD14, which is a specific marker of monocyte/macrophage and not expressed on the wild-type FBL-3 cells, was also detected on the surface of FBL-3-OSM$^+$ cells. The results suggested that OSM gene transfer could increase the immunogenicity of FBL-3 cells and promote their differentiation into macrophage-like cells. The data outline a promising approach to OSM gene therapy of leukemia mediated by recombinant adenovirus.

Key words: Oncostatin M, Gene transfer, Erythroleukemia, Immunogenicity, Costimulatory molecules, CD14.

Oncostatin M(OSM),a newly identified 28-KD glycoprotein, is a pleiotropic cytokine mainly produced by activated monocytes, macrophages and T lymphocytes, which shares structural and functional similarities with leukemia inhibitory factor(LIF) and interleukin-6.[1] OSM is a distinct member of a cytokine family whose member include interleukin-6, LIF, granulocyte colony-stimulating factor, and myelomonocytic growth factor. It can inhibit the proliferation of cultured solid tumor cell lines such as human melanoma, lung, ovarian and stomach tumor cells, or leukemia cells. It can induce the differentiation of the myeloblastic M1 murine leukemia cells into macrophage-like cells *in vitro*.[2,3] In recent years cytokine gene-modified tumor vaccine is a new approach to immunotherapy of cancer .In this study, FBL-3 erythroleukemia cells were transfected with human OSM(hOSM) gene by recombinant adenovirus, then the immunological properties of hOSM gene-transfected FBL-3 cells (FBL-3-OSM$^+$) were investigated.

MATERIALS AND METHODS

Materials

Recombinant human Oncostatin M and mouse anti-human OSM monoclonal antibody were purchased from R&D(Minneapolis);FITC-labeled Anti-CD14, anti-CD80(B7-1), anti-I-A^{b,d} (major

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histocompatibility complex ,MHC-II) , anti-CD95 (ICAM-1) and anti-H-2K^{b}MoAb were supplied by ParMingen; ^{3}H-TdR was production of Amersham.

Cell Lines and Recombinant Adenovirus

FBL-3 cell , Friend virus-induced erythroleukemia cell line of C57BL/6 (H-2^b) origin, was gifted by Dr. Wei Chen (University of Washington, Seattle). 293 (CRL1573, ATCC), kindly provided by Dr.Thomas Blankenstein(MDC,Berlin), is a human embryonic kidney cell line transformed with Ad5 E1A and E1B genes and supporting propagation of E1-deleted recombinant adenoviruses. The above cell lines were cultured in RPMI1640 and DMEM (Gibco, BRL) supplemented with 10% heat-inactivated FCS, 2mM glutamine, 100U/ml penicillin, and 100μg/ml streptomycin, respectively. Recombinant adenovirus vector harboring hOSM cDNA was kindly provided by Dr. Hirofumi Hamada(Department of Molecular Biotherapy Research, Japanese Foundation of Cancer Research). Recombinant adenovirus vector harboring LacZ reporter gene has been previously described.[4]

Adenovirus-mediated Oncostatin M Gene Transfection into FBL-3 Cells

As described elsewhere, 1×10^5 FBL-3 cells were allowed to grow to confluent in 25cm^2 tissue culture vessels in the complete RPMI1640 medium. After washed with PBS solution, the culture was supplemented in small volume of serum free RPMI 1640 containing 100 MOI of recombinant adenovirus harboring human OSM gene or LacZ report gene. 2 hours later, the gene transfection medium was removed, and the complete RPMI 1640 medium was added. The OSM gene-transfected FBL-3 cells were further used in the following studies.

Measurement for OSM Secretion in the Supernatants of Gene-Transfected FBL-3 Cells

The FBL-3-OSM$^+$ cells were seeded in a 6-well microplate (1×10^6 cells /well), then the supernatants were collected at different times. The concentration of OSM was determined using a ELISA method.[5]

Assay for Cell Proliferation

To investigate the effect of recombinant human OSM on the proliferation of erythroleukemia cell line, 10^4 /well FBL-3 cells were cultured in each well of 96-well flat-bottomed microplates for 3 days with either culture medium alone or various concentration of recombinant OSM (0.5ng,1ng,2ng, 5ng or 10ng/ml, respectively). During the last 12 hours of the 72-hour culture, cells were incubated with 1μCi (37kBq) of (methyl-^{3}H) thymidine, and proliferation was assessed by thymidine incorporation.

To investigate the proliferative ability of FBL-3 cells after gene transfection, FBL-3-OSM$^+$ cells, LacZ report gene-transfected FBL-3 cells (FBL-3-LacZ) and wild-type FBL-3 were cultured in each well of 96-well flat-bottomed microplates for 3 days with a cell concentration of 10^4 per well in 0.1ml culture medium . ^{3}H-TdR (1μCi/well) was added at the last 12 hours of 72-hour culture, the cells were collected on a glass filter using an automatic cell harvester, the radioactivity was measured in liquid scintillation counter (Wallac 1409) and proliferation was assessed by thymidine incorporation.

Assay for Clonal Formation

The cells(500/ml) of each experimental groups were cultured in 24 well dishes containing 1ml DMEM with a final concentrations of 20% horse serum and 0.3% agar. Cultures were incubated at 37 ℃ in a fully humidified atmosphere of 5%CO_2. Colonies (clones of more than 40 cells) were scored using dissection microscope after 7 days of incubation.[6]

Determination of Surface Molecules Expressed on FBL-3-OSM$^+$ Cells

The expression of cell surface molecules were determined by direct immunofluorescence with flow cytometry. 1×10^6 cells per experimental condition were supplemented in 0.5ml of phosphate buffered saline (PBS), and 10 μl of FITC-labeled anti-CD14, anti-Fas, anti-B7-1, anti-I-A^{b,d}, anti-ICAM-1or anti-H-2K^b monoclonal antibody was added, respectively. The cells were incubated for 45 min at 4 ℃, and were washed twice with PBS, then the cells were analyzed on a FACScan (Becton Dickinson).

Statistical Analyses

Student's *t*-test was used to test the significant

confidence.

RESULTS

OSM Secretion of FbL-3 Cells Transfected with OSM Gene

2 hours after the gene transfection, the FBL-3-OSM$^+$cells were stained with X-gal. It was confirmed that LacZ report gene could be transfected into FBL-3 cells by recombinant adenovirus with efficiency over 96%. 4 hours after transfected with hOSM gene, hOSM could be detected in the supernatant of FBL-3-OSM$^+$ cells and its secretion peaked at 24 hours. Furthermore, hOSM could be detected in the supernatant of FBL-3OSM$^+$ cells even 7 days after gene transfection , as shown in Figure 1.

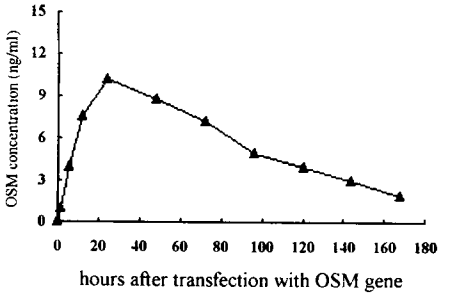

Fig. 1.The level of hOSM secreted by hOSM gene-transfected FBL-3 cells *in vitro*.

The Proliferation of Fbl-3 Cells Transfected with OSM Gene

To study the proliferative ability of OSM gene-transfected FBL-3 cells, FBL-3-OSM$^+$ cells were incubated in culture medium or wild-type FBL-3 cells were cultured in medium with various concentrations of recombinant human OSM at 37 ℃ for 3 days. As shown in Figure2, 2 μg/ml of recombinant OSM could significantly inhibit the proliferation of FBL-3 cells, and the proliferation of FBL-3-OSM$^+$ cells was obviously suppressed. The results confirmed that OSM can inhibit the proliferation of FBL-3 cells, and the proliferative ability of OSM gene transfected FBL-3 cells was greatly reduced(Figure3).

Clonal Formation of FBL-3 Cells Transfected with OSM Gene

In order to further investigate the growth characteristic of OSM gene-transfected FBL-3 cells, soft agar assay has been used. As shown in Figure4, the clonal formation of wild-type FBL-3 cells decreased when cultured in soft agar containing 5ug/ml of recombinant OSM. Meanwhile, the clonal formation of FBL-3 cells transfected with OSM gene reduced significantly.

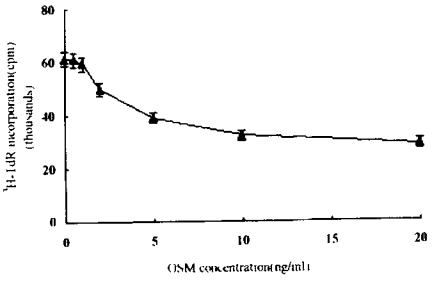

Fig. 2. The proliferation of wild-type FBL-3 cells treated with recombinant hOSM *in vitro*.

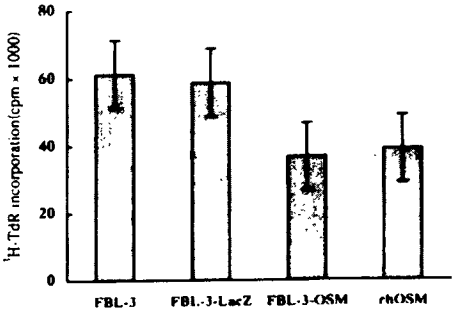

Fig. 3. The proliferation of hOSM gene-transfected FBL-3 cells *in vitro*.

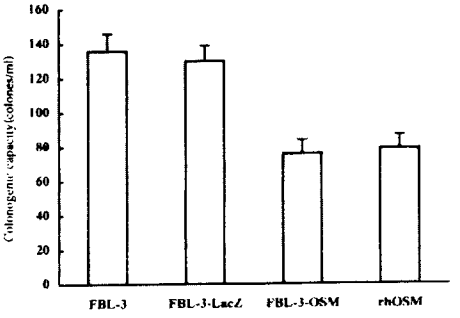

Fig. 4. Clonogenic capacity of FBL-3 cells transfected with OSM gene in soft agar culture.

CD14 and Fas Expression on the FBL-3 Cells Transfected with OSM Gene

CD14 is a specific marker of monocyte /macrophage. In order to determine whether or not the FBL-3 cells were induced to differentiate into macrophage-liked cells after transfection with OSM gene, CD14 and Fas were determined with anti-CD14 or anti-Fas monoclonal antibody by flow cytometry. As shown in Figure5, wild-type FBL-3 cells didn't express any CD14 and express Fas protein at low level. But high expression of CD14 and Fas protein could be detected on the surface of OSM gene-transfected FBL-3 cells. The results indicated that FBL-3 cells could differentiate into macrophage-liked cells after transfection with OSM gene.

Expression of MHC Molecules and Co-Stimulatory Molecules on FBL-3 Cells Transfected with OSM Gene

24 hours after transfected with Ad-OSM, the immunological characteristics of FBL-3-OSM$^+$ cells were further analysed. FBL-3-OSM$^+$ cells were found to express higher levels of B7 and ICAM-1 molecules compared with wild-type FBL-3 cells. In addition, FBL-3-OSM$^+$ cells expressed higher level of MHC class I molecules(H-2K^b) but expression of MHC class II molecules (Ia) remained unchanged, as shown in Figure 6.

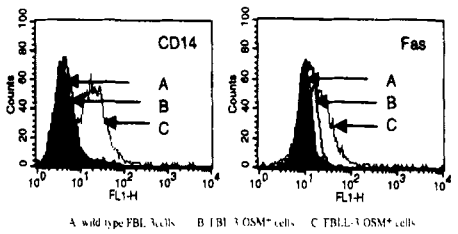

Fig. 5. FACS analysis of CD14 and Fas expression on FBL-3 cells transfected with OSM gene.

DISCUSSION

OSM shares structural and functional similarities with LIF and interleukin-6, and acts on a wide variety of cells and elicits a multitude of biologic effects.[7,8] Although numerous studies have been performed previously to elucidate its biologic effects, little is known about characteristics of leukemia cells transfected with OSM gene. In this study, FBL-3 erythroleukemia cells were transfected with human OSM(hOSM) gene by recombinant adenovirus, then the immunological properties of hOSM gene-transfected FBL-3 cells (FBL-3 - OSM$^+$) were investigated.

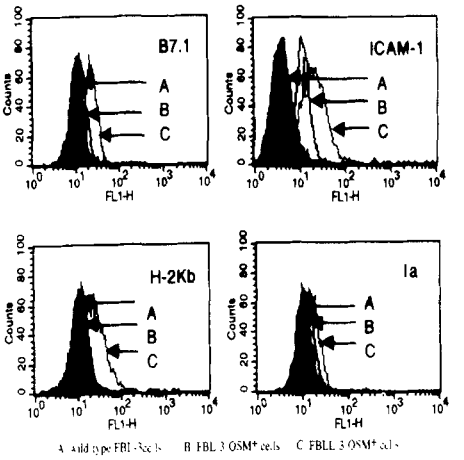

Fig.6. FACS analysis of H-2K^b, Ia, ICAM-1 and CD80 expression on FBL-3 cells transfected with OSM gene.

Previous study has shown that OSM can inhibit the growth of myeloblastic M1 murine leukemia cells and can induce them to differentiate into macrophage-like cells. In this study, our result showed that OSM also can suppress the proliferation of erythroleukemia cells in vitro. When the wild-type FBL-3 cells were cultured in medium with 5ug/ml of recombinant OSM at 37 ℃ for 3 days, the FBL-3 cells proliferate poorly. Meanwhile, the proliferation of FBL-3-OSM$^+$ was very weak. By soft agar assay ,we found that clonal formation of wild-type FBL-3 cells decreased when cultured in soft agar containing 5 μg/ml of recombinant OSM, and the clonal formation of FBL-3-OSM$^+$ cells was also significantly reduced. The results suggested that recombinant human OSM can inhibit the proliferation of FBL-3 cells, and OSM gene transfected FBL-3 cells exhibited the weak proliferative ability and decreased tumorigencity.

CD14 is a specific marker of monocyte or macrophage, and tumor cells usually don't express this molecule. In this study, we also have not found CD14 molecule expression on the wild-type FBL-3 cells. But,FBL-3-OSM$^+$ cells express high level of CD14. The data indicated that OSM gene transfection

could induce FBL-3 cells to differentiate into $CD14^+$ cells, or macrophage - liked cells. Another fact supported this suggestion. FBL-3-OSM^+ cells also expressed much higher level of Fas protein. Fas protein, a member of tumor necrosis factor receptor (TNFR) family, is expressed on activated lymphocytes and in various tissues including the thymus, liver, heart and kidney. It is one of the ways to induce cell's apoptosis when the Fas protein bind with Fas ligand.[9] Fas mediated apoptosis posses three basic factors, i.e abundant Fas expression on cells surface, Fas and Fas ligand interaction and closed in anti-apopotosis program by the cell. High level of Fas protein expression on FBL-3 cells after OSM gene transfection provided the critical factor governing Fas mediated apoptosis. These results offered important foundation to further study the relation between leukemia cells and monocyte/macrophages. The results also proved that OSM directly acts on the erythroleukemia cells and induces erythroleukemia to differentiate into $CD14^+$ cells.

Alternatively, OSM gene-transfected FBL-3 cells could express higher levels of CD80, ICAM-1 and $H\text{-}2K^b$ molecules when compared with LacZ report gene transfected one. However, we could determine expression of MHC Ia molecule on OSM gene transfected FBL-3 cells but its expression remained unchanged when compared with wild-type FBL-3 cells. ICAM-1, a member of immunoglobulin superfamily, is usually expressed on white blood cells, macrophages, dendritic cells and many tumor cells, its ligand is LFA-1. CD80 and MHC class II molecules are important relative molecules in T lymphocyte activation. It is generally accepted that stimulation of antigen-MHC complex alone isn't sufficient for T-cell activation. Additional costimulatory signals must be provided. However, the poor immunogenicity of many tumor cells may be, in a large part, a consequence of failure to express costimulatory molecules.Previous studies have demonstrated that antitumor immunity can be enhanced by the provision of costmulatory signals.In this study,our results showed that the OSM gene-transfected FBL-3 cells could express higher level of CD80,ICAM-1 and MHC class I molecules which would contribute to stimulate the activation of T lymphocytes , and induce the potent specific immune response. In view that tumor cells are regarded as non-professional antigen presenting cells, it is very important to express high level of adhesion molecules or costimulatory molecules to enhance its immunogenicity and then activate T lymphocytes. Our results outline a promising approach to OSM gene therapy of leukemia mediated by recombinant adenovirus.

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