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STUDY OF ENHANCED IMMUNOGENECITY OF B7-1 GENE TRANSFECTED HUMAN HELA CELL LINE

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Although cervical carcinoma cells may express the human papillomavirus protein E6 and E7, they fail to induce an effective specific cytotoxic T lymphocyte response. Recent studies suggest that expression of CD-80 (B7-1) on tumor cells is effective to induce antitumor immune responses.[1,2] In our study, CD-80 gene was transfected into human Hela cell line with a CD-80 expression plasmid (B7-1*pcDNA$_3$) by electroporation, then the immunogenecity of the modified Hela cell was tested in TLMC (tumor lymphocyte mixed culture) system. [^{3}H]thymidine lymphocyte proliferation assays showed that the response of human peripheral blood lymphocytes (PBLS) to CD-80 positive Hela cells demonstrated a substantial increase in cell proliferation compared to the response to control cells. Cocultivation of allogeneic PBLs with CD-80 positive tumor cells for three days can induce an increased secretion of IL-2. Our results demonstrate an immunostimulatory effect of CD-80 expression on cervical cancer cells, which provides a basis for the development of a therapeutic tumor vaccine.

Key words: B7-1 gene, Hela cell line, CD-80, Immunogenecity

Full activation of naive T lymphocyte requires not only ligation of the TCR complex, but also participation of at least one "second signal".[3,4] While the first signal provided by TCR initiates the process of Ag-specific T cell activation, the second signal provided by costimulator factors are critical for optimal cytokine production, T cell expansion and effector function.[5] Lack of costimulation can lead T cells to a state of unresponsiveness upon Ag stimulation (anergy). One of most important costimulatory pathway is the interaction of B7-1 (CD-80) and B7-2 (CD-86) with CD28 molecule on T cells.[6,7] Though the human cervical carcinoma, Hela cell line may express specific papillomavirus antigen E6 and E7, it can not provide the second signal strong enough to activate T cells without expressing B7-1 and B7-2 molecules. To augment the immunogenecity of cervical carcinoma cells, we transfected Hela cell with the CD80 expression plasmid (pcDNA$_3$). The result demonstrated that the immunogenicity of Hela cell was enhanced by transfection of B7-1 plasmid. Both the proliferation assay and the cytokine detection indicated that the transfected Hela cells are more effective to activate T lymphocytes.

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MATERIALS AND METHODS

Cells

Hela cells, a human cervical carcinoma cell line. Obtained from ATCC. This cell line was grown in RPMI 1640 complete medium (RPMI 1640 supplemented with 10% bovine calf serum, 2mM L-glutamine, 100 U of penicilline/streptomycin, and 2×10^{-5}M β-mercaptoethanol). CTLL-2 is a gift from Dr. Ma BL, Shanghai Second Medical University.

Regents and Monoclonal Antibodies

CD80 (B7-1) expression plasmid (B7-1$^+$pc DNA_3) and control plasmid (B7-1$^-$pcDNA_3) were gifts from Lieping Chen (Bristol-Myers Squibb Pharmaceutial Research Institute, USA).

FITC-conjugated anti-CD80(B7-1) monoclonal antibody was purchased from Ancell (Bayport, MN, USA). MTT (Fluka) G418 (Gibco) Alamar blue (Biosource international corporation).

Transfection of B7-1

5×10^6 Hela cells were electroporalized. after adding 10μg B7-1$^+$pcDNA_3 plasmid was transfected, the cells were incubated on ice for 5 min. Then the cell was expanded in medium containing 400μg/ml G418. A clone with high expression of CD80 molecule was obtained by limited dilution method. B7-1$^-$pcDNA_3 plasmid was transfected as a control.

Flow Cytometric Analysis of B7-1

B7-1$^+$Hela cells were detected by FACS.

TLMC (Tumor lymphocyte mixed culture)

PBLs(10^5) from healthy donors were cocultured separately with B7-1$^+$Hela, B7-1$^-$Hela or Hela tumor cells (10^4) treated with mytomycin in a round-bottomed microtiter plate in RPMI 1640 supplemented with 10%FCS, 2mM glutamine, and antibiotics (100IU/ml penicillin/streptomycin) in a total volume of 200μg for 6 days. [^{3}H] Thymidine 1μCi/ml was added 16 hr prior to termination of the incubation. The culture were harvested. [^{3}H] Thymidine incorporation was measured in a liquid scintillation spectrometer.

Measurement of IL-2

1×10^5 PBLs/well were cultured separately with 1×10^4/well B7-1$^+$Hela, B7-1$^-$Hela or Hela for 72h. Supernatants were collected and evaluated for IL-2 levels in a standard CTLL-2 bioassay.

RESULTS

Expression of B7-1 Molecule

A clone of high expression of B7-1 molecule (83.71%) on Hela cells has been obtained, but Hela cells transfected with control plasmid or parental cells didn't express B7-1 molecules (Figure 1).

TLMC (Tumor and lymphocyte mixed culture)

Proliferation response of PBLs to B7-1 Hela cells was significantly stronger than that to B7-1$^-$Hela cells or parental cells although exist an individual difference (Table 1).

Cytokines Production

Figure 2 showed the result of IL-2 production in different TLMC systems. The production of IL-2 was remarkably increased by stimulation with B7-1$^+$ Hela cells.

Table 1. Proliferation response of PBLs to different Hela cells (TLMC)

Responder	($\bar{x}\pm s$) cpm Stimulator			
	CD80$^+$ Hela	CD80$^-$Hela	Parental Hela	PBLS*
1	4040 ± 485 (1.56)	935 ± 140 (0.36)	1122 ± 148 (0.43)	2595± 467
2	1507 ± 120 (6.50)	207 ± 31 (0.89)	331 ± 36 (1.43)	232± 21
3	1386 ± 135 (5.75)	554 ± 44 (2.30)	602 ± 89 (2.50)	241± 22
4	6336 ± 976 (14.08)	2013 ± 294 (4.47)	1897 ± 218 (4.22)	450± 66
5	23685 ± 2576 (64.01)	1450 ± 185 (3.91)	1630 ± 184 (4.40)	370±55
6	6690 ± 682 (2.53)	1657 ± 149 (0.63)	1732 ± 208 (0.66)	2644± 259
	Hela+medium 89	CD80$^+$Hela+medium 78	CD80$^-$Hela+medium 67	

* PBL alone ( )index Index=(PBL + tumor cell) (cpm)/(PBL alone) (cpm)

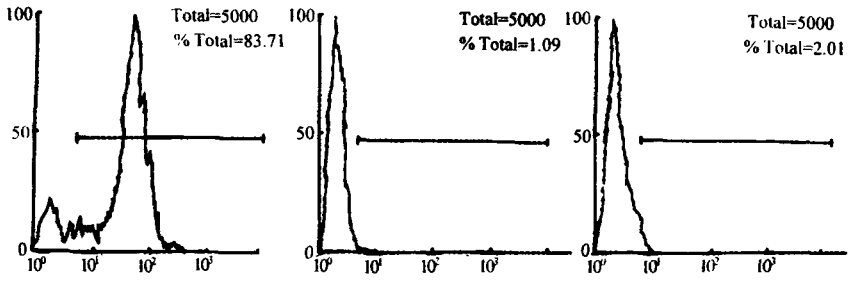

Fig. 1. Flowcytometric analysis of CD80 (B7-1)
A. CD80$^+$ Hela cell B. CD80$^-$Hela cell C. Parental Hela cell

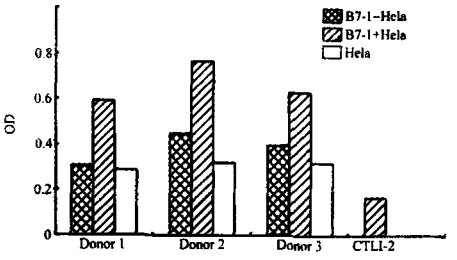

Fig. 2. The production of IL-2 in the TLMC supernatant (n=3).

Three donor's PBLs were used. The CTLL-2 cultured in RPMI 1640 served as a control. $P<0.01$

DISCUSSION

A poor immunogenicity of tumor cells has been regarded as one mechanism which enables tumor cells to escape host immune surveillance. Although some tumor cells do express tumor-associate antigen, they fail to induce an efficient immune response without costimulatory molecules such as CD80. Our studies showed this can be reversed by transfecting tumor cells with CD80 expression plasmid. Using the $pcDNA_3$ transfection system, B7-1 molecule can be stable expressed on tumor cells. In agreeent with other reports, B7-1 gene modified Hela cells can induce a stronger allogeneic reaction.

A full activation of T cell always showed a strong proliferation response of T lymphocyte to stimulator tumor cells as well as having a high level IL-2 production. Our results demonstrated that a stronger porliferation and increased IL-2 porduction of PBLs by stimulating with B7-1$^+$ Hela cells. These findings are confident with other results. TNF was also detected in the TLMC supernatant.The production of TNF didn't increase significantly.

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