Establishment of a cervical cancer model via inoculating SiHa Cells into humanized severe combined immunodeficient mice

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Summary

Purpose of investigation: To establish a human papillomavirus (HPV) 16 positive cervical cancer model in the humanized severe combined immunodeficient (SCID) mouse.

Methods: A HPV16 positive cervical carcinoma cell line (SiHa) was transplanted subcutaneously into SCID mice (SiHa-SCID); human peripheral blood lymphocyte (Hu-PBL) was transplanted intraperitoneally (Hu-PBL-SCID), Hu-PBL was transplated intraperitoneally and SiHa subcutaneously (Hu-PBL-SiHa-SCID), and, PBS was transplanted subcutaneously (PBS-SCID) as a control. The biological and immunological features were investigated.

Results: The transplanted tumor grew slowly and no metastasis was found. The survival time of Hu-PBL-SiHa-SCID was significantly longer than that of SiHa-SCID. HPV16 DNA could be detected in all of the tumor tissues, but not in peripheral blood and organ tissues. Human serum IgG levels in Hu-PBL-SCID and Hu-PBL-SiHa-SCID were significantly elevated following immunoreconstructed time elongating, and significantly higher in Hu-PBL-SiHa-SCID than those in Hu-PBL-SCID. The numbers of human $CD3^+$, $CD4^+$ and $CD8^+$ T cells were significantly increased in the peripheral blood and spleen of Hu-PBL-SiHa-SCID and Hu-PBL-SCID mice, and significantly higher in Hu-PBL-SiHa-SCID than those of Hu-PBL-SCID mice. The weight of the spleen was significantly increased in Hu-PBL-SiHa-SCID. Tumor infiltrating lymphocytes (TILs) and human $CD4^+$ T cells were detected in Hu-PBL-SiHa-SCID but not in SiHa-SCID mice. The spleen cells of Hu-PBL-SiHa-SCID mice displayed significantly stronger cytotoxicity to target cells than those of SiHa-SCID mice. No graft-versus-host disease (GVHD) was found in either Hu-PBL or Hu-PBL-SiHa-SCID mice.

Conclusion: A HPV16 positive cervical carcinoma model has been successfully established in SCID mice. This model can perfectly simulate the biological features of spontaneous human cervical cancer, and present anti-tumor immune response after the human immune system is reconstructed.

Key words: Cercival carcinoma; HPV; SCID mice; Animal model.

Introduction

Cervical cancer is the most common cancer in developing countries. An estimated 500,000 new cases are diagnosed worldwide with 250,000 of those women destined to die of the disease [1, 2]. A lot of evidence has shown that both the incidence and mortality rate can be reduced by the use of cervical screening programs. In the United States, it was estimated that widespread screening for cervical cancer with the Papanicolaou smear resulted in a 70% decline in mortality from cervical cancer in the past 50 years [3]. However, cervical cancer remains still the second leading cause of cancer death among women in developing countries. The major causative agent of this disease is considered to be human papillomavirus (HPV) infection. Over the past 15 years, epidemiological data have identified the fact that there is a consistently strong relationship between HPV infection and cervical neoplasia, and HPV DNA has been detected in more than 99.7% of cervical cancer and its precursors [4]. The use of HPV typing shows that HPV 16 is a predominant etiological agent and presents in approximately 50% of all the disease [5]. Therefore, HPV 16 has become a target for the development of a HPV vaccine [6]. In addition to the infectious factor, immunosuppression is another risk factor for the development of cervical cancer. In studies of patients with renal transplants, a relative risk of 13.6% has been reported for the development of cervical cancer in situ in transplant recipients compaed with women in the general population [7]. It has become widely accepted that there is also an association between cervical disease and infection with HIV. Screening for HIV-infected and uninfected women documented a 5.6-fold higher prevalence of biopsy-confirmed cervical intraepithelial neoplasia in the HIV-infected group than in the comparable group of HIV-seronegative women [8]. Moreover, HPV infections are more prevalent and tend to be more persistent in HIV-infected women [9].

Considering that HPV infection and immune status of the host play a crucial role in the development of cervical cancer, it should be very important to investigate the relationship between HPV infection and host immunity. However, routine laboratory methods for culturing HPV in vitro are not available, unless a suitable animal models is used. The earliest animal model for cervical cancer showed low tumorigenecity. The nude-mouse transplan-

Revised manuscript accepted for publication July 24, 2006

tation system is commonly used for growing xenografted human tumor, with a higher tumorigenecity rate [10, 11]. However, it does not appear to be an ideal animal model to accurately simulate the features of human spontaneous tumor because humoral immunity still exists in nude mice. Severe combined immunodeficient (SCID) mice make research of the animal transplantation system for human tumor move forward. The main characteristic of SCID mice is lack of both T and B lymphocytes [12]. Therefore, SCID mice have become a better animal model for transplantation of human tumors. It was reported that various kinds of human tumors have been successfully inoculated into SCID mice [13-15]. Moreover, SCID mice have also been used for reconstruction of the human immune system because of their deficiency of both humoral and cellular immunity. Moiser *et al.* [16] first established a stable humanized model in 1988. Thereafter, humanized SCID mice have been considered to be the most available animal model for investigating the relationship between tumor and host immunity.

Although models for transplanting different tumors into humanized SCID mice were established [17, 18], not one for cervical cancer was reported. In this study, we transplanted HPV-16 positive SiHa cells subcutaneously into SCID mice, and successfully established an ideal animal model for human cervical cancer, which simulated perfectly the biological features of spontaneous human cervical cancer and presented anti-tumor immune response after the human immune system was reconstructed via inoculating intraperitoneally human peripheral blood cells into SCID mice.

Material and Methods

Animals

CB-17 scid/scid mice were purchased from Shanghai Laboratory Animal Center, Chinese Academy of Sciences. All mice were maintained under specific pathogen-free conditions by housing in a flexible film, positive pressure isolator. Only nonleakage female mice (i.e., less than 1 μg/ml of total murine serum immunoglobulin by enzyme-linked immunosorbent assay), aged four to six weeks, were used for the experiment.

Tumor cells

The human cervical cancer cell line SiHa was originally derived from ATCC. The established cell line was maintained in vitro in RPMI 1640 medium, supplemented with 10% (v/v) fetal calf serum (FCS) and 1% (v/v) of a standard penicillin/streptomycin solution. Culture was maintained at 37°C in a fully humidified atmosphere containing 5% CO_2. SiHa cells were harvested by trypsinization and washed twice with Hanks buffer solution, and the final concentration suspended in PBS was designed to be $5x10^7$/ml for transplantation.

Human peripheral blood lymphocytes (PBLs)

Briefly, peripheral venous blood was obtained from a healthy female donor and PBLs were isolated by centrifugation over Histopaque 1.077 (Sigma, Poole, UK). The final concentration suspended in PBS was designed to be $8x10^7$/ml. PBLs ($4.0x10^6$ per mouse) were intraperitoneally transplanted into SCID mice for reconstruction of the human immune system [19].

Grouping

Thirty-two nonleakage female CB-17 scid/scid mice were randomly divided into four groups. PSB-SCID (5 mice): 0.2 ml PBS were subcutaneously transplanted into two sides of the axilla and back, as a control group. Hu-PBL-SCID (5 mice): $4.0x10^7$ PBLs in 0.5 ml sterile PBS were intraperitoneally transplanted, as a humanized group. SiHa-SCID (11 mice): $1.0x10^7$ SiHa cells in 0.2 ml sterile PBS per each site were subcutaneously transplanted into two sides of the axilla and back, as a transplantation group. Hu-PBL-SiHa-SCID (11 mice): PBLs were intraperitoneally transplanted, and SiHa cells subcutaneously transplanted after 24 hours of PBL transplantation, as a humanized transplantation group. SiHa-SCID and Hu-PBL-SiHa-SCID were further randomly divided into two groups: in group one (6 mice) the mice were executed on the 90th day by extirpating the eyeballs to detect the immunological status, and in group two (5 mice) to observe the biological features.

Cell kinetics

After transplantation, the tumors were measured in two dimensions (length **a (mm)** and breadth **b (mm)**) by calipers every five days. Tumor volume (v) was calculated according to the formula: $\mathbf{v\ (mm^3)} = 0.4 \times \mathbf{ab^2}$.

Biological features

General aspect: Psychosis, activity, appetite, body weight, nutritional status, color of fur, and growth of transplanted tumor were examined every days.

Gross examination: SCID mice were killed and anatomized on the 90th day. The shape, texture and activity of the transplanted tumor, and metastases to the liver, intestine, spleen, lung, kidney, ovary, fallopian tuber, uterus and omentum were examined. The diameter of transplanted tumor was measured.

Survival time: SCID mice in two groups (5 mice) of SiHa-SCID and Hu-PBL-SiHa-SCID were raised until they spontaneously died.

Histological examination: The tissues were fixed in 10% formalin solution, embedded in paraffin, cut serially, stained with hematoxylin/eosin, and examined under the microscope.

Primary cell culture of transplanted tumor: The tumor tissues were cut into pieces of about 0.5 cm in diameter and trypsinized. Cells were cultured in RPMI 1640 medium supplemented with 10% (v/v) fetal calf serum (FCS) and 1% (v/v) of a standard penicillin/streptomycin solution. Culture was maintained at 37°C in a fully humidified atmosphere containing 5% CO_2. Cultured cells were observed by inverted phase contrast microscope, and HE stained cells were observed microscopically.

HPV DNA detection by polymerase chain reaction (PCR)

Primers HPV16-E6-E7: upstream: 5'-CAGTTACTGCGACGTGAGGT-3', downstream: 5'-CTCCTCCTCTGAGCTGTCAT-3' (product length 458 bp). Primers HPV16-L1: upstream: 5'-GCCTAGTGAGGCCACTGTCT-3', downstream: 5'-ATGGCTGACCACGACCT ACC-3' (product length 323 bp) were designed by primer designer software referring to the whole sequence of HPV16 and synthesized by Shanghai Sangon Biological Engineering & Technology Service Co. Ltd.

PCR reation: 1) HPV16 DNA in peripheral blood: DNA was prepared for PCR according to the UNIQ-10 virus DNA extractive kit (Sangon, Shanghai). Each PCR reaction mixture (50 μl) contained 10 μl template, 10 x PCR buffer 5 μl, 25 mM $MgCl_2$ 4 μl, 10 mM dNTP Mix 1 μl, 5 u/μl Taq DNA polymerase 0.3 μl (1.5U), and 10 μmol/l primers each 1 μl. SiHa cell template was used as a positive control, ovarian carcinoma $SKOV_3$ cells

template as a negative control, and sterile water as a blank control. 2) HPV16 DNA in translanted tumor and organ tissues: hydroxybenzene and chloroform were used to extract DNA as templates. Each PCR reaction mixture (50 μl) contained 1 μl template. The content of PCR reaction mixture was the same as above. Reaction parameter: 94°C 5 min, 94°C 1 min, 52°C 1 min, 72°C 1 min, 35 cycles, 72°C 10 min. All PCR products were separated by electrophoresis on a 2% agarose gel and visualized by ethidium bromide fluorescence.

Immunological determination

Detection of human immunoglobulin in blood plasma: 200 μl peripheral blood of each SCID mouse was obtained by cutting the tail every five days and on the 90th day. Human immunoglobulin levels were determined by magnetism-enzyme-linked assay.

Detection of human CD3+, CD4+, CD8+ T cells: After SCID mice were killed, the spleens were immediately obtained and dissociated into single-cell suspension using wire mesh under sterile conditions. Spleen cells and peripheral blood lymphocytes were analyzed by indirect immunofluorescence flow cytometry using biotinylated mouse monoclonal antibodies specific to human lymphocyte surface markers (CD3, CD4 and CD8) in conjunction with streaptavidin-fluorescein isothiocyanate (FITC) conjugate (Caltaglab, American).

Detection of tumor infiltrating hymphocytes (TILs): Transplantated tumor tissues were stained with hematoxylin/eosin, and TILs were counted under microscope. The human CD4+ T cells in spleen and tumor tissue were detected by SP immunohistochemistry. Antibodies were purchased from Neomarkers and the staining procedure followed the manufacturer's instructions.

Spleen weight: The spleen of each SCID mouse was weighed when it was sacrificed.

CTL assay: LDH release assay was used. K_{562} cells (1.0 x 10^4/well), as target cells, were cultured for 24-48 hours. Effector cells were spleen cells from SCID mice in SiHa-SCID and Hu-PBL-SiHa-SCID. The effector: target cell ratios were 50:1 and 100:1. Incubation time was four hours at 37°C, 5% CO_2. Citric acid acted as a stop solution and he absorbance was at 490 nm wavelength. Cytotoxicity (%)=$(A_{Experimental}-A_{Target\ Spontaneous})/(A_{Target\ Maximum}-A_{Target\ Spontaneous})$ x 100%.

Diagnosis of graft-versus-host disease (GVHD): Changes in weight, skin rash, ruffled fur, or diarrhea of humanized mice were observed. Lymphocyte infiltration in the liver, lung and intestine was histologically examined. The diagnostic criteria of GVHD were according to the literature [20].

Results

Latent period and rate of tumorigenecity

The latent periods of tumorigenecity in SiHa-SCID and Hu-PBL-SiHa-SCID mice were 15 to 18 days, with no significant difference. The rates of tumorigenecity in both groups were 100%.

General aspect of the tumor-bearing mice

The general aspect of Hu-PBL-SiHa-SCID and SiHa-SCID mice was similar. Briefly, the transplanted tumor grew slowly during the latent period. Thereafter it could be palpated with an irregular tubercular and movable appearance. After 50 days, the growth of the tumor was remarkably rapid. The local surface skin presented red, ruptured or scabbed. After 80 days, the tumor tubercle became fixed. The spirit of the tumor-bearing mice was sagged with a slow moving, dull looking, and blunt response. The color of the fur was bleak. Some of them presented hematuria and thinness. There was no significant difference in the body weight between, before, and after tumor transplantation. However, the growth of transplanted tumor was significantly slower and the mean diameter of the tumor significantly smaller in Hu-PBL-SiHa-SCID mice than those in SiHa-SCID mice (Figures 1 and 2).

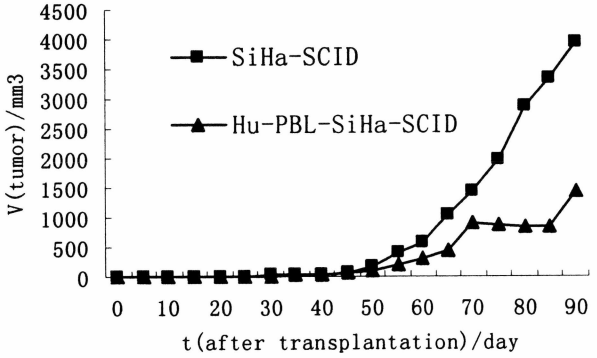

Figure 1. — Growth curve of transplanted tumor in SCID mice.

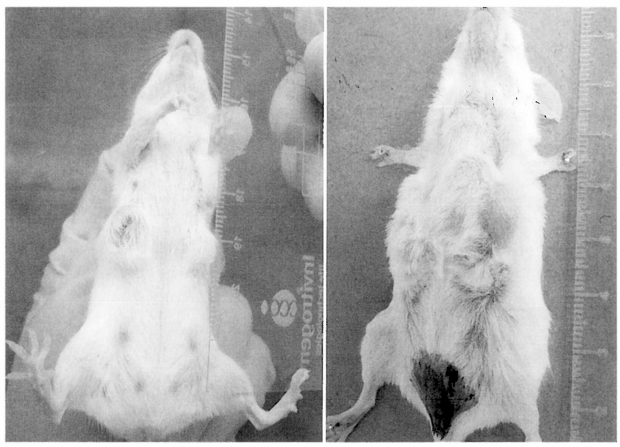

Figure 2. — Gross appearance of the tumor-bearing mice. The tumor (arrow) in the SiHa-SCID mouse was bigger than that in the Hu-PBL-SiHa-SCID mouse (left: Hu-PBL-SiHa-SCID mouse; right: SiHa-SCID mouse).

Survival time

The mean survival time in the Hu-PBL-SiHa-SCID group was longer than that in the SiHa-SCID group (136.2 ± 6.3 days vs 97.8 ± 3.7 days, $t = 11.75$, $p = 0.000$).

Gross and microscopic appearance

The transplanted tumor presented a hard texture and intact capsule with abundant surface blood vessels. Some of them appeared colliquative and necrosed. The tumor projected toward the abdominal cavity and adhered with the peritoneum, but no metastasis including liver, intestine, spleen, lung, kidney, uterus, ovary, fallopian tuber, omentum, and no ascites were found. The tumor tissues in Hu-PBL-SiHa-SCID and SiHa-SCID mice presented typical squamous cell carcinoma under microscope, but cell grade in Hu-PBL-SiHa-SCID mice was significantly lower than that in SiHa-SCID mice (Figure 3).

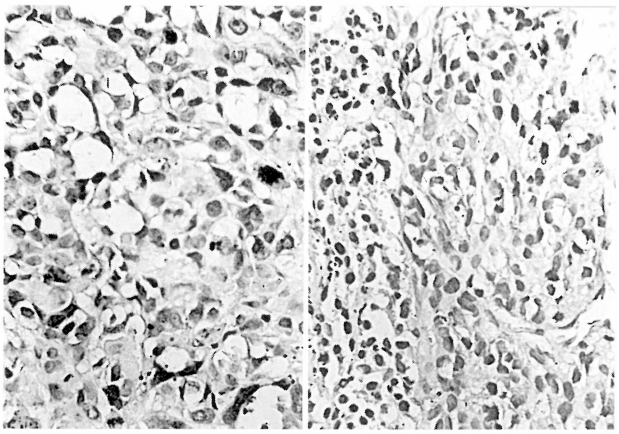

Figure 3. — Histological appearance of transplanted tumor (x 400, HE). Left: SiHa-SCID mouse; Right: Hu-PBL-SiHa-SCID mouse.

HPV-16 virus detection

HPV-16 DNA could be detected in all the transplanted tumor tissues, but not in the peripheral blood and organ tissues including the liver, intestine, spleen, lung, kidney, uterus, ovary, fallopian tuber, and omentum (Figures 4 and 5).

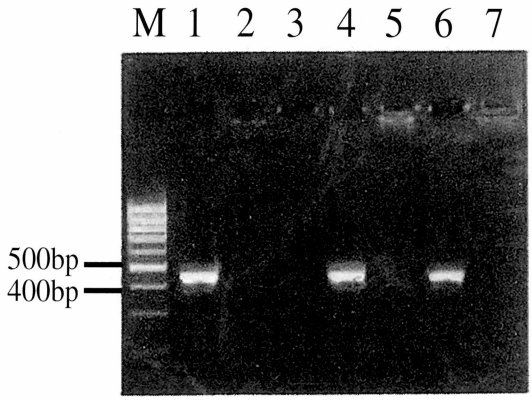

Figure 4. — Detection of HPV16 E6E7 in tumor tissue by PCR. M. DNA marker; 1. Positive control: 458bp; 2. Negative control; 3. Vacant control; 4. Group SiHa; 5. Group Hu-PBL; 6. Group Hu-PBL-SiHa; 7. Group PBS.

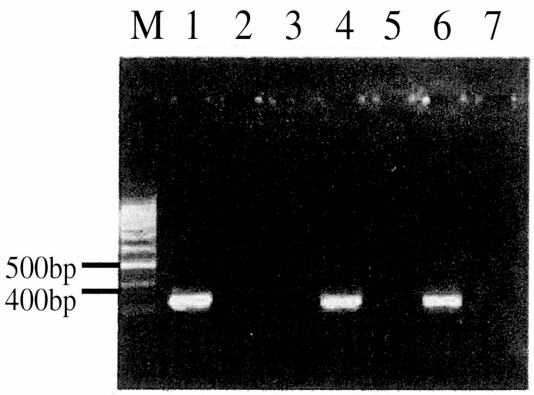

Figure 5. — Detection of HPV16 L1 in tumor tissue by PCR. M. DNA marker; 1. Positive control: 323bp; 2. Negative control; 3. Vacant control; 4. Group SiHa; 5. Group Hu-PBL; 6. Group Hu-PBL-SiHa; 7. Group PBS.

Primary cell culture of transplanted tumor

Cultured cells from transplanted tumors did not present morphologic changes via HE staining when compared with the non-transplanted cells (Figure 6).

Human serum IgG levels

The level of human serum IgG was gradually elevated in all the humanized SCID mice following the time of PBL transplantation prolonging, and was significantly higher than that in non-humanized mice ($p < 0.05$). Furthermore, the human serum IgG level in the Hu-PBL-SiHa-SCID group was significantly higher than that in the Hu-PBL-SCID group, while only very low levels of human serum IgG were detected in the SiHa-SCID and PBS-SCID groups with no significant difference. Although the level of human serum IgG in the humanized group was significantly decreased on the 90th day, it could still be detected, and was significantly higher compared with the non-humanized group (Table 1).

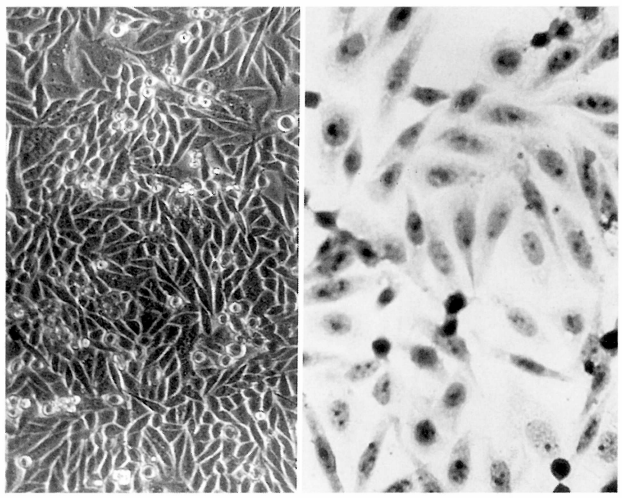

Figure 6. — Primary cell culture (x 400). Left: cultured live cell; Right: HE staining.

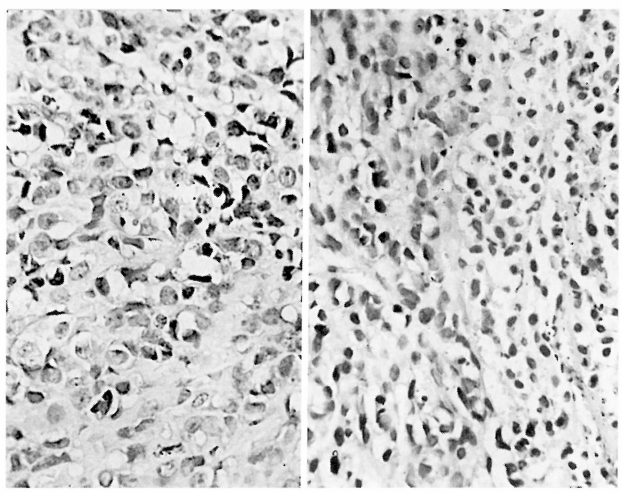

Figure 7. — TILs in tumor tissue (x 400, HE). Left: No TILs observed in SiHa-SCID. Right: obvious TILs (arrow) observed in Hu-PBL-SiHa-SCID.

Table 1. — *Human serum IgG levels in SCID mice (μg/ml, $\bar{X}$ ± S).*

Group	n	Day 0	Day 5	Day 10	Day 15	Day 20	Day 25	Day 30	Day 90
PBS	5	0.19± 0.12	0.20± 0.11	0.20± 0.12	0.24± 0.13	0.27± 0.14	0.27± 0.15	0.23± 0.12	0.26± 0.07
Hu-PBL-SCID	5	0.18± 0.12	0.98± 0.20$^{\Delta}$	95.32± 16.40$^{\Delta}$	282.85± 46.96$^{\Delta}$	465.36± 63.13$^{\Delta}$	689.42± 92.17$^{\Delta}$	851.74± 97.95$^{\Delta}$	256.74± 58.57$^{\Delta}$
SiHa-SCID	11	0.17± 0.11	0.21± 0.12$^{\blacktriangle}$	0.22± 0.11$^{\blacktriangle}$	0.23± 0.12$^{\blacktriangle}$	0.25± 0.15$^{\blacktriangle}$	0.23± 0.14$^{\blacktriangle}$	0.24± 0.15$^{\blacktriangle}$	0.24± 0.06$^{\blacktriangle}$
Hu-PBL-SiHa-SCID	11	0.17± 0.12	1.39± 0.25$^{\bullet\star}$	345.02± 31.12$^{\bullet\star}$	589.77± 56.88$^{\bullet\star}$	1166.68± 243.03$^{\bullet\star}$	2049.71± 468.21$^{\bullet\star}$	2651.04± 460.91$^{\bullet\star}$	1225.86± 306.83$^{\bullet\star}$

Day 5, 10, 15, 20, 25, 30, 90 $^{\Delta}$compared with PBS, t = 7.655, 12.970, 13.457, 16.473, 16.719, 19.438, 9.792, p = 0.000, 0.000, 0.000, 0.000, 0.000, 0.000, 0.001; $^{\blacktriangle}$compared with PBS, all $p \geq 0.05$; $^{\bullet}$compared with PBS, t = 9.937, 36.745, 34.378, 15.918, 14.517, 19.075, 13.248, all p = 0.000; *compared with Hu-PBL, t = 3.200, 16.698, 10.493, 8.931, 6.325, 12.348, 10.079, p = 0.006, 0.000, 0.000, 0.000, 0.000, 0.000, 0.000.

Human CD3+, CD4+ and CD8+ T cells

Human CD3$^+$, CD4$^+$ and CD8$^+$ T cells were detectable in pheripheral blood and spleen tissues of both Hu-PBL-SCID and Hu-PBL-SiHa-SCID mice, but not of PBS-SCID and SiHa-SCID mice. Furthermore, all the human T cells in Hu-PBL-SiHa-SCID mice were significantly higher than those in Hu-PBL-SCID mice (Table 2).

Table 2. — *Human CD3$^+$, CD4$^+$, CD8$^+$ T cell of SCID mice (median IQR).*

Group		CD3$^+$	CD4$^+$	CD8$^+$
Hu-PBL	Peripheral blood	0.90 (0.64)	0.51 (0.88)	0.45 (0.79)
	Spleen	0.69 (0.27)	0.13 (0.04)	0.17 (0.09)
Hu-PBL-SiHa	Peripheral blood	4.12 (0.45)$^{\Delta}$	2.39 (1.15)$^{\Delta}$	1.49 (0.38)$^{\Delta}$
	Spleen	1.35 (0.22)$^{\blacktriangle}$	0.26 (0.05)$^{\blacktriangle}$	0.90 (0.17)$^{\blacktriangle}$

$^{\Delta}$CD3$^+$, CD4$^+$, CD8$^+$ in Hu-PBL-SiHa compared with those in Hu-PBL of peripheral blood, using the Mann-Whitney test, z = 2.745, 2.739, 2.739; p = 0.006, 0.006, 0.006; $^{\blacktriangle}$CD3$^+$, CD4$^+$, CD8$^+$ compared with Hu-PBL in the spleen, using the Mann-Whitney test, z = 2.739, 2.751, 2.745; p = 0.006, 0.006, 0.006.

Human tumor infiltrating lymphocytes (TILs)

Human TILs and CD4$^+$ T cells in transplanted tumor tissues were more obvious in the Hu-PBL-SiHa-SCID group than those in the SiHa-SCID group (Figures 7 and 8).

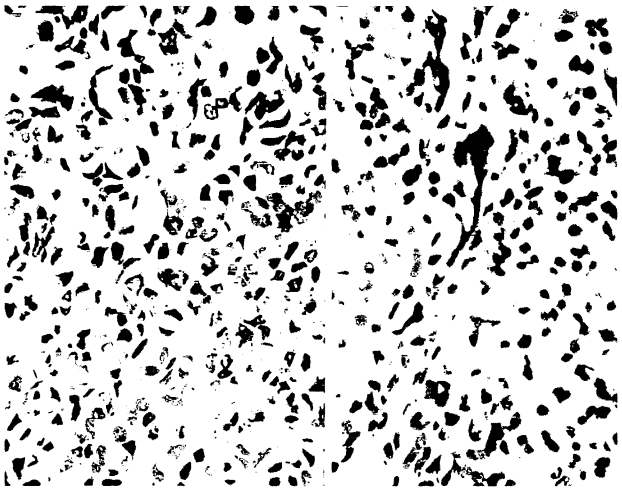

Figure 8. — Human CD4+ T cells (x 400, SP). Left: No human CD4+ T cells were observed in tumor tissue of SiHa-SCID. Right: The membrane of human CD4+ T cells stained yellow were observed in Hu-PBL-SiHa-SCID.

Human CD4+ T cells in spleen tissue

Human CD4$^+$ T cells in spleen tissues were observed in all the humanized SCID mice, but not in non-humanized SCID mice (Figure 9).

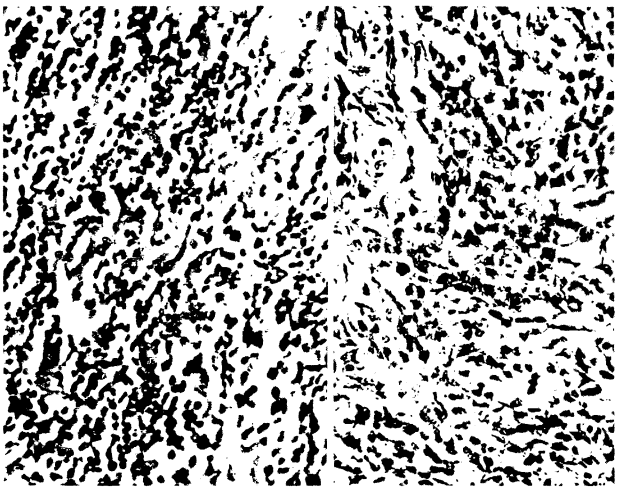

Figure 9. — Human CD4+ T in spleen tissue (x 400, SP). Left: Group PBS. Right: Human CD4+ T cells stained yellow were observed in Group Hu-PBL.

Spleen weight

Spleen weight in SiHa-SCID, Hu-PBL-SCID and Hu-PBL-SiHa-SCID mice was significantly higher than that in the PBS-SCID group when mice were sacrificed on the 90th day. Furthermore, spleen weight in Hu-PBL-SiHa-SCID mice was significantly higher than that in Hu-PBL-SCID mice (Table 3).

Cytotoxicity of spleen cells

Cytotoxicity of spleen cells in the Hu-PBL-SiHa-SCID group was significantly increased compared to the Hu-PBL-SCID and SiHa-SCID groups (Table 4).

Table 3. — *Mean weight of spleens in SCID mice (mg, $\bar{X}$±S).*

Group	PBS	Hu-PBL	SiHa	Hu-PBL-SiHa
Weight	50.44±7.61	96.80±8.73$^{\Delta}$	266.07±43.89$^{\blacktriangle}$	460.18±56.78$^{\bullet\star}$

$^{\Delta}$compared with PBS group, t = 8.949, p = 0.000; $^{\blacktriangle}$compared with PBS, using modified t test, t' = 11.823, p = 0.000; $^{\bullet}$compared with PBS, t' = 17.488, p = 0.000; *compared with Hu-PBL, t' = 15.458, p = 0.000.

Table 4. — *Cytotoxicity of spleen cells of SCID mice (%, $\bar{X}\pm S$).*

Effector/target rate	Hu-PBL	SiHa	Hu-PBL-SiHa
50:1	1.48% ± 0.79%	2.52% ± 0.81%	8.16% ± 1.11%$^{\Delta\blacktriangle}$
100:1	3.52% ± 1.23%	4.48% ± 1.21%	13.56% ± 2.14%$^{\bullet\star}$

$^{\Delta}$compared with Hu-PBL, t = 11.289, p = 0.000; $^{\blacktriangle}$compared with SiHa, t = 10.053, p = 0.000; $^{\bullet}$compared with Hu-PBL, t = 9.235, p = 0.000; *compared with Hu-PBL, t = 9.029, p = 0.000.

GVHD

No clinical presentation of GVHD was found in any humanized mice, such as hunched back, ruffled fur, thinness, or diarrhea, and no histological appearance was observed, such as lymphocyte infiltration into the liver and intestinal crypts.

Discussion

Squamous carcinoma of the human cervix usually originates at the squamocolumnar junction. The lesion is frequently associated with carcinoma in situ, usually progressing over 10-20 years. It is generally acepted that carcinoma in situ precedes invasive carcinoma of the cervix. The basement membrane is considered to be a natural barrier that prevents carcinoma cells from invading the cervical stroma. In case the malignant process breaks through the basement membrane, invasion may progress. The spread of invasive carcinoma is also a slow progress. It may first spread to the adjacent vaginal fornices or to the parametrial tissues. Dissemination usually follows an orderly sequence. Regional lymphatic or hematogenous spread may occur, but only after local metastasis.

The SCID mice model established in our study perfectly simulated the natural process of human cervical carcinoma. The tumorigenecity was 100% through subcutaneous injection of HPV-16 positive SiHa cells into the mice. The cytological appearance appeared to be unchanged before and after transplantation, indicating that cellular morphologic features, probably as well as biological behavior, were not affected by the transplantation process. The growth and spread of transplanted tumor was shown to be slow. The latent period of tumorigenecity was as long as 15-18 days and the grossly palpable tumor occurred on the 50th day of transplantation. The tumor capsule remained intact and did not break through the peritoneum. No ascites, intraperitoneal and hematogenous metastases were found. HPV-16 DNA could not be detected in any of the peripheral blood and organ tissue including the liver, intestine, spleen, lungh, kidney, ovary, fallopian tube, uterus and omentum.

The development of cervical cancer is associated with host immunosuppression. Research on host immune status facilities understanding the development and progress of the disease. The SCID mice, in which we reconstructed human immune function, provided an available carrier for this kind of research. In our experiments, human T3, T4 and T8 cells could be detected in peripheral blood and spleen tissues of SCID mice after they were intraperitoneally injected into the mice. The level of human IgG in peripheral blood could be detected on the fifth day after human PBL transplantation, gradually elevated thereafter and maintained to the 90th day. It was reported that spleen weight is one of the signs for immune reconstruction because of infiltration of human PBLs in the spleen [21]. The spleen weight of the humanized mice in the study was also significantly increaseed when compared with non-humanized mice. Our findings indicate that human immunity is able to be reconstructed in SCID mice via intraperitoneal inoculation of human PBLs. It is generally accepted that GVHD of the recipient may occur when the xenograft is immune cells [22]. However, no clinical presentation or histological appearance was found in any of the humanized SCID mice in the study according to the diagnostic criteria [20], suggesting that numbers and concentration of inoculated immune cells might be available in our experiment.

In addition to the above results, our findings also showed that the number of T cells and the level of human IgG in Hu-PBL-SiHa-SCID mice were significantly higher than those in SiHa-SCID mice. TILs could be obviously observed in Hu-PBL-SiHa-SCID mice, but not in non-humanized SiHa-SCID mice. CTL assay presented increased cytotoxicity of spleen cells in Hu-PBL-SiHa-SCID mice. Analysis on survival time showed to be prolonged in Hu-PBL-SiHa-SCID mice, with slower growth and smaller size of transplanted tumors. The results suggest that transplanted human tumor cells activate the reconstructed human immune system and initiate anti-tumor immune response, therefore tumor growth is declined and survival time of the host is prolonged. Taking our results altogether, an ideal animal model of cervical cancer can be established via transplantating HPV-16 positive SiHa cells into SCID mice. This model can perfectly simulate the biological features of spontaneous human cervical cancer, and present an anti-tumor immune response after the human immune system is reconstructed.

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