Clin Exp Immunol 1995; 101:494–501

# The development of a novel immunotherapy model of human ovarian cancer in human PBL-severe combined immunodeficient (SCID) mice

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(*Accepted for publication 5 May 1995*)


## SUMMARY

The reported ability of SCID mice to accept xenografts of both human tumours and peripheral blood lymphocytes (PBL) provides the potential for the development of novel immunotherapy models in these animals. This study describes the development of a novel small animal model of human ovarian cancer. This was achieved by engrafting a human ovarian cancer cell line (Ovan-4) into the peritoneal cavity of immunodeficient SCID and immune reconstituted human PBL-SCID mice. When transplanted to SCID mice this cell line exhibited growth characteristics similar to the clinical disease observed in patients with implantation of metastatic nodules onto the interior surface of the peritoneal wall. Reconstituted human PBL-SCID mice challenged with identical numbers of Ovan-4 cells exhibited a significant increase in survival time, suggesting a role for cells of the human immune system in preventing the development of this type of malignancy *in vivo*. Furthermore, vaccination of human PBL-SCID mice against Ovan-4 produced tumour-specific human antibodies in the serum of these animals. Animals reconstituted with CD8-depleted PBL exhibited increased serum immunoglobulin levels and produced enhanced anti-Ovan-4 activity after vaccination. Subsequent challenge of these animals with Ovan-4 revealed a further increase in survival time. These results suggest that human antibodies may have a role in immunity against ovarian cancer and could be of therapeutic value in this type of disease.


**Keywords** human PBL-SCID mice ovarian cancer vaccination

## INTRODUCTION

SCID mice are profoundly lacking in functional, mature T and B lymphocytes [1–3] and it has been demonstrated that these animals readily accept xenografts, leading to the development of the SCID-human mouse [4]. As these animals readily accept grafts of both human lymphocytes to create human PBL-SCID mice [5] and human tumour cells [6], engraftment of both these populations to the same animal provides an *in vivo* model for analysing immune effector mechanisms active against human malignant disease.

In particular, this study addressed human ovarian cancer, as a small animal model of this type of malignancy had already been developed [7]. This model essentially involves the growth of human ovarian tumour xenografts and cell lines in the peritoneal cavity of immunodeficient athymic 'nude' mice, and has been useful for the pre-clinical testing of new therapies [8,9]. However, problems reported with this model include growth of xenografts as ascitic rather than solid peritoneal tumours which is the pattern more commonly observed in the human disease [7]. Given these deficiencies with the nude mouse model and the fact that a poor prognosis is still associated with this disease (i.e. 5 year survival rate of only 22% due to the difficulty in achieving early diagnosis) [10], we endeavoured to develop a novel *in vivo* model of this disease which would be more representative in terms of growth characteristics by propagating ovarian tumour cells in the peritoneal cavity of SCID mice. To assess the role of human lymphocytes in the pathophysiology of this disease we also transplanted ovarian tumour cells to immune reconstituted human PBL-SCID mice.

As there is considerable interest in developing immunotherapeutic treatments of malignant disease [11,12] and as we have previously shown that antigen-specific humoral immune responses can be induced in human PBL-SCID mice by employing a suitable adjuvant system and manipulation of the T cell subset populations within these animals [13], we employed similar methodology in this study in an attempt to vaccinate reconstituted animals against the ovarian (Ovan-4) tumour. Human PBL-SCID mice were immunized with Ovan-4 membranes entrapped within liposomes as an immunological adjuvant [14] and screened for the presence of serum anti-Ovan-4 human antibodies. Furthermore, the effectiveness of this procedure in inducing an anti-tumour immune response in

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these animals was verified by comparing the survival of vaccinated animals with non-immunized human PBL-SCID mice after transfer of identical numbers of viable tumour cells.

## MATERIALS AND METHODS

*Cell culture*

All tissue culture media and reagents were purchased from GIBCO (Paisley, UK). The human ovarian cell line Ovan-4 was originally derived in our laboratory from a freshly resected tumour specimen subsequently diagnosed histologically as a very poorly differentiated adenocarcinoma of the ovary. The established cell line was maintained *in vitro* in 75-$cm^2$ flasks in RPMI 1640 medium containing 2 mM glutamine, supplemented with 10% (v/v) fetal calf serum (FCS) and 1% (v/v) of a standard penicillin/streptomycin solution. Cultures were maintained at 37°C in a fully humidified atmosphere containing 5% $CO_2$. Ovan-4 grows as an adherent monolayer, and cultures approaching 90% confluence were harvested with 0·02% (w/v) EDTA in 10 mM PBS (138 mM NaCl, 2·7 mM KCl) pH 7·2 and subcultured or used as described. All tumour cell suspensions were >95% viable as determined by trypan blue exclusion before i.p. inoculation.

*Animals*

C.B.17 SCID mice were maintained in pathogen-free conditions by housing in flexible film, positive pressure isolators (Moredun Isolators, Edinburgh, UK) in the University of Glasgow Animal Facility, Glasgow Royal Infirmary. Breeding pairs of SCID mice were initially obtained from B & K Universal (Hull, UK). Only non-leaky (i.e. total murine serum immunoglobulin of less than 1 $\mu$g/ml by ELISA), adult female SCID mice were used in experiments. Groups (eight per group) of female SCID and human PBL-SCID mice were inoculated intraperitoneally with 0·5 ml of tumour cell suspension in sterile PBS ($10^6$ cells). All animals receiving tumour cells intraperitoneally were monitored on a daily basis for tumour burden, abdominal distension, weight loss, cachexia or other abnormality. Once these life-threatening symptoms became markedly manifest or if weight loss of the animal was >10% of total body weight, the animal was killed by cervical dislocation.

*Creation of human PBL-SCID mice*

SCID mice were reconstituted with human PBL as previously described [13]. Briefly, venous blood was obtained from a single normal donor and PBL isolated by centrifugation over Histopaque 1077 (Sigma, Poole, UK). PBL ($40 \times 10^6$ per mouse) were injected intraperitoneally into SCID mice (eight per group).

Cell suspensions depleted of CD8-expressing lymphocytes were prepared as previously described [13] by employing a MACS cell separator [15] and high gradient separation column in conjunction with anti-Leu-2a (CD8) magnetic microbeads (Miltenyl Biotec, Berglsch-Gladbach, Germany). Briefly, $10^8$ mononuclear cells were treated with 20 $\mu$l MACS CD8 microbeads and incubated at 4°C for 20 min. After incubation this mixture was then applied to the top of a separation column and washed with sterile PBS/1% bovine serum albumin (BSA). Cells eluted from the bottom of the column were treated as the $CD8^-$ fraction and those retained in the column as the $CD8^+$ fraction. $CD8^+$ cells were recovered from the column by eluting outside the magnetic field of the MACS separator by vigorous washing with PBS/1% BSA. Purity of cell fractions was confirmed by flow cytometric analysis before transfer, i.e. lymphocytes were analysed by indirect immunofluorescence staining using biotinylated mouse MoAbs specific for human lymphocyte surface markers (CD3, CD4, CD8, CD19, CD45RB) in conjunction with streptavidin–FITC conjugate (Dako Ltd., High Wycombe, UK). Analysis of the CD8dL population consistently revealed <5% detectable CD8-expressing lymphocytes, whereas the CD8-enriched fraction consisted of >95% $CD8^+$. CD8dL ($40 \times 10^6$ per SCID mouse) were injected (eight mice per group). As a control group, purified $CD8^+$ cells were added back to the reconstituting CD8-depleted fraction before transfer, i.e. $30 \times 10^6$ CD8dL were mixed with $10 \times 10^6$ purified $CD8^+$ cells and adjusted to a volume of 0·5 ml in PBS before i.p. inoculation.

*Preparation of Ovan-4 membrane antigen*

Cell membranes were prepared by treating confluent flasks of Ovan-4 cells with 0·02% EDTA/PBS to remove attached cells, washed in PBS and pelleted by centrifugation. Lysis buffer (1 ml; 0·15 M NaCl, 0·01 M Tris, 0·01 M $MgCl_2$, 0·001 M phenylmethylsulfonyl fluoride (in ethanol), 2 U/ml aprotinin, 0·05% octylglucoside pH 7·2) was added to each 0·1 ml of cell pellet and incubated at 20°C for 15 min followed by centriguation at 100 000 *g* for 60 min. The protein concentration in the supernatant was determined by the method of Bradford [16] before entrapment of antigen within liposomes. Liposomes were prepared according to the method previously described by Van Rooijen [17]. Briefly, 75 mg of phosphatidylcholine and 11 mg of cholesterol were dissolved in 20 ml chloroform in a 500-ml round-bottomed flask. The organic phase was removed by low vacuum rotary evaporation and the resulting lipid film that forms on the interior of the flask after evaporation was dispersed in 10 ml sterile PBS containing 20 mg Ovan-4 membrane antigen. The suspension was maintained at room temperature for 2 h, sonicated for 3 min and maintained for a further 2 h at room temperature to allow liposome swelling. The liposomes were then washed twice in sterile PBS to remove any untrapped antigen by centrifuging at 100 000 *g* for 40 min at 16°C. The method of Bradford was used to assay entrapped protein content after lysis of liposomes with propanol.

*Immunization schedule*

Two days after immune reconstitution animals were vaccinated intraperitoneally with 100 $\mu$g Ovan-4 membrane antigen entrapped within liposomes. All animals received booster doses of antigen 14 and 28 days later, respectively. At 42 days post-reconstitution animals were challenged intraperitoneally with $10^6$ Ovan-4 cells as previously described.

*Determination of human immunoglobulin levels in human PBL-SCID mice*

Serum samples from individual mice were screened for the presence of human immunoglobulins using a standard sandwich ELISA as previously described [13]. All antibodies and reagents used in this assay were purchased from Sigma. Briefly, immulon 3 microtitre plates (Dynatech Laboratories Ltd., Billingshurst, UK) were coated with goat anti-human polyva-

lent immunoglobulin (I9631) at 20 $\mu$g/ml by overnight incubation at 4°C, washed with PBS/0·05% Tween-20 three times, followed by a blocking step employing 1% BSA in PBS (1 h 37°C). The plates were then incubated with diluted serum samples (1:20, in triplicate wells) for 2 h at 37°C, washed with PBS/0·05% Tween-20 and then incubated with either alkaline phosphatase-conjugated goat anti-human:polyvalent immunoglobulin ($\alpha$, $\gamma$, $\mu$ chain specific; A5034) IgG ($\gamma$ chain specific, A3150) or IgM ($\mu$ chain specific, A9794)). Samples of purified human immunoglobulins (Sigma) were included in the assay for construction of the standard curve. After further washing, 100 $\mu$l of *p*-nitrophenyl-phosphate (1 mg/ml in 0·1 M diethanolamine pH 10·6) were added for colour development. Absorbance at 405 nm was measured using a BioRad 450 microplate reader (BioRad, Hemel Hempstead, UK) and serum immunoglobulin concentrations determined from the standard curve.

*Determination of antigen-specific antibody*

Microtitre plates were coated with Ovan-4 cell membrane preparation (2·5 $\mu$g/well) overnight at 4°C. Following blocking with 1% BSA/PBS, plates were incubated with diluted (1:100) serum samples for 2 h at 37°C. Specific, bound IgG and IgM was detected with the appropriate conjugated antibody using the same ELISA protocol as described above. Samples were assayed in triplicate and expressed as absorbance units (OD 405 nm). Tissue culture supernatants were harvested and used in the ELISA protocol undiluted. Supernatants from the human Epstein–Barr virus (EBV)-transformed B cell line secreting antibody (IgG) with specificity for the human ovarian cancer-associated antigen termed 14Cl [18] were employed as a negative control in the ELISA.

*Histopathology of tumours and major organs*

On dissection each animal was macroscopically examined for the presence of solid tumour and tumour nodules at the original injection site and in any other organs. Tumour tissue and all major organs (i.e. lungs, liver, kidneys, intestines, brain and ovaries) were fixed immediately in 10% buffered neutral formalin for a minimum period of 48 h. Representative blocks of tissue were then processed and embedded in paraffin wax before sectioning. Cut 4-$\mu$m sections were subsequently stained by the standard haematoxylin and eosin (H&E) method.

*Immunocytochemistry*

Frozen or paraffin sections were stained for the expression of human lymphoid cell surface markers using antibodies reactive with human CD3, IgM and EBV latent membrane protein in conjunction with the indirect immunoperoxidase staining technique. All antibodies were purchased from Dako and used at the manufacturer's recommended dilutions.

*Statistical analysis*

Human serum immunoglobulin levels expressed as means ± s.d. were compared by Student's *t*-test. Survival times of groups of tumour-bearing animals were compared by the Mann–Whitney *U*-test.

## RESULTS

*Pathology of Ovan-4 tumour growth in SCID mice*

Transfer of $1 \times 10^6$ Ovan-4 cells to the peritoneal cavity of SCID mice resulted in successful growth of solid tumour in all recipient animals. Examination of the peritoneal cavity of tumour-bearing mice revealed the tumour to be distributed generally on the peritoneal wall and mesenteric membranes. Microscopic examination of implanted Ovan-4 tumour nodules revealed that most of the tumour cells were large and undifferentiated, with large, frequently multiple nuclei, consistent with the original diagnosis of very poorly differentiated adenocarcinoma. This tumour was frequently observed attached to and invading the local musculature (Fig. 1a). In cases of advanced disease, growth was also observed on other organs such as the stomach, pancreas, intestine, kidney and liver (Fig. 1b). Growth of tumour nodules was consistently observed on the peritoneum and on the peritoneal surface of the diaphragm with tumour penetrating through the musculature to invade the subcutaneous or thoracic areas in some cases. No demonstrable blood-borne metastasis was observed outside the peritoneal cavity (i.e. tumour could not be detected by macroscopic or histological examination of other major organs), and histological examination revealed tumour nodules growing on rather than through the peritoneum.

*Survival of tumour-bearing SCID and human PBL-SCID mice*

To determine whether the presence of human lymphocytes had any effect on tumour development within these animals, we ascertained the relative survival times of human PBL-SCID and SCID mice after i.p. transfer of Ovan-4 cells. Consistent with a previous report by us [13], all reconstituted animals produced detectable levels of human immunoglobulin. These levels increased over time and remained detectable for the duration of experiments (Fig. 2). Reconstitution with $40 \times 10^6$ PBL resulted in mean serum immunoglobulin levels in excess of 12 mg/ml, whereas reconstitution with CD8dL produced significantly higher immunoglobulin levels in excess of 20 mg/ml ($P \leqslant 0{\cdot}005$, all time points). Animals reconstituted with $CD8dL^+ + CD8^+$ cells produced human immunoglobulin levels that were comparable to the PBL-reconstituted group. Ovan-4 cells were transplanted to untreated SCID and human PBL-SCID mice (42 days after reconstitution) and monitored as before for tumour development. The mean survival time of SCID mice receiving $1 \times 10^6$ Ovan-4 cells was 18 days (Fig. 3). Human PBL-SCID animals subsequently transplanted with the same number of Ovan-4 cells also developed solid tumour masses. However, there was a significant increase in the mean survival times of the SCID–human (i.e. PBL and CD8dL reconstituted) groups receiving tumour cells compared with immunodeficient animals ($P \leqslant 0{\cdot}0005$, Mann–Whitney). The mean survival times of human PBL-SCID mice receiving tumour cells was increased to 25 days (PBL) and 28 days (CD8dL) respectively. Comparison of the PBL-reconstituted group with the CD8dL group revealed a smaller although still significant difference in mean survival time ($P < 0{\cdot}025$, Mann–Whitney). This possibly reflects the enhanced engraftment of the CD8dL mice with human lymphocytes as evidenced by their significantly higher total serum immunoglobulin levels.

These results demonstrate the successful establishment of a novel model of human ovarian cancer in terms of the growth

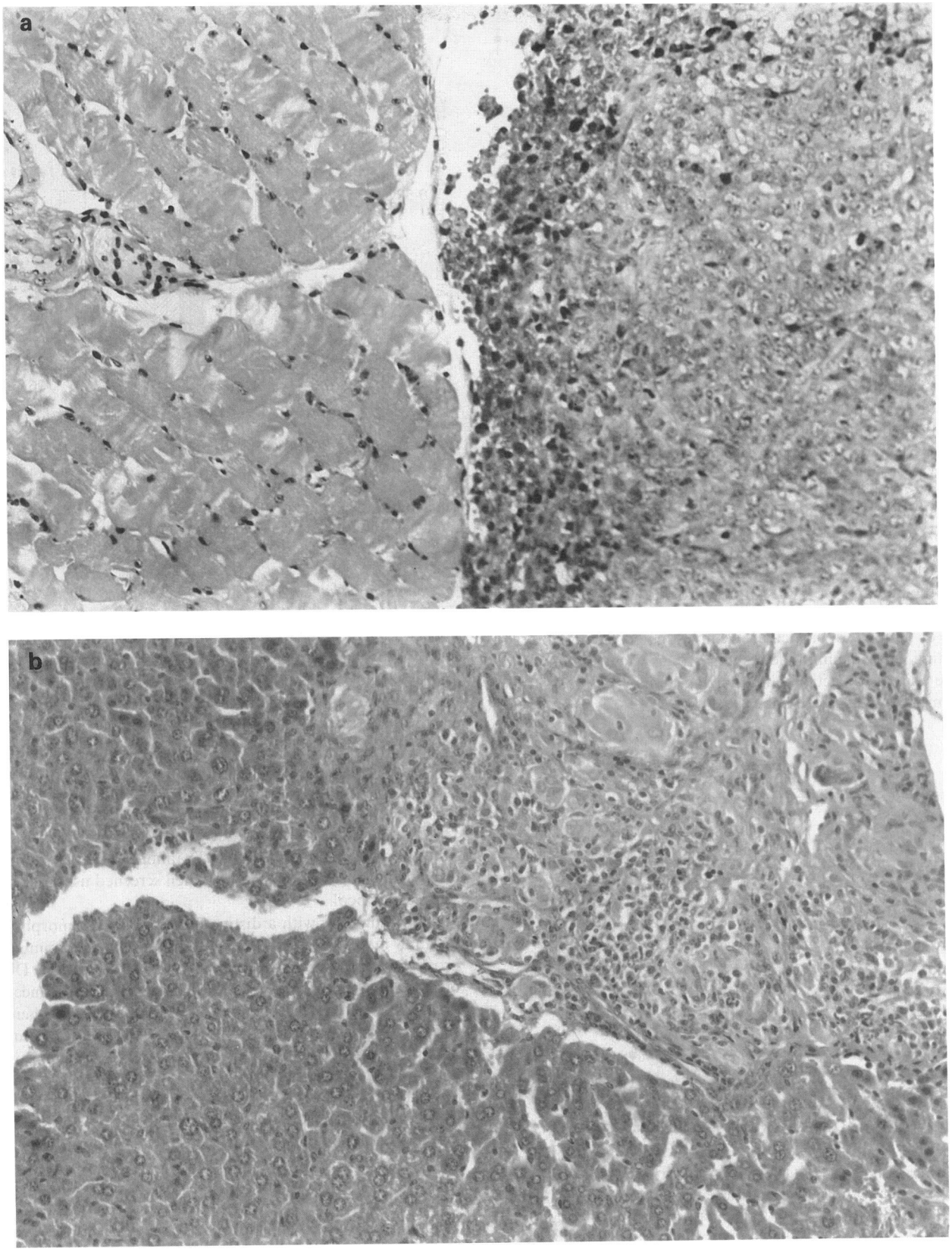


**Fig. 1.** Histopathology of human Ovan-4 tumour excised from SCID mice injected intraperitoneally with $10^6$ tumour cells 18 days previously. (a) Ovan-4 grows as a solid tumour in the peritoneal cavity and attaches to local musculature. The tumour morphology is consistent with a poorly differentiated adenocarcinoma of the ovary. (b) In advanced disease, tumour cells commonly attach to and infiltrate the liver parenchyma. (Paraffin section, H & E, × 150.)

 

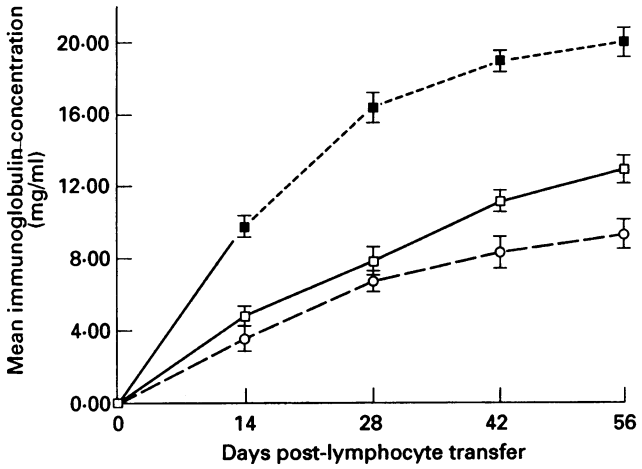


**Fig. 2.** Human immunoglobulin concentrations measured in human PBL-SCID mouse serum after reconstitution with $40 \times 10^6$ peripheral blood lymphocytes (PBL) (□), CD8dL (■) or $CD8dL^+$ CD8 cells (○). The reconstitution of SCID mice with CD8dL produced significantly higher serum immunoglobulin levels than mice reconstituted with either PBL ($P \leqslant 0{\cdot}005$) or $CD8dL^+CD8^+$-enriched lymphocytes ($P \leqslant 0{\cdot}005$). Results shown are mean immunoglobulin concentrations ±1 s.d.

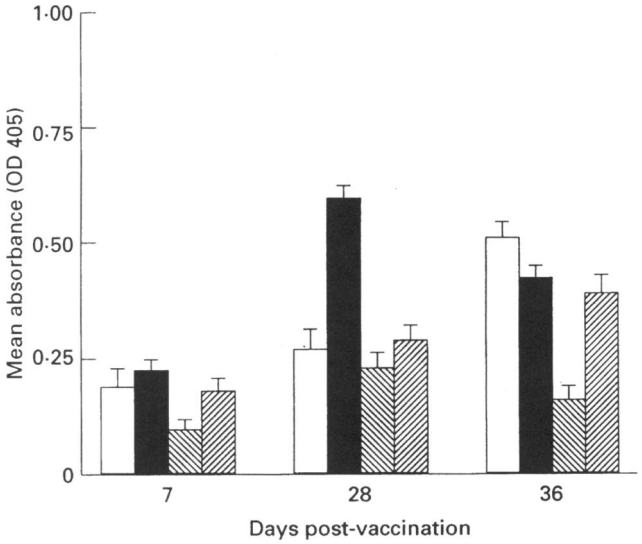


**Fig. 4.** Induction of anti-Ovan-4 antibodies by vaccination of human PBL-SCID mice. Serum samples were obtained from vaccinated animals at the indicated time points and screened for the presence of human IgG (CD8dL, □; PBL, ▨) and IgM (Cd8dL, ■; PBL, ▨)-specific antibodies by ELISA. Values shown are mean absorbances ±1 s.d.

characteristics of Ovan-4 within SCID mice, and in conjunction with the human PBL-SCID mouse provide a suitable model for further investigations into the human immune response to this type of tumour. In particular, we investigated the possibility of eliciting a specific immune response to growing tumour within these animals by actively immunizing human PBL-SCID mice against the Ovan-4 cell line.

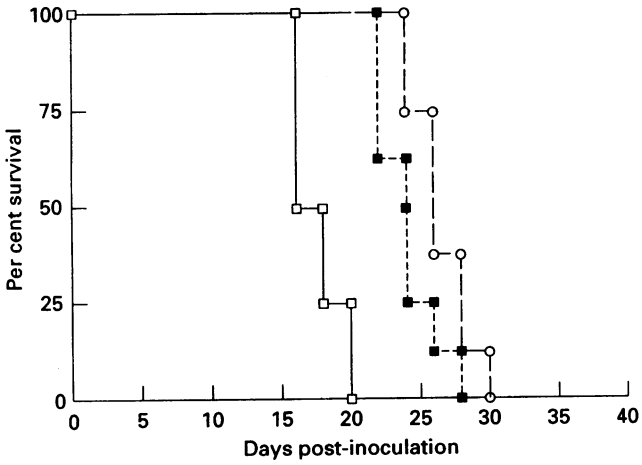


**Fig. 3.** Survival of SCID and human PBL-SCID mice after i.p. transfer of Ovan-4 tumour cells. Groups of female, SCID (□) or human PBL-SCID mice (PBL, ■; CD8dL, ○) were injected intraperitoneally with $10^6$ tumour cells on day 0 and monitored on a daily basis for tumour growth and morbidity. Survival of human PBL-SCID mice (PBL and CD8dL) was significantly longer than the immunodeficient SCID group ($P < 0{\cdot}0005$, Mann–Whitney *U*-test). The CD8dL group also exhibited significantly longer survival than the PBL group ($P < 0{\cdot}025$, Mann–Whitney *U*-test) with mean survival times of 28 and 25 days, respectively.

*Vaccination of human PBL-SCID mice against Ovan-4 tumour*

In an attempt to induce a specific human immune response to the Ovan-4 tumour *in vivo*, we vaccinated human PBL-SCID mice with Ovan-4 membrane preparations entrapped within liposomes and screened serum samples from these animals for the presence of tumour-binding antibodies. All reconstituted groups generated some anti-Ovan-4 binding antibody in their serum after vaccination, but only the CD8dL-reconstituted group appeared to produce initially a predominantly IgM response with higher levels of specific human IgG detected at later time points, suggesting development of a specific immune response and affinity maturation (Fig. 4). Serum samples from non-immunized human PBL-SCID animals did not exhibit any appreciable anti-Ovan-4 activity when screened using the same ELISA protocol.

Solid tumours with a distinct lymphoblastoid morphology became manifest in the CD8dL-reconstituted, immunized group at approximately 10 weeks post-reconstitution. Development of these tumours in CD8dL-reconstituted mice has previously been observed by us [13], and immunohistochemical staining of these tumours confirmed expression of human immunoglobulin and EBV latent membrane protein, consistent with their characterization as B cell lymphomas of human origin. In some Ovan-4-transplanted animals these malignant lymphoblastoid cells could be observed infiltrating the developing ovarian tumour (Fig. 5). These lymphoid tumours were readily established as *in vitro* cultures, and subsequent analysis of culture supernatants by ELISA revealed anti-Ovan-4-specific human antibodies of both IgM and IgG isotypes (Fig. 6). The ELISA protocol was shown to be specific for the detection of anti-Ovan-4 antibodies, as supernatants from the human EBV-transformed B cell line secreting antibody (IgG) with specificity for the human ovarian cancer-associated antigen termed 14Cl (as previously described by us [18]) consistently tested negative for anti-Ovan-4 reactivity in this assay.

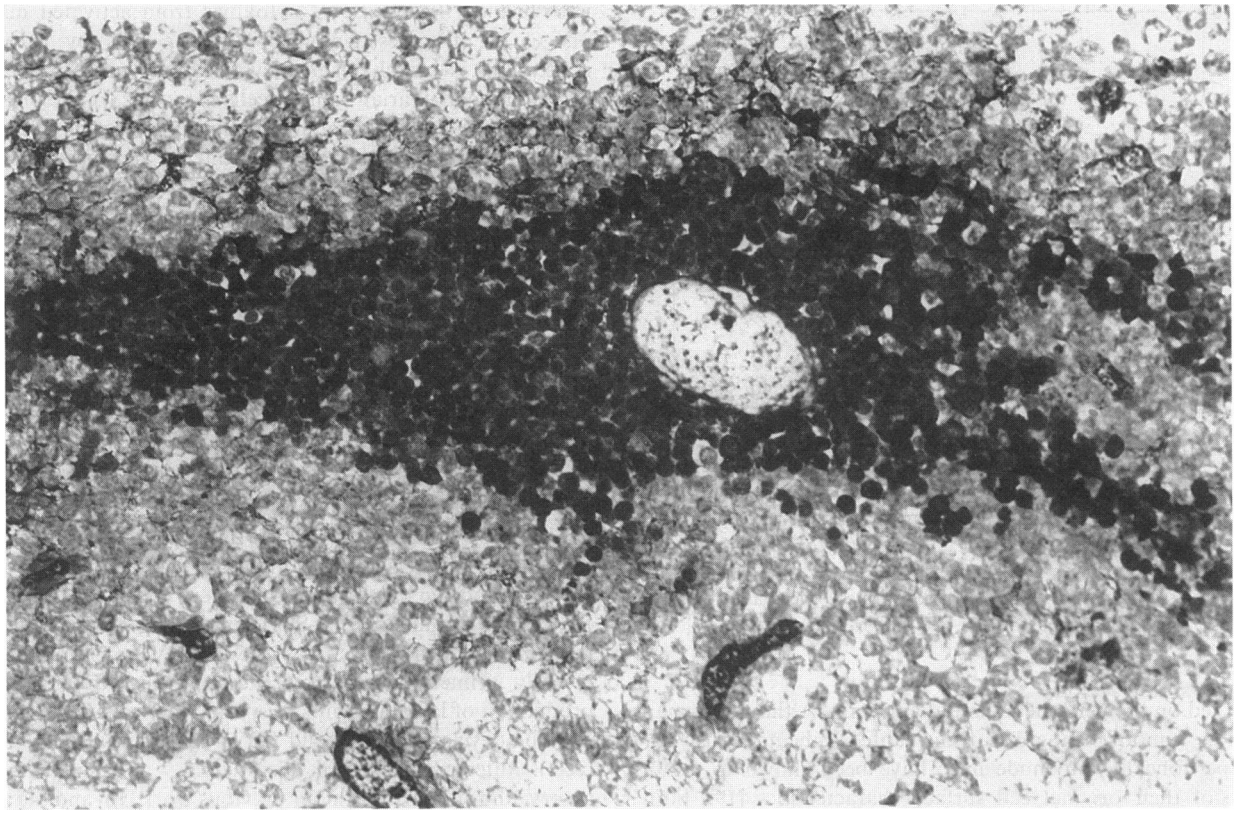

**Fig. 5.** Human lymphoblastoid tumour cells excised from vaccinated tumour-bearing human PBL-SCID (CD8dL) mice. Lymphoma cells expressing surface human immunoglobulin (dark staining) can be seen infiltrating the developing intraperitoneal ovarian tumour. (Paraffin section, immunoperoxidase × 160.)

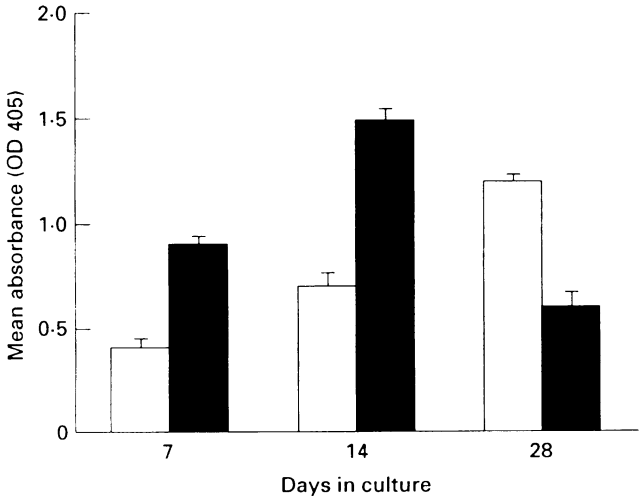


**Fig.6.** Secretion of anti-Ovan-4-specific antibodies by human lymphoblastoid tumours derived from vaccinated, CD8dL-reconstituted SCID mice. Supernatants obtained from confluent cultures were screened for the presence of Ovan-4-specific IgG (□) and IgM (■) by ELISA. Values shown are mean absorbances ±1 s.d. Supernatants from the human, Epstein–Barr virus (EBV)-transformed B cell line secreting IgG antibodies specific for the human ovarian cancer-associated antigen 14C1 [18] consistently tested negative in this assay.

*Growth of Ovan-4 in vaccinated human PBL-SCID mice*

To assess the functional nature of the immune response induced in human PBL-SCID animals by vaccination, immunized animals were challenged with live Ovan-4 cells on day 42 post-reconstitution and subsequent survival determined. Vaccinated human PBL-SCID mice created by transfer of CD8dL showed a further significant increase in survival ($P \leqslant 0{\cdot}0005$) compared with both non-vaccinated human PBL-SCID groups and vaccinated PBL and $CD8dL^+CD8^+$ reconstituted groups (Fig. 7). It should be noted that the CD8dL-reconstituted group of animals produced significantly higher levels of circulating immunoglobulin post-lymphocyte transfer (Fig. 2), and the development of B cell tumours in these animals makes accurate interpretation of the data more difficult. Vaccinated human PBL-SCID animals created by transfer of $CD8dL^+ + CD8$ cells exhibited mean survival times comparable to immunized PBL mice (25 days and 26 days, respectively), which is consistent with the similar pattern of reconstitution (in terms of immunoglobulin levels) observed in these two groups.

## DISCUSSION

This study demonstrates the successful growth of the human ovarian cancer cell line Ovan-4 in female SCID mice. The growth pattern of established Ovan-4 tumour within SCID mice mimics the pathological characteristics of human ovarian cancer, with the growth of solid tumour nodules consistently

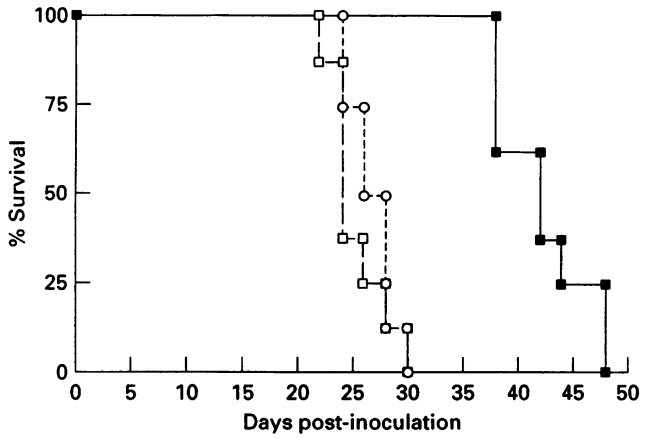


**Fig. 7.** Growth of Ovan-4 in vaccinated human PBL-SCID mice. Groups of vaccinated SCID-human mice (PBL, □; CD8dL, ■; CD8dL$^+$ + CD8, ○) were injected intraperitoneally with $10^6$ tumour cells on day 0 and monitored on a daily basis for tumour burden. Survival of human PBL-SCID mice reconstituted with CD8dL was significantly longer than the other reconstituted, vaccinated tumour-bearing groups ($P < 0.0005$, Mann-Whitney $U$-test).

observed on the peritoneum and diaphragm. Although human ovarian tumour xenografts transplanted subcutaneously or intraperitoneally into nude mice have been shown to retain some of their original cell surface characteristics [19], both xenografts and many cell lines in common usage suffer from the disadvantage that they grow as semi-solid ascitic tumours in the peritoneal cavity of nude mice, and this phenomenon can make the interpretation of data problematic. For example, Malik *et al.* [20,21] demonstrated that treatment of an ascitic xenografted tumour with tumour necrosis factor-alpha (TNF-$\alpha$) prolonged the life of treated animals. However, this may have been due to a change in tumour morphology, as TNF-$\alpha$-treated tumours implanted onto the peritoneal wall and grew more slowly. In this study we observed the more natural pattern of solid tumour growth without the addition of exogenous cytokines, and either there are other undefined host immune factors involved in the development of these tumours within the SCID environment or the Ovan-4 cell line secretes cytokines such as TNF-$\alpha$. We have not yet examined the latter possibility, but it has recently been suggested that ovarian cancer may be a cytokine-propelled disease [22], with the production of cytokines such as TNF-$\alpha$, either by the tumour itself or by other cells such as macrophages, responsible for the implantation and continued growth of this type of malignancy *in vivo*.

In addition to the similarity between the tumour growth pattern observed in our model and in the natural disease, the SCID mouse also accepts xenografts of human PBL, providing a novel model for studying anti-tumour immunity *in vivo*. In particular, this study shows that human PBL-SCID mice have a significantly increased survival time after challenge with tumour cells, and that human antibodies capable of recognizing Ovan-4 can be induced in these animals by vaccination. Further work is required to elucidate which anti-tumour immune mechanisms are important in these animals. For example, the transfer of purified lymphocyte subpopulations may delineate which effector cell populations are important in providing protection. Possible non-specific effector mechanisms include the release of cytokines from activated macrophages, or the destruction of tumour cells by activated neutrophils, a mechanism previously shown to be active against human ovarian tumour cells *in vitro* [23]. The presence of circulating human immunoglobulins in the serum of human PBL-SCID animals may also account for increased survival, possibly via antibody-dependent cellular cytotoxicity (ADCC) mechanisms, although this is unlikely in unvaccinated animals given the non-specific nature of the antibodies. In vaccinated animals, however, there was a further increase in survival time suggesting antibody-mediated suppression of tumour growth. This vaccination effect was only significant in the CD8dL group, and the relative contribution of Ovan-4-reactive antibody and the absence of CD8$^+$ T cells to the increased survival of these animals remains to be determined. However, we have previously shown that CD8dL-reconstituted animals can be specifically vaccinated against antigens such as ovalbumin [13] and that the humoral response in such animals is significantly enhanced by the absence of CD8$^+$ T cells. As CD8$^+$ T cells are thought to be the major lymphocyte population controlling EBV infection *in vivo* [24], and as there is evidence of a lytic EBV cycle in human PBL-SCID mice [25], we hypothesized that this enhancing effect was due to EBV transformation of recently primed B cells (by vaccination) with subsequent production of large amounts of specific human immunoglobulin. The development of solid EBV$^+$ tumours in the CD8dL-reconstituted mice used in this study is consistent with this theory, and supports both our previous work [13] and the work of others who have reconstituted SCID mice with PBL from EBV$^+$ donors [25]. These secondary tumours were occasionally observed to permeate the ovarian tumour masses, and the observed increase in survival could be explained by physical disruption of the ovarian tumour. Alternatively, as these lymphoblastoid cells were shown to produce antibodies against Ovan-4 when propagated *in vitro*, combined with the fact that CD8dL animals had enhanced serum anti-Ovan-4 titres, it is possible that the production of specific antibodies by these malignant B cells was responsible for the increase in survival. Although these two hypotheses are not mutually exclusive, creation of human PBL-SCID animals with lymphocytes from EBV$^-$ donors should avoid the development of these secondary tumours and permit a clearer evaluation of the role of antibody in the pathogenesis of ovarian cancer. It should be noted, however, that recovery of EBV-transformed human B cells from these animals with specificity for tumour may be advantageous for the production of therapeutic, anti-tumour human MoAbs. These studies are unique in that previous reports [26,27] on the production of human MoAbs by immunization of human PBL-SCID mice have involved immunization with antigens from infectious agents rather than using human tumour to stimulate immunity. Given the demand for anti-cancer MoAbs of human origin and the current problems associated with the production of such agents [28,29], we suggest this model may prove useful in future cancer studies.

In the anti-human ovarian cancer immune responses modelled here, vaccination with Ovan-4 antigen was required for increased survival. However, it is also possible that the presumed allogeneic relationship between the tumour and reconstituting lymphocytes could account for the observed protection. Given this caveat, there was no immunohistochemical evidence that human T cells had infiltrated growing

tumours, but given the presumed allogeneic nature of the model, T cell involvement must be a possibility. Further studies using autologous tumour cells and lymphocytes will help to elucidate this point. Finally, although specific binding of anti-Ovan-4 antibodies could be detected in our ELISA, further analysis of the specificity of these antibodies will determine whether they are genuinely tumour-specific. Testing against other ovarian cell lines or surgical tumour specimens will reveal the nature of these antibodies.

In conclusion, these results demonstrate that the SCID mouse is a suitable host for the growth of human ovarian tumours and provides a unique model for investigations into the interaction of the human immune system with this type of cancer. This model may also be useful in the future development and assessment of drug treatments, novel immunotherapies, and particularly in the production of anti-cancer human monoclonal antibodies.

## ACKNOWLEDGMENTS

W.W. was in receipt of a Medical Research Council Studentship for the duration of this work, and this study was also generously supported by Tenovus Scotland.

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