The detection of human cells in a murine tissue is a frequently arising problem after xenotransplantation of normal or malignant cells. The formation of micrometastases of solid tumours, dissemination of leukaemia cells or the engraftment potential of haematological cells are examples for which a sensitive proof of the human-specific origin of cells is necessary to address biological, diagnostic or therapeutic questions.

In literature, for that purpose tissue-(cytokeratins) or tumour associated markers like mucins (Finn et al, 1995) or adhesion molecules (Giavazzi et al, 1994) are described. Also human-specific surface markers like HLA-DR (Goan et al, 1999) or human endogenous retroviral sequences (Ailles et al, 1999) are used. Others, artificially transfected cells with marker genes like lacZ (Hayashi et al, 1996; Krüger et al, 1999) or green fluorescent protein (Yang et al, 2000) for a detection of the specific gene product in the in vivo xenotransplantation system.

These methods are at least partially hampered by the fact that tumour markers are only (over-) expressed in a certain proportion of known malignancies. Immunohistochemical methods for the detection of surface markers are relatively insensitive and difficult to standardise. Furthermore, the use of artificial markers is frequently restrained by the low transfection efficacy of the cells. Therefore, we decided to develop a sensitive PCR assay which is universally applicable either for the detection of human cells in murine blood and organs or for a quantification of such cells via real-time (TaqMan) PCR. We targeted for that purpose a highly repetitive α-satellite DNA sequence of the centromer region of human chromosome 17 (Warburton et al, 1991). The sensitivity and specificity of that method was investigated and used for: (i) the detection of micrometastases of a human breast carcinoma xenograft; (ii) the estimation of dissemination of a human acute
lymphatic leukaemia (ALL) model; (iii) the proof of distribution of mature lymphocytes and (iv) the evaluation of the engraftment potential of human blood stem cells.

**MATERIALS AND METHODS**

**Extraction of DNA**

Genomic DNA of mouse blood and organs was extracted using the QiaAmp blood and tissue kit respectively (Qiagen, Hilden, Germany). Briefly, the DNA of lysed cells was adsorbed onto a silica matrix, washed and eluted with QIAamp elution buffer by centrifugation.

**Conventional DNA PCR**

The presence of human-specific DNA within the blood and organs of transplanted mice was confirmed by polymerase chain reaction (PCR) amplifying an 850-bp fragment of the z-satellite region of the human chromosome 17 using primers corresponding to the primer pair 17a1/17a2 as described by Warburton et al (1991). The primers were elongated to 25 nucleotides each for use at higher annealing temperatures. The sequences are shown in Table 1. For PCR, the AmpliTaq-Gold polymerase and related reagents from Perkin Elmer (Applied Biosystems GmbH, Weiterstadt, Germany) were used. The PCR reaction mixture contained 200 µM each of the respective nucleotides, 250 nM of each primer, 2 mM MgCl2 and 250 ng of genomic DNA template. Following an initial DNA denaturation and Taq activation at 94°C for 10 min, 35 1-min cycles of denaturation at 94°C and annealing/extension at 72°C for 10 min. Amplified DNA fragments were electrophoresed through 1.75% agarose gels and subsequently visualised under ultraviolet light after staining with ethidium bromide. Genomic DNA samples from both a human breast carcinoma line (MaCa 3366) as a positive control and NOD/SCID mouse liver tissue as a negative control were processed in parallel. Routinely used PCR was evaluated by scoring band intensities expressed by one plus to three plus. One plus corresponded to very weak bands and three plus corresponded to very intense bands comparable to that of the positive control with 100% human cells.

**Quantitative real-time (TaqMan) PCR**

In parallel, we developed a TaqMan-chemistry based real-time PCR using an optimised primer/probe-combination, which effectively amplifies a shorter (467 bp) fragment of the z-satellite-DNA-region (Table 1). The exonuclease probe was 5′-labelled with the reporter fluorescent dye TAMRA (6-carboxy-fluoresceine) and carried the non-fluorescent quencher dye TAMRA. Probe extension during PCR was blocked by a 3′-phosphate. The standardised PCR was performed in a Perkin Elmer 7700 Sequence Detection System (TaqMan) in 96-well microtiter plates with a final volume of 50 µl using similar conditions as for the conventional PCR described above. Additionally, the PCR-Mix contained 10 nM of the TaqMan-Probe. A total of 45 cycles was performed without a final elongation step. Each sample was tested twice in parallel. Serial dilution steps of human mammary carcinoma cells (MCF-7) in mouse ascitis cells (P388) served to construct the TaqMan-calibration curves. These control cells were obtained from in vivo-tumour lines established and permanently passaged in our laboratory.

**Experimental animals**

For the in vivo experiments immunodeficient SCID or NOD/SCID mice (6–8 weeks of age) were used. While SCID mice lack mature B- and T-lymphocytes due to a recombining gene deficiency (Bosma et al, 1983) NOD/SCID mice are additionally characterised by a deficit in NK-cells and functioning macrophages (Shultz et al, 1995). Balb/c-SCID mice were purchased from Bundesgesundheitsamt (Berlin, Germany) NOD/SCID mice from Jackson Laboratories (Bar Harbour, USA).

All mice were held under sterile and standardised conditions (20°C room temperature, 50% relative humidity, 12 h-light-dark rhythm) in laminar flow shelves with autoclaved food and bedding (Sniff, Soest, Germany) and acidified tap water (pH 4) ad libitum. All mice were tested for leaseness and only mice with murine IgG levels below 100 ng ml^-1 serum were used for the experiments. All experiments were performed according to the German Animal Protection Law and with permission from the responsible local authorities.

**Detection of micrometastases**

Cells of the human breast carcinoma MDA-MB 435 were taken from cell culture flasks and admixed with matrigel (Basement Membrane Matrix, Becton Dickinson, Bedford, USA) shortly before injection into the mammary fat pad (mfp) of female NOD/SCID mice. Six animals each received 10⁶ to 1 cell in a log dilution assay. Once per week tumour size was measured with a calliper-like instrument and tumour volumes \( v = (w^2l)/2 \) were calculated. Further, blood was taken from the tail vein once per week.

After 4 weeks mice were sacrificed, liver, lung and tumour from the mfp area were taken and stored at -80°C for PCR analysis.

**Leukaemic dissemination**

Female Balb/c-SCID mice (five per group) received 10⁶, 10⁷ or 10⁸ cells of a human paediatric ALL (ALL-SCID 2, Fichtner et al, 1999) intraperitonally (i.p.). Every 2–3 weeks blood was taken from the tail vein and stored for PCR analysis. Mice were checked at least twice per week for leukaemia development. Body weight was measured once per week and related to the initial value (body weight change, BWC) in per cent. The mice were sacrificed at moribund stage and gross examined.

**Distribution of lymphocytes**

Three female NOD/SCID mice per group received 5 x 10⁷, 1 x 10⁷ or 0.5 x 10⁷ lymphocytes from auffy coat after Ficoll gradient separation (Lymphocyte separation medium, Life Technologies, Eggenstein, Germany). The cell mixture contained 5% CD4⁺, 5% CD8⁺, 7% CD56⁺, 10% HLA-DR⁺ cells as determined by FACS analysis.

The lymphocytes were admixed with 10 U ml⁻¹ heparin in order to prevent aggregation and injected intravenously (i.v.) into the tail vein. Half of the mice received a total body irradiation of 2 Gy (¹³¹Cs source) 24 h before lymphocyte administration in order to disrupt remaining circulatory macrophages in the circulation. After 4 weeks mice were sacrificed and different organs were

---

**Table 1** Oligonucleotides and probes used for PCR

| Name | 5′–3′ sequence | Localisation⁴ |
|------|----------------|--------------|
| Cr17_1a⁴ | GGC ATA ATT TCA GCT GAC TAA ACA G | 15,39 |
| Cr17_2b⁴ | TTC CGT TTA GTT AGG TGC AGT TAT C | 867,891 |
| Cr17_4b⁴ | AAA GGT CCA CCA GTA GTC | 472,494 |
| TMatt_probe | 6FAM CAC GTT TGA AAC ACT CTT | 109,137 |
| XT TTG CAG GATC p | (X=Tamra) |

¹Forward primer; ²reverse primer for the conventional PCR; ³reverse primer for TaqMan PCR; ⁴nucleotide positions correspond to the sequence HSSATA17, GeneBank Acc.-No. 13882; further explanations are given in the text.
analysed for human-specific DNA. In some mice a graft vs host disease (GVHD) was diagnosed by the appearance of ruffled fur, an icteric view of the skin and signs of inflammation in the head region. Livers of those mice were additionally examined by a pathologist for GVHD symptoms.

Engraftment of blood stem cells

Mononuclear cells (MNC) originating from cord blood or from peripheral blood after stem cell mobilisation were injected i.v. into pre-irradiated (2 Gy) female NOD/SCID mice (2–5 per group). CD34 positive progenitors were obtained by magnetic separation as described elsewhere (Goan et al, 1996). The mice additionally received a co-transplant consisting of a rat fibroblast cell line stably transfected with the human interleukin-3 gene in order to supplement a species-specific haematopoietic growth factor (Goan et al, 1996). After at least 5 weeks the murine blood was analysed for human DNA. In the case of a positive PCR-signal mice were sacrificed 2–3 weeks later. Human haematopoietic cells (CD45+, HLA-I+) were analysed by flow cytometry (FACS) or immunohistochemistry (IH); for details see Goan et al (1996).

RESULTS

Sensitivity and specificity of PCR method

Figure 1 shows that at least a rough visual semi-quantitative evaluation of dilution mixtures of human in murine cells is possible by conventional DNA-PCR. While the murine P388 lymphatic leukaemia revealed no positive signal, as few as one human MCF-7 cell (breast carcinoma line) in 10^6 of mouse cells was detectable. However, as our dilution experiments showed, the PCR method with 35 cycles seemed to reach a plateau phase. The differences between band intensities of logarithmic dilution steps were too small to perform a reliable densitometric analysis (data not shown). In contrast, the use of lower cycle numbers allowed a more reliable evaluation, but caused a disappearance of weak bands. Thus, for a routine screening we decided to perform a visual subjective estimation of band intensities characterised by one to three plus.

TaqMan assay for real-time quantification of human DNA

Since certain xenotransplantation experiments require an exact quantification of human cells in mouse organs, we developed in parallel a TaqMan-chemistry based real-time PCR approach amplifying a smaller fragment of the same genome localisation and using similar reaction and cycle conditions. Serial dilutions of human MCF-7 cells in mouse P388 cells tested with each PCR reaction served to construct the TaqMan-calibration curves. We always
obtained a linear correlation between DNA-amount, respectively cell numbers, and the CT values over the whole detection range from 50 to 0.001% human cells (with some slight deviations in the highest 100% and the lowest 0.0001% dilution step). Calibration results are presented in Figure 2. Figure 3 shows the TaqMan results of mouse liver and lung of three animal groups.
of the micrometastasis experiment with the human breast carcinoma xenograft MDA-MB 435 as described below (Table 2). The columns are related to the agarose gel bands of amplification products and the resulting visual estimation of band intensities of corresponding samples as obtained by conventional PCR.

**Detection of micrometastases**

The human breast carcinoma line MDA-MB 435 is highly metastasising after orthotopic inoculation into the mfp of immunodeficient mice (Price et al., 1990). Lung and lymph node metastases are usually macroscopically detectable after a relatively long growth period when the primary tumour reaches a volume of more than 1.5 cm³.

In our assay system (Table 2 and Figure 3) only 10 cells per mouse were sufficient to detect human DNA in lung and liver of mice, while at that point of time (30 days after inoculation) no palpable tumour in the mfp-region was detectable. Higher cell numbers led to an increase in primary tumour size and a corresponding magnification of the PCR signal. Surprisingly, at no time point human DNA as indicator for circulating tumour cells could be found in the murine blood. This suggests either a predominant lymphogenic pathway of metastasis or indicates that cell numbers in circulation were below the detection limit of the assay. The PCR method presented here allows a very sensitive detection and quantification of micrometastases. It can be taken as surrogate marker for the monitoring of response of therapeutic interventions.

**Leukaemic dissemination**

Table 3 shows the results of blood analysis of SCID mice at several time points after inoculation of cells of a paediatric leukaemia, ALL-SCID 2. While at day 50 transiently human DNA was detected in five out of five mice receiving 10⁶ cells, three out of five mice with 10⁴ and only one out of five mice with 10² cells, at later time points (day 97) a positive PCR signal was only obtained in three out of five mice at the highest cell dose. At a delayed time, after 140 days, when the animals were sacrificed due to a moribund stage only in those mice with detection of human DNA at an advanced stage the leukaemia became manifest. The leukaemia development in the peritoneum was accompanied by a concomitant increase in body weight. It can be concluded that the determination of human DNA by the PCR method presented here, allows an early evaluation of leukaemic dissemination before manifestation of the disease. This enables the initiation of therapeutic interventions in the xenograft system at a stage being comparable with minimal residual disease in patients.

**Distribution of lymphocytes**

The distribution pattern and survival rate of mature lymphocytes after i.v. injection into NOD/SCID mice can be taken from Table 4. At least 10⁷ cells had to be inoculated to obtain a positive PCR signal in bone marrow, thymus, lymph node and lung, while the five-fold cell number apparently led to a broad distribution of lymphocytes to all tissues analysed. There was a clear difference between irradiated and non-irradiated mice indicating that a sublethal pre-irradiation leading to a long-standing depletion of macrophages (data not shown) is a precondition for a survival of i.v. inoculated mature lymphocytes.

The positive take rate of 5 × 10⁷ or 10⁷ lymphocytes was accompanied by the appearance of GVHD symptoms in one out of three or two out of three mice, respectively. Our results confirm the limited survival potential of mature lymphocytes in the conditioned murine host. Oppositely, the PCR method enables the evaluation of lymphocyte distribution and survival in experiments investigating immunotherapeutic approaches against cancer.

**Engraftment of blood stem cells**

The administration of cord blood mononuclear or CD34 positive (>90% of cell mixture) cells to NOD/SCID mice led in two out of three independently performed experiments (Table 5 I and II) to positive PCR signals in the blood of mice at 7–11 weeks after transplantation. When blood and bone marrow of those mice were analysed for haematopoietic cells (CD45, HLA-I) by FACS and IH a high percentage of human cells could be found, especially in the bone marrow (Figures 4 and 5). In blood, 0.9 to 6.6% human CD45 positive cells were found confirming the PCR results obtained several weeks before. In one example of experiment II, after 7 weeks no human DNA in the blood was detected. Testing the same mice after 11 weeks, a positive signal was obtained. That suggests a relatively long time period being necessary before establishment of a human haematopoiesis, lead-

### Table 3

| Group | Mouse | Cell number | PCR result on day | Body weight gain (%) | Leukaemia development on day 140 |
|-------|-------|-------------|-------------------|----------------------|----------------------------------|
| A     | 1     | 10⁹        | – – + + + +      | 29                   | +                                |
| B     | 1     | 10⁷        | – – + + + +      | 20                   | –                                |
| C     | 1     | 10⁷        | – – + + + +      | 16                   | –                                |

Five Balb/c-SCID mice per group obtained 10⁷, 10⁶, or 10² cells of the paediatric leukaemia ALL-SCID 2 i.p. At the indicated times, blood was taken from the tail vein and analysed for human DNA. Leukaemia development was diagnosed in sacrificed mice by gross examination.

---

**Experimental Therapeutics**

**PCR quantification of human cells in mice**

M Becker et al

---

**British Journal of Cancer (2002) 87(11), 1328–1335 © 2002 Cancer Research UK**

---

### Table 2

Detection of micrometastases

| Cell number | Tumour volume mm³ (d30) | PCR result | PCR result |
|-------------|-------------------------|------------|------------|
|             | Liver                   | Lung       | mfp area   | Blood     |
| 1           | 0                       | – – – –    | – –        |
| 10²         | 6 ± 5                   | ++ ++ (+)  | – –        |
| 10³         | 200 ± 99                | ++ ++ ++   | – –        |
| 10⁴         | 402 ± 153               | ++ ++ ++   | – –        |
| 10⁵         | 914 ± 259               | ++ ++ +++  | – –        |
| 10⁶         | 1181 ± 560              | ++ ++ +++  | – –        |

Six female NOD/SCID mice per group were inoculated into the mammary fat pad (mfp) with the indicated cell numbers of the human MDA-MB 435 breast cancer carcinoma admixed with matrigel. Blood was taken once per week. After 30 days mice were sacrificed and their liver, lung and mfp-area were analysed for human DNA. The size of the primary tumour was measured with a caliper-like instrument and the volumes were calculated.
**Figure 4**  FACS analysis of bone marrow of mice 12 weeks after transplantation of cord blood MNC (A) or sorted CD 34 cells (B) from experiment II, Table 4. FL-1=CD 19+ FITC, FL-2=CD 45+ PE.

**Table 4**  Distribution of human lymphocytes

| Group | Body irradiated (2 Gy) | Cell number | PCR results |
|-------|------------------------|-------------|-------------|
|       |                        |             | Blood       | Bone marrow | Liver | Spleen | Thymus | Lymph node | Lung | Heart | Stomach | Gut | Skin | GVHD |
| A     | +                      | 5×10^7      | +++         | +++         | +++    | +++    | +++    | +++        | +++  | +++   | +++    | +++ | +++  | 1/3  |
| B     | +                      | 1×10^7      | ++          | +           | +      | +      | -      | -          | -    | -     | -      | -   | -    | 2/3  |
| C     | +                      | 0.5×10^7    | -           | -           | -      | -      | +      | -          | -    | -     | -      | -   | -    | 0/3  |
| D     | -                      | 5×10^7      | -           | +           | -      | -      | -      | +          | -    | -     | -      | -   | -    | 0/3  |
| E     | -                      | 1×10^7      | -           | -           | -      | -      | +      | -          | -    | -     | -      | -   | -    | 0/3  |
| F     | -                      | 0.5×10^7    | -           | -           | -      | -      | +      | -          | -    | -     | -      | -   | -    | 0/3  |

Lymphocytes of a healthy donor were injected i.v. into three NOD/SCID mice per group. Mice of groups A–C were sublethally irradiated with 2 Gy. After 29 days mice were sacrificed; blood, bone marrow and different organs were analysed for human DNA; mean values are presented. Graft vs host disease (GVHD) was visible by ruffled fur, icteric skin and inflamed eyes and confirmed by a pathologist.
ing to a detectable number of cells in the peripheral blood of mice.

In experiment III engraftment of CD34 cells completely failed probably due to insufficient cell numbers. This led to completely negative results both in PCR, in FACS and IH.

The administration of $8 \times 10^6$ CD34 cells gained from peripheral blood of patients undergoing mobilising chemotherapy (experiment IV) also revealed human-specific signals in PCR, which were confirmed by FACS and IH analysis of blood and bone marrow cells. On contrary, a lower cell number of $3 \times 10^5$, only led to a positive PCR result but failed to show the presence of human cells by FACS and IH. This suggests the expected higher sensitivity of the PCR method compared to the other two techniques. All together, it can be concluded that the detection of human DNA in the peripheral blood of mice xenotransplanted with haematopoietic cells can be taken in the vast majority of cases as an indicator for a positive engraftment.

**DISCUSSION**

After xenotransplantation of human cells into mice, it is frequently necessary to detect those cells as early as possible and at a relatively low level.

This problem for instance arises when metastasising models of human solid tumours are used (Giavazzi et al, 1986; Fodstad et al, 1988; Morikawa et al, 1988; Shoemaker et al, 1991) and their growth and dissemination potential has to be evaluated for an estimation of efficacy of therapeutic approaches.

Even more complicated is the situation when haematological malignancies as models in immunodeficient mice are used (Uckun, 1996). Here, frequently an early diagnosis of only few disseminated cells is warranted to start therapeutic interventions in a clinically-related manner as early as possible and before the disease becomes manifest and incurable.

Modern experimental gene- and immunotherapeutic approaches against cancer occasionally use transected or modified haematological cells. In those pre-clinical studies it is unavoidable to characterise the survival and distribution potential of these cells (Licht et al, 1998).

And last but not least, several projects deal with the engraftment potential of blood stem cells for the enabling of high-dose-chemotherapy protocols. Questions of interest in this area are the potential of ex vivo-expansion protocols of CD34 positive cells (Moebst et al, 1999) or the role of stroma and endothelial cells for the engraftment of stem cells (Goan et al, 2000).

For that purpose, the necessity arose to develop an analytical method which is sensitive, specific and universally applicable to detect human cells in immunodeficient mice. Shoemaker et al (1992) described the use of repetitive human Alu DNA sequences in a dot blot assay which allowed the quantitative detection of metastases of a human melanoma in several tissues of nude mice.

We decided to develop a human specific PCR method targeting highly repetitive sequences of the $\alpha$-satellite DNA of the centromere region of human chromosome 17. A 850 bp fragment was amplified with primers which were varied according to Warburton et al (1991). A BLAST-search of this human sequence fragment in the EMBL rodent DNA database as well as in the ENSEMBLE whole mouse genome browser (EBI, Hinxton, Cambridge, UK) revealed no significant similarity with any mouse DNA sequence. Thus, these sequences seem to be completely absent in the mouse genome and the method shows high specificity for human tissues with a complete lack of positive signals for murine tissues. As shown by logarithmic dilution series, we were able to detect one human cell in $10^6$ murine cells. However, with 35 cycles necessary to reach the high sensitivity the method allowed only a rough semi-quantitative estimation. Therefore, we developed in parallel a quantitative real-time PCR approach based on TaqMan methodology, targeting a smaller fragment of the same genome localisation. The resultting standardised TaqMan assay provides the possibility for a quantification of human cells in the mouse from 100% up to 0.0001% (one human cell in $10^6$ mouse cells).

The method described herein was used for the detection of micrometastases of a breast carcinoma model. It proved its high sensitivity by detecting human DNA in lung and liver of NOD/SCID mice after orthotopic transplantation of only 10 cells and at an early time point when no primary tumour was palpable. The lack of positive signal in the blood of mice even at injection of up to $10^6$ cancer cells suggests the predominance of a lymphogenic route of dissemination leading to probably only few or shortly surviving cells in the blood circulation.

On the contrary, the manifestation of an ALL in SCID mice demanded at least $10^6$ cells and took several months. But even in this case, the PCR method allowed an about 50-days earlier detection of human cells in the blood of engrafted mice. That fact makes the xenotransplantation model suitable for the study of minimal residual disease and for the early commencement of therapeutic interventions.

Similar to others (Martino et al, 1993) we also stated that the survival of human mature lymphocytes gained from peripheral

**Table 5** Engraftment of blood stem cells

| Experiment | Source | Cells           | Cell number | PCR        | % Human CD45 in blood** | % Human CD45 in bone marrow** | % Human CD45 in bone marrow** |
|------------|--------|----------------|-------------|------------|-------------------------|-------------------------------|-------------------------------|
| I Cord blood | MNC     | $10^7$         | +++ (9)     | (9)        | 6.6                     | (9)                           | 64.6                          |
| II Cord blood | MNC     | $2 \times 10^7$ | ++ (7)      | (7)        | 1.5                     | (9)                           | 17.1                          |
| III Cord blood | CD34 | $10^7$         | +++ (11)    | (11)       | 3.9                     | (12)                          | 59.2                          |
| IV BPC CD34 | $3 \times 10^5$ | ++ (7)      | (7)        | (7)        | 0.9                     | (12)                          | 37.8                          |
|            | CD34    | $8 \times 10^6$ | + (8)       | (8)        | 1.9                     | (9)                           | 34.5                          |

**Immunohistochemistry, †FACS, n.t.=not tested. Blood stem cells originating from different sources (cord blood, BPC-blood progenitor cells) were injected either as mixture of mononuclear cells (MNC) or enriched CD34 positive cells i.v. to sublethally irradiated (2 Gy) NOD/SCID mice (2 – 5 per group). At the indicated times blood was analysed for human-specific DNA. Some weeks later mice were sacrificed, CD45 positive or HLA-I positive cells were determined in blood or bone marrow by flow cytometry (†) or immunohistochemistry (‡).
blood is rather limited, needing more than 10^7 cells to be transplanted and requiring a conditioning of mice by sublethal total body irradiation. Those conditions have to be known for preclinical studies using immunotherapeutic agents (vaccines, antibodies).

Oppositely, haematopoietic stem cells isolated from cord blood or from cytapheresates of patients after mobilising chemotherapy under our conditions successfully engrafted in the majority of cases. Here, a good correlation of PCR, FACS and IH results could be shown.

In the past, imaging techniques have been developed for detection and quantification of tumours based on genetically engineered tumour cells expressing reporters such as beta galactosidase, green fluorescent protein or the newly emerging technology based on luciferase reporters (Rice et al., 2001). These methods have the relative disadvantage that they require transformation of tumour cells with an expression vector to enable detection and in the case of luciferase, infusion of the luciferin substrate intravenously. Such genetic manipulation could potentially alter phenotypes of relevance to experimental work. However, the imaging approaches are relevant to the current report in that both techniques could profitably be used in concert. For example, experiments using luciferase or GFP methods could be confirmed and generalised using our universally applicable PCR approach.

In summary, the PCR method described herein allows a specific and sensitive detection of human cells in xenotransplantation systems. The method is relatively simple, well-standardisable, non-radioactive, and except for the technical device relatively inexpensive. However, because of its high sensitivity, the method requires a very clean laboratory work to avoid any contamination with human genomic DNA. When performed as a real-time PCR using TaqMan methodology, it allows a reliable quantification of human cells in mouse organs over a wide dilution range. It can be used as an universally applicable surrogate marker for the quantitative evaluation of engraftment, proliferation and dissemination of malignant or normal cells in immunodeficient hosts.

ACKNOWLEDGEMENTS

The excellent technical support of Mrs Monika Becker and Mrs Margit Lemm is gratefully acknowledged.

REFERENCES

Ailles LE, Gerhard B, Kawagoe H, Hogge DE (1999) Growth characteristics of acute myelogenous leukaemia progenitors that initiate malignant hematopoeisis in nonobese diabetic/severe combined immunodeficient mice. Blood 94: 1761 – 1772

Bosma GC, Custer RP, Bosma MJ (1983) A severe combined immunodeficiency mutation in the mouse. Nature 301: 527 – 530

Fichtner I, Goan SR, Becker M, Baldy C, Borgmann A, von Stackelberg A, Henze H (eds) (1999) Transplantation of human haematopoietic or leukemic cells into SCID and NOD/SCID mice. In Relevance of tumor models for antitumor drug development, Vol 54, Fiebig, HH, Burger, AM (eds) pp 207 – 217. Freiberg, Germany: Karger

Finn OJ, Jerome KR, Henderson RA, Pecher G, Domenech N, Magarin-Blander J, Barrat-Boyes SM (1995) MUC-1 epithelial tumor mucin-based immunity and cancer vaccines. Immunol Reviews 145: 61 – 89

Fodstad O, Aaradal S, McNemar N, Nesland JM, Pihl A (1988) A new experimental metastasis model in athymic nude mice, the human malignant melanoma LOX. Intl J Cancer 41: 442 – 449

Giavazzi R, Campbell DE, Jessup JM, Cleary K, Fidler IJ (1986) Metastatic behavior of tumor cells isolated from primary and metastatic human colorectal carcinomas implanted into different sites in nude mice. Cancer Research 46: 1928 – 1933

Giavazzi R, Nicolotti MI, Chirivi RG, Hemingway J, Bernasconi S, Allavena P, Gearing AJ (1994) Soluble intercellular adhesion molecule-1 (ICAM-1) is released into the serum and ascites of human ovarian carcinoma patients and in nude mice bearing tumour xenografts. Europ J Cancer 30A: 1865 – 1870

Goan SR, Schwarz K, von Harsdorf S, von Schilling C, Fichtner I, Junghann I, Just U, Herrmann F (1996) Acute myelogenous leukaemia progenitors retrovirally transduced with the human IL-3 gene initiate and sustain multilineage human hematopoiesis in vivo. Blood 96: 3971 – 3978

Hayashi M, Tomida M, Hozumi M, King IC, Sartorelli AC (1996) Detection of in vivo differentiation of murine WEHI-3B D+ leukemia cells transfected with the lac-Z marker gene using two-color flow cytometry. Leuk Res 20: 333 – 341

Krüger A, Schirrmacher V, Khokha R (1999) The bacterial lacZ gene: An important tool for metastasis research and evaluation of new cancer therapies. Cancer and Metastasis Reviews 17: 285 – 294

Licht T, Gottesman MM, Pastan I (1998) Retroviral transfer of the multidrug transporter to murine hematopoietic stem cells. Methods in Enzymology 292: 546 – 557

Martino G, Anastasi J, Feng J, Mc Shan C, de Groot L, Quintans J, Grimaldi LME (1993) The fate of human peripheral blood lymphocytes after transplantation into SCID mice. Eur J Immunol 23: 1023 – 1028

Moś B, Goan SR, Junghann I, Winkler J, Fichtner I, Herrmann M, Becker M, de Lima-Hahn E, Henschler R (1999) Differential kinetics of primitive hematopoietic cells assayed in vitro and in vivo during serum-free suspension culture of CD34+ blood progenitor cells. Stem Cells 17: 152 – 161

Morikawa K, Walker SM, Nakajima M, Pathak S, Jessup JM, Fidler IJ (1988) Influence of organ environment on the growth, selection, and metastasis of human colon carcinoma cells in nude mice. Cancer Res 48: 6863 – 6871

Price JE, Polyzos A, Zhang RD, Daniels LM (1990) Tumorigenicity and metastasis of human breast carcinoma cell lines in nude mice. Cancer Research 50: 717 – 721

Rice BW, Cable MD, Nelson MB (2001) In vivo imaging of light-emitting probes. J Biomed Opt 6: 432 – 440

Shultz LD, Schweitzer PA, Christianson SW, Gott B, Schweitzer IB, Tennent B, McKenna S, Mobbraiten L, Rajan TV, Greiner DL, Leiter EH (1995) Multiple defects in innate and adaptive immunologic function in NOD/LttSz-scid mice. J Immunol 154: 180 – 191

Shoemaker RH, Dykes D, Plowman J, Harrison SD, Griswold DP, Abbott BJ, Mayo JG, Fodstad O, Boyd MR (1991) Practical spontaneous metastasis model for in vivo therapeutic studies using a human melanoma. Cancer Research 51: 2837 – 2841

Shoemaker RH, Smythe AM, Wu L, Balaschak MS, Boyd MR (1992) Evaluation of metastatic human tumor burden and response to therapy in a nude mouse xenograft model using a molecular probe for repetitive human DNA sequences. Cancer Research 52: 2791 – 2796

Uckun FM (1996) Severe combined immunodeficient mouse models of human leukemia. Blood 88: 1135 – 1146

Warburton PE, Greig GM, Haaf T, Willard HF (1991) PCR amplification of chromosome-specific alpha-satellite DNA: Definition of centromeric STS markers and polymorphic analysis. Genomics 11: 324 – 333

Yang M, Baranov E, Jiang P, Sun FX, Li XM, Li L, Hasegawa S, Bouvet M, Al-Tuwaijri M, Chishima T, Shimada H, Asahina AR, Penman S (2000) Whole-body optical imaging of green fluorescent protein-expressing tumors and metastases. PNAS 97: 1206 – 1211