Technical Advance

Leukocytes Expressing Green Fluorescent Protein as Novel Reagents for Adoptive Cell Transfer and Bone Marrow Transplantation Studies

Denise J. Manfra, Shu-Cheng Chen,
Tong-Yuan Yang, Lee Sullivan,
Maria T. Wiekowski, Susan Abbondanzo,
Galya Vassileva, Petronio Zalamea,
Donald N. Cook, and Sergio A. Lira

From the Department of Immunology, Schering-Plough Research Institute, Kenilworth, New Jersey

Transgenic mice expressing green fluorescent protein (GFP) were generated to provide a source of labeled leukocytes for cell transfer studies. The transgene comprises the GFP coding region under the transcriptional control of the chicken β -actin promoter and human cytomegalovirus enhancer. Mice expressing this GFP transgene were generated in the B6D2 and in the 129SvEv backgrounds. Flow cytometric analysis of cells from the blood, spleen, and bone marrow of these transgenic mice revealed that most leukocytes, including dendritic cells and memory T cells, express GFP. In allogeneic cell transfers, donor GFP⁺ splenocytes were detected in the spleen and mesenteric lymph nodes of recipient mice within 2 hours after transfer and for at least 9 days thereafter. In syngeneic experiments using 129-derived GFP⁺ donor splenocytes, donor cells were detected in multiple tissues of 129 recipients from 2 hours to 3 weeks after transfer. In bone-marrow transplantation experiments using irradiated allogeneic recipients, the percent of GFP⁺ donor cells in recipients at 3 weeks was comparable to that seen in similar tissues of GFP⁺ donor mice. These data demonstrate that GFP⁺ transgenic mice provide a ready source of GFP-expressing primary cells that can be easily monitored after their transfer to recipient animals. (*Am J Pathol* 2001, 158:41–47)

In cell trafficking and bone-marrow transplantation studies, it is essential that donor cells can be distinguished from those of the recipient. Procedures to accomplish this include labeling of cells with fluorescent dyes,^{1–3} digoxigenin,² or radioisotopes³ before their introduction to recipient animals. There are several inherent disadvantages to these methods. First, variability in the labeling

process may preclude comparison of data from different experiments. Second, most cells labeled by these methods are only useful for short-term studies because the label is diluted as the cells proliferate.¹ Third, labeling procedures can affect cellular functions such as cell proliferation, signaling, and DNA replication.¹ Finally, some methods, using digoxigenin, congenic cell surface markers,^{4–6} or LacZ^{7,8} require additional steps involving use of secondary antibodies or immunohistochemical reactions for analysis. These steps preclude their direct use in intravital microscopy studies. Thus, cells constitutively expressing a fluorescent protein that does not require additional steps for detection, such as green fluorescent protein (GFP), would be useful reagents for cell trafficking and transplantation studies.

Previously, Okabe and co-workers⁹ generated GFP-expressing mice using a ubiquitous promoter, and showed that GFP is expressed in multiple tissues and cells. Here, we describe the generation of similar GFP transgenic mice, show that all analyzed leukocyte subtypes express GFP, and that these cells are useful for cell trafficking and bone marrow transplantation studies.

Materials and Methods

Transgene Construction and Microinjection

The plasmid containing the CMV-EGFP transgene was graciously provided by Dr. M. Okabe (Osaka University, Osaka, Japan) and was described previously.⁹ Plasmid (100 μ g) was digested with *Bam*HI and *Sal*I for 24 hours at 37°C. The digested DNA was centrifuged on a continuous 10 to 40% sucrose gradient using a Beckman ultracentrifuge with a SW51 rotor at 36K at 25°C for 20 hour.¹⁰ Fractions containing the transgene were identified by gel electrophoresis, pooled, and desalted using a Centricon 100 column (Millipore Corp., Bedford, MA).

Accepted for publication September 28, 2000.

Address reprint requests to Dr. Sergio A. Lira, Department of Immunology, Schering-Plough Research Institute 2015 Galloping Hill Rd. Kenilworth, NJ 07033. E-mail: sergio.lira@spcorp.com.

Generation of GFP Transgenic Mice

The transgene was resuspended in microinjection buffer (5 mmol/L Tris-HCl, pH 7.4, 5 mmol/L NaCl, 0.1 mmol/L ethylenediaminetetraacetic acid) to a final concentration of 1 to 5 ng/ml, and microinjected into C57BL/6J × DBA/2 F2 eggs (The Jackson Laboratory, Bar Harbor, ME). Injected eggs were transferred into oviducts of ICR (Sprague-Dawley) foster mothers, according to published procedures.¹¹ At birth, transgenic founders were identified by the green fluorescence of their skin under a long-wave UV lamp. By 10 days of life, a small piece of tail was clipped for DNA analysis, and the presence of the transgene in their DNA was confirmed by a polymerase chain reaction-based screening assay using the following primers: (5'-TTCAAGGACGACGGAAGTA-3') and (5'-GGCGGTCTACGAACTCCAG-3'). As an internal control for the amplification reaction, primers for the endogenous ZP3 gene were used (5'-CAG CTC TAC ATC ACC TGC CA-3'; 5'-CGCAGTGCTCCTCATCTGACTTGT-3'). These primers amplify a 363-bp segment of the EGFP transgene and a 511-bp segment of the ZP3 gene. The polymerase chain reactions were done under the following conditions: 95°C, 30 seconds; 60°C, 30 seconds; 72°C, 60 seconds for 30 cycles. Because of the complete concordance between the green skin phenotype and the genotyping results, the UV hand-held lamp was routinely used to identify GFP+ transgenic mice. Transgenic animals were kept under specific pathogen-free conditions.

Generation of Embryonic Stem-Derived Transgenic Mice Expressing GFP

CMV-EGFP DNA was linearized with *Sall*. DNA (20 µg) was mixed with 10⁷ 129SvEv-derived embryonic stem cells in 0.8 ml of phosphate-buffered saline, placed in a 0.4 cm Bio-Rad cuvette and electroporated using a Bio-Rad Gene Pulser (Bio-Rad, Richmond, CA) set at 0.24 kV volts and 500 µF capacitance. Electroporated cells were seeded at a density of ~10⁶ cells/60-mm tissue culture plate and cultured for 3 days in the presence of irradiated mouse embryo fibroblasts. From a total of 5 × 10⁷ electroporated embryonic stem cells, 10 GFP-expressing embryonic stem cell colonies were identified using a fluorescence microscope. Three of these colonies were expanded into cell lines, which were used to generate chimeric mice by microinjection into C57BL/6 blastocysts. Two of the injected embryonic stem cell lines yielded chimeras that transmitted the GFP gene to their offspring, as determined by visual inspection of the pups under a UV lamp. One of these two lines of mice exhibited a level of green fluorescence in blood leukocytes comparable to that seen in the mouse line generated by transgenesis. This line was used in the studies presented here.

Histology

Mice were sacrificed and tissues were freshly frozen in freezing media. GFP fluorescence of frozen sections (5 to

8 µm) was analyzed using a Nikon E800 microscope with a GFP filter cube. No fixation conditions were used because the GFP fluorochrome is sensitive to fixation.

Flow Cytometry

Single cell suspensions were prepared from individual tissues by passage through a 100-µmol/L nylon cell strainer (BD Biosciences-Labware, Bedford, MA) in RPMI medium containing 10% fetal calf serum. Cells (10⁵ to 10⁶) were incubated with 5 µg/ml Fc block (BD Pharmingen, Los Angeles, CA) and 300 µg/ml of mouse IgG (Pierce Chemical Co, Rockford, IL). Cells were directly analyzed for GFP expression or stained with the directly conjugated primary monoclonal antibody in phosphate-buffered saline, 1% bovine serum albumin, 0.1% sodium azide for 20 minutes at 4°C in the dark. Just before acquisition, 20 µl of 5 µg/ml of propidium iodide (Calbiochem-Novabiochem, San Diego, CA) was added to each sample and events were acquired on a Becton-Dickinson FACScan (Becton-Dickinson). Data were analyzed using CellQuest Software version 3.1. Monoclonal antibodies to detect the following mouse cell subtypes were purchased from Pharmingen (BD Pharmingen): T cells: CD3 (145-211C), CD4 (RM4-5), CD45RB (16A), and TCRαβ (H57-597); dendritic cells: CD11c (HL3); NK cells: Pan NK (DX5); macrophages: Mac-1 (M1/70); and B cells: CD45R/B220 (RA3-6B2).

Expansion of Spleen and Bone Marrow-Derived Immature Dendritic Cells

Spleens were harvested, injected with 100 U/ml of collagenase-D (Roche Molecular Biochemicals, Indianapolis, IN), minced, and incubated in Hanks' balanced salt solution containing 400 U/ml of collagenase-D for 30 minutes at 37°C. After incubation, the cells were collected by passage through a 100-µm nylon cell strainer, centrifuged, and resuspended in 1 ml of 37.5% bovine serum albumin in Hanks' balanced salt solution buffer. This cell preparation was then overlaid with 2 ml of Hanks' balanced salt solution and centrifuged for 30 minutes at 2,200 rpm at 4°C. Cells at the interphase were collected and plated in 24-well plates at 2 × 10⁶ cells/well. Dendritic cells were expanded for 7 days in RPMI medium containing 5% fetal calf serum, 1× penicillin-streptomycin, and 20 ng/ml granulocyte-macrophage colony-stimulating factor (GM-CSF; Schering-Plough, Kenilworth, NJ) as previously described.¹² Cells were washed and fresh medium was added on days 2, 4, and 6. Bone marrow cells were isolated from the tibia and femur by flushing with RPMI medium containing 10% fetal calf serum. Single cell suspensions were prepared by passage through a 100-µmol/L nylon cell strainer (BD Biosciences-Labware). After lysis of red blood cells, bone marrow cells were collected by centrifugation and resuspended in RPMI containing 10% fetal calf serum. Bone marrow cells were cultured as described for the splenocytes.

Figure 1. Expression of GFP in splenocytes. GFP fluorescence is detected in splenocytes from B6D2Tg and 129Tg mice but not in splenocytes from control mice. RCN, relative cell number.

Adoptive Transfer and Bone Marrow Transplantation Studies

For adoptive transfer studies, spleens were isolated from B6D2Tg or 129Tg mice. Single cell suspensions were prepared from the tissues by passage through a 100 μ mol/L nylon cell strainer (BD Biosciences-Labware) in RPMI medium containing 10% fetal calf serum. Cells (2×10^7) were injected into the lateral tail vein of nonirradiated B6D2 or 129 (Taconic Farms, Germantown, NY). At various time points after transfer, recipient mice were sacrificed and the tissues were analyzed for the presence of GFP⁺ cells.

For transplantation studies, bone marrow cells were isolated from B6D2Tg mice. Single cell suspensions were prepared as described above. Cells (5×10^7) were transferred into B6D2 F1 recipient mice that received 1,100 rads from a Cesium 137 irradiator.

Results

Generation of GFP Transgenic Mice in a Mixed Genetic Background

Mice expressing GFP were generated in the C57BL/6 $\times$ DBA2/J background (B6D2) using the CMV-EGFP transgene, which has been previously described.⁹ In this transgene, GFP is expressed from a human CMV enhancer-chicken β -actin promoter cassette, which directs expression to virtually all tissues, with particularly high expression seen in muscle and pancreas.⁹ Transgenic founder pups were initially identified by the fluorescence of their skin when placed under a hand-held UV light. Subsequently, their genotypes were confirmed by a polymerase chain reaction-based screening strategy. In addition to skin, these transgenic mice had high levels of GFP fluorescence in skeletal muscle, pancreas, and heart. The expression of GFP in these organs had no apparent deleterious effect on the health of these transgenic mice because they developed normally, were fertile, and had a normal life span. Six GFP founder mice

were bred to generate lines. The transgenic line with the highest levels of fluorescence in peripheral blood cells was selected for further analysis. This line will be referred to throughout the text as B6D2Tg.

Generation of GFP Transgenic Mice in an Inbred Genetic Background

To facilitate adoptive transfer studies between syngeneic mice, GFP transgenic mice were also generated in the 129 background. The CMV-EGFP transgene was transfected into embryonic stem cells derived from 129 mice, and colonies of cells expressing the transgene were identified under UV light. These cells were expanded and injected into host blastocysts. Chimeric male mice were bred to 129 females, and their offspring were screened for transgene expression by fluorescence under UV light. Two individual embryonic stem cell lines yielded chimeras that transmitted the GFP transgene to their offspring. The transgenic line with the highest level of fluorescence in peripheral blood cells was selected for further analysis. This line will be referred to throughout the text as 129Tg.

GFP Is Expressed in Multiple Leukocyte Subtypes

Flow cytometry was used to compare the level of GFP expression in splenocytes derived from B6D2Tg and 129Tg mice. Comparable levels of fluorescence were seen in these transgenic lines, whereas no fluorescence was observed in nontransgenic mice (Figure 1). Further analysis revealed that >95% of cells in all lineages analyzed, including B, T, NK, and dendritic cells, macrophages, and neutrophils, express GFP (Figure 2). The relative number of these various cell types did not differ between control and transgenic mice, indicating that expression of GFP in these cells did not affect cell development.

Some less abundant cell lineages, such as dendritic cells, are often expanded *in vitro* before their introduction

Figure 2. Expression of GFP in leukocyte subsets in the spleens of transgenic mice. Single cell suspensions were prepared from the spleens of B6D2Tg mice, stained with the indicated antibodies and analyzed by flow cytometry. The percentages of GFP⁺ lineage⁺ and GFP⁺ lineage⁺ cells in the transgenic mice and the percentage of lineage⁺ cells in nontransgenic mice are indicated in the corresponding quadrant. Similar results were obtained in 129Tg mice.

into recipient mice. To determine whether dendritic cells from GFP Tg mice would retain their fluorescence after culture *in vitro*, we prepared dendritic cells from spleen and bone marrow, cultured them for 6 days in the presence of GM-CSF,¹² and then assessed their fluorescence. Both spleen- and bone marrow-derived dendritic cells fluoresced brightly after their expansion *in vitro* (Figure 3), suggesting that these cultured cells might be useful in dendritic cell trafficking studies.

Adoptive Transfer of GFP+ Splenocytes to Wild-Type Mice

Fluorescence microscopy was used next to determine whether donor GFP+ splenocytes could be detected after their transfer into allogeneic recipient mice. GFP+

Figure 3. Expression of GFP in spleen- and bone marrow-derived dendritic cells. Dendritic cells were expanded *in vitro* for 7 days in the presence of GM-CSF. Bone marrow-derived dendritic cells (**top**) and spleen-derived dendritic cells (**bottom**) were photographed using fluorescence microscopy.

cells could be clearly detected in the white pulp of spleens of donor B6D2Tg mice (Figure 4A). Whereas only background fluorescence was detected in mice receiving nontransgenic splenocytes (Figure 4B), strong fluorescence was seen in spleens of mice that received transgenic splenocytes (Figure 4C). We next used flow cytometry to examine the tissue distribution of GFP+ donor cells after their transfer into allogeneic recipients. GFP+ cells were detected in multiple tissues, including spleen and mesenteric lymph nodes within 3 hours after transfer, and they remained detectable for at least 9 days after transfer (Figure 5A). Similar results were obtained when blood and bone marrow samples were analyzed (data not shown).

To compare the longevity of donor cells in allogeneic and syngeneic recipients, GFP+ donor cells from 129 transgenic mice were transferred into wild-type 129 recipients. As seen with the allogeneic cell transfers, GFP+ cells were detected in spleen and mesenteric lymph nodes of recipient mice within 2 hours of transfer (Figure 5B). In addition, GFP+ cells were detected in Peyer's patches, blood, and bone marrow (data not shown). However, in syngeneic transfers, donor cells remained detectable for at least 3 weeks after transfer. To determine whether GFP+ lineage subtypes could be detected after transfer, we used CD4 and CD45RB staining (Figure 6A). CD4 cells can be subdivided into memory and naïve

Figure 4. Adoptive transfer experiments using GFP+ cells. Splenocytes from B6D2Tg and control mice were injected intravenously into B6D2 nontransgenic mice. Two hours after transfer, the spleens were harvested, and fresh frozen sections were prepared and analyzed by fluorescence microscopy. Shown are representative spleen sections from a B6D2Tg mouse (**A**), and from recipients of B6D2 nontransgenic (**B**) and B6D2Tg transgenic (**C**) splenocytes.

cells based on expression of CD45RB. Both memory (CD45RBlo) and naïve (CD45RBhi) T cells were readily detected for at least 1 week after transfer (Figure 6B). Thus, GFP-expressing transgenic mice can be used as a source of memory and naïve T cells for trafficking studies.

To determine whether GFP+ T cell subsets could be detected after transfer, we used CD4 and CD45RB staining. CD4 cells can be subdivided into memory and naïve cells based on expression of CD45RB. As seen in Figure 6A, CD4⁺CD45RBlo memory and CD4⁺CD45RBhi naïve cells can be clearly distinguished in recipient mice. To determine whether some of these cells were derived from donor mice, we gated on memory (CD45RBlo) and naïve (CD45RBhi) T cells and analyzed these individual populations for expression of GFP. This analysis revealed that GFP+ cells represented a small but detectable percentage of memory and naïve T cells for at least 1 week after transfer (Figure 6B). Thus, GFP-expressing transgenic

mice can be used as a source of memory and naïve T cells for trafficking studies.

Transplantation of GFP+ Bone Marrow Cells into Lethally-Irradiated Mice

Successful transplantation requires survival and engraftment of the transferred cells. To determine whether GFP+ cells would provide a convenient and quick method for monitoring successful transplantation, bone marrow cells of GFP transgenic mice were injected into lethally irradiated mice, and the presence of donor cells in lymphoid tissues was monitored throughout time. At 24 hours after transfer, GFP+ cells represented between 0.4 to 6% of total cells in the blood, spleen, mesenteric lymph nodes, and bone marrow (Figure 7). After this time, the relative number of GFP+ cells in these tissues increased and by 1 to 3 weeks after transfer, the percentage of GFP+ cells was comparable to that seen in the same tissues of the donor transgenic mice. To investigate the extent of engraftment at a later time point (22 weeks), a wide variety of tissues including blood, bone marrow, spleen, lung, liver mesenteric lymph nodes, Peyer's patches, ears, small and large intestine colon, and brain were examined for GFP expression. GFP+ cells represented from 70 to 90% of all CD45+ cells in every tissue analyzed except brain, which had only 40 to 50% of the CD45+ leukocytes expressing GFP (data not shown). These data demonstrate that donor GFP+ hematopoietic stem cells can reconstitute the leukocyte populations of most tissues.

Figure 5. Relative number of GFP+ cells in the spleen and mesenteric lymph nodes of control mice after injection of GFP+ cells. **A:** Relative number of GFP+ cells in allogeneic recipients (representative of two experiments, $n = 3$ mice per point). **B:** Relative number of GFP+ cells in syngeneic recipients. Bars represent average $\pm$ SD (representative of three experiments, $n = 3$ mice per point).

Discussion

In this report, we describe the characterization of mice expressing GFP in virtually all tissues and cells. Two transgenic lines expressing GFP under the direction of

Figure 6. Detection of memory and naïve GFP⁺ T cells in syngeneic recipients. GFP⁺ splenocytes were transferred to recipient mice. **A:** Expression of CD4 and CD45RB define memory (M: CD45RB^{lo}) and naïve (N: CD45RB^{hi}) T cell populations in the spleen of recipient mice. **B:** Cells were analyzed for expression of GFP⁺ cells after gating on CD4⁺CD45RB^{lo} and CD4⁺CD45RB^{hi} populations. GFP⁺ cells are detected within both memory and naïve CD4⁺ populations, at 2 hours and 1 week after transfer.

the CMV enhancer- β -actin promoter were studied; one in a mixed genetic background (B6D2), and the other in an inbred background (129). Both lines of transgenic mice express GFP in multiple tissues, as previously reported by others for similar transgenic mice.^{9,13,14} Despite high levels of GFP expression in all major hematopoietic cell lineages, the relative proportions of these leukocyte subsets in transgenic mice were indistinguishable from those of nontransgenic littermates. Thus, GFP expression does not adversely affect the development of the major leukocyte lineages. These data support and extend the experiments reported by others, who showed that GFP did not affect the development, function, or survival of T and B cells.^{13,15–18}

The broad expression pattern of GFP in the transgenic mice suggests that a variety of leukocyte subsets derived from these mice can be used in cell trafficking studies. These cells have several advantages over cells from conventional mice that are labeled *ex vivo*. For example,

they continue to fluoresce even after having undergone extensive proliferation, and no cofactors, substrates, or antibodies are required for their detection. These features make GFP-expressing cells excellent reagents for use in cell-trafficking and transplantation studies.

In addition to the major classes of leukocytes, GFP is also expressed in minor cell populations such as dendritic cells. Dendritic cells are potent antigen-presenting cells and therefore play a key role in initiating immune responses. Recent studies have revealed that multiple subsets of dendritic cells exist, but much remains to be learned about the developmental history of these cells and their migration patterns *in vivo*. GFP-expressing dendritic cells can be used to facilitate these studies. Interestingly, GFP expression is retained in cultured dendritic cells, suggesting that the dendritic cells that have been manipulated or amplified *in vitro* can be monitored after their transfer to recipient animals.

We have also shown GFP expression can be used to track the migration patterns of cells within a defined cell lineage. That is exemplified by our experiments studying naïve and memory T cells. These cells can be tracked *in vivo* for at least 1 week after transfer. Other cells that can also be studied with this system include T helper cells that have been polarized *in vitro* so that they produce either Th1- or Th2-associated cytokines. The ability to monitor these various cell lineages *in vivo* provides a means to investigate their trafficking patterns, life span, and biological function in both physiological and pathological states.

In bone marrow transplantation experiments, full reconstitution of most tissues, including bone marrow,

Figure 7. Time course of engraftment of GFP⁺ bone marrow cells. The relative number of GFP⁺ cells was determined in blood, bone marrow, mes.LN, and spleen of recipient mice. Bars represent average $\pm$ SD ($n = 3$ mice per point).

blood, spleen, and lymph node was obtained within 3 weeks after transfer and by 6 months, 70 to 90% of the CD45+ cells were reconstituted in all tissues analyzed except for the brain. These data are consistent with previous studies of reconstitution in various tissues, including the central nervous system.^{19,20} In those studies, GFP+ cells were detected at various levels in different regions of the brain, but at lower levels than in other peripheral tissues such as the spleen. Our data and those of others support the use of GFP+ cells to study the reconstitution potential of hematopoietic cells in various tissues and models.

Genetic crosses between GFP+ mice and mice bearing gene targeted mutations or other transgenes will provide a means for investigating the role of various genes in cell migration. For example, it is well established that chemokines and chemokine receptors mediate cell migration *in vitro*. However, the role of chemokine receptors in cell trafficking to specific tissues and their positioning within these tissues is less well understood. Crosses between 129 GFP+ mice and 129-derived mice bearing chemokine receptor transgenes or targeted mutations will facilitate detailed studies of chemokine receptor function *in vivo*.

GFP+ mice will also be very useful in cancer studies. Crosses between GFP+ transgenic mice and genetically defined mice that develop tumors will permit the generation of GFP+ tumor cells that can be used to study various aspects of tumor biology, including metastasis. Similarly, in transplant experiments, the biology of both host and graft tissue can be more easily studied with cells having a long-lived, reliable marker such as GFP.

In summary, the transgenic GFP mice reported here will provide a readily available source of donor leukocytes—and other cell types—whose trafficking patterns can be easily followed by fluorescence microscopy or by flow cytometry. These cells will be useful for a wide range of *in vivo* studies, including those involving cell trafficking, cell longevity, and the effect of experimental drugs.

Acknowledgments

We thank Dr. M. Okabe and Dr J. Miyazaki for sharing with us the CMV-EGFP construct used for generation of the transgenic mice; and Azra Misra for her assistance in isolating and expanding bone marrow-derived dendritic cells.

References

- Weston SA, Parish CR: New fluorescent dyes for lymphocyte migration studies. Analysis by flow cytometry and fluorescence microscopy. *J Immunol Methods* 1990, 133:87–97
- Berlin-Rufenach C, Otto F, Mathies M, Westermann J, Owen MJ, Hamann A, Hogg N: Lymphocyte migration in lymphocyte function-associated antigen (LFA)-1-deficient mice. *J Exp Med* 1999, 189: 1467–1478
- Buzoni-Gatel D, Debbabi H, Moretto M, Dimier-Poisson IH, Lepage AC, Bout DT, Kasper LH: Intraepithelial lymphocytes traffic to the intestine and enhance resistance to *Toxoplasma gondii* oral infection. *J Immunol* 1999, 162:5846–5852
- Nagoshi M, Sadanaga N, Joo HG, Goedegebuure PS, Eberlein TJ: Tumor-specific cytokine release by donor T cells induces an effective host anti-tumor response through recruitment of host naive antigen presenting cells. *Int J Cancer* 1999, 80:308–314
- Richters CD, van Gelderop E, du Pont JS, Hoekstra MJ, Kreis RW, Kamperdijk EW: Migration of dendritic cells to the draining lymph node after allogeneic or congenic rat skin transplantation. *Transplantation* 1999, 67:828–832
- Kang BY, Lim YS, Chung SW, Kim EJ, Kim SH, Hwang SY, Kim TS: Antigen-specific cytotoxicity and cell number of adoptively transferred T cells are efficiently maintained *in vivo* by re-stimulation with an antigen/interleukin-2 fusion protein. *Int J Cancer* 1999, 82:569–573
- Kennedy DW, Abkowitz JL: Mature monocytic cells enter tissues and engraft. *Proc Natl Acad Sci USA* 1998, 95:14944–14949
- Kapasi ZF, Qin D, Kerr WG, Kosco-Vilbois MH, Shultz LD, Tew JG, Szakal AK: Follicular dendritic cell (FDC) precursors in primary lymphoid tissues. *J Immunol* 1998, 160:1078–1084
- Okabe M, Ikawa M, Kominami K, Nakanishi T, Nishimune Y: 'Green mice' as a source of ubiquitous green cells. *FEBS Lett* 1997, 407: 313–319
- Mann JR, McMahon AP: Factors influencing production frequency of transgenic mice. *Methods Enzymol* 1993, 225:771–781
- Hogan B, Beddington R, Costantini F, Lacy E: Manipulation of the Mouse Embryo. Plainview, Cold Spring Harbor Laboratory Press, 1994
- Inaba K, Inaba M, Romani N, Aya H, Deguchi M, Ikehara S, Muramatsu S, Steinman RM: Generation of large numbers of dendritic cells from mouse bone marrow cultures supplemented with granulocyte/macrophage colony-stimulating factor. *J Exp Med* 1992, 176:1693–1702
- Ikawa M, Kominami K, Yoshimura Y, Tanaka K, Nishimune Y, Okabe M: Green fluorescent protein as a marker in transgenic mice. *Dev Growth Differ* 1995, 37:455–459
- Ikawa M, Yamada S, Nakanishi T, Okabe M: 'Green mice' and their potential usage in biological research. *FEBS Lett* 1998, 430:83–87
- Kawakami N, Sakane N, Nishizawa F, Iwao M, Fukada SI, Tsujikawa K, Kohama Y, Ikawa M, Okabe M, Yamamoto H: Green fluorescent protein-transgenic mice: immune functions and their application to studies of lymphocyte development. *Immunol Lett* 1999, 70:165–171
- Bierhuizen MF, Westerman Y, Visser TP, Wognum AW, Wagemaker G: Green fluorescent protein variants as markers of retroviral-mediated gene transfer in primary hematopoietic cells and cell lines. *Biochem Biophys Res Commun* 1997, 234:371–375
- Ramiro AR, De Yebenes VG, Trigueros C, Carrasco YR, Toribio ML: Enhanced green fluorescent protein as an efficient reporter gene for retroviral transduction of human multipotent lymphoid precursors. *Hum Gene Ther* 1998, 9:1103–1109
- Persons DA, Allay JA, Riberdy JM, Wersto RP, Donahue RE, Sorrentino BP, Nienhuis AW: Use of green fluorescent protein as a marker to identify and track genetically modified hematopoietic cells. *Nat Med* 1998, 4:1201–1205
- Ono K, Takii T, Onozaki K, Ikawa M, Okabe M, Sawada M: Migration of exogenous immature hematopoietic cells into adult mouse brain parenchyma under GFP-expressing bone marrow chimera. *Biochem Biophys Res Commun* 1999, 262:610–614
- Wu YP, McMahon E, Kraine MR, Tisch R, Meyers A, Frelinger J, Matsushima GK, Suzuki K: Distribution and characterization of GFP(+) donor hematogenous cells in Twitcher mice after bone marrow transplantation. *Am J Pathol* 2000, 156:1849–1854