Published in final edited form as:

J Reprod Immunol. 2011 August ; 90(2): 188–194. doi:10.1016/j.jri.2011.05.004.

Maternal background strain influences fetal-maternal trafficking more than maternal immune competence in mice

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Abstract

The objective of this study was to determine if fetal-maternal cell trafficking is affected by maternal immune competence and/or parental background strain using fluorescence-activated cell sorting (FACS). In our experience the sensitivity of FACS allows for the detection of 5 fetal in 10⁷ maternal cells and assessment of cell surface phenotype. Wild-type C57BL/6J (n=18), FVB/NJ (n=15), and immunodeficient B6129S7-Rag1^{tm1Mom}/J (n=16) female mice were mated to C57BL/6J males homozygous for the green fluorescent protein (GFP) transgene. Single cell suspensions of maternal lung, liver, spleen, bone marrow, and blood were analyzed between late gestation (day e16–18) and one day post-partum for the number of GFP-positive fetal cells in relation to 10⁷ maternal cells and the percentage of GFP-positive cells that expressed the surface markers CD11b, CD29, CD34, CD44, or CD105. The highest relative proportions of GFP-positive fetal cells were observed in maternal lungs and livers from immunocompetent allogenic females. Among congenic matings, fetal cell microchimerism was higher in immunodeficient compared with immunocompetent females. Maternal strain and strain differences between the mother and father statistically significantly affected both the numbers of fetal cells and the relative distribution of cell types in maternal organs. The highest relative proportion of fetal cells was observed in allogenic matings with immunocompetent females. Since allogenic matings are more similar to those that occur in humans, future studies using animal models of microchimerism should consider incorporating this type of experimental design.

Keywords

fetal cell trafficking; mouse model; background strain

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1. Introduction

Fetal cell microchimerism (FCM) is a term that describes the presence of fetal cells in maternal blood and organs during pregnancy and post-partum. It has been studied in both humans and animal models (Johnson and Bianchi 2004; Lissauer et al. 2007; Dutta and Burlingham 2009). In humans, most studies on FCM have used the Y chromosome as a marker of fetal cells, thereby limiting the study subject population to women who have had sons (Johnson et al. 2001). Because of this and other inherent limitations of human studies, our laboratory began to use a murine model of FCM in which males that are transgenic for the green fluorescent protein (GFP) are used for breeding. The model we initially used incorporated hemizygous males with the transgene present on an autosome, so its inheritance was independent of fetal gender. Fifty percent of the offspring were GFP-positive (Khosrotehrani et al. 2004; 2005). With this model, we showed that the frequency of FCM was highest in the maternal lung, peaked just prior to parturition, and declined rapidly post-partum (Khosrotehrani et al. 2005; Fujiki et al. 2008a). Following this work, we incorporated a strain that was homozygous for the *Gfp* transgene, resulting in 100% fetal cell detection. This strain was used earlier by other investigators to study fetal cell microchimerism in the maternal mouse brain (Tan et al. 2005). In a prior study from our group using this homozygous transgenic model, we demonstrated that fetal cells in maternal organs expressed a variety of cell surface antigens, including CD34, CXCR4 (CD184), SLAMF1 (CD150), CD45, integrin alpha M (CD11b), integrin beta 1 (CD29), CD44, and endoglin (CD105) (Fujiki et al. 2009).

The role of immune competence and background strain in FCM was first studied in the mouse by Bonney and Matzinger (1997). Using PCR amplification of a Y chromosome sequence, they showed that fetal cell trafficking occurs in only 20% of wild-type pregnancies, and that early in pregnancy immune compromised (SCID) and normal females have equivalent levels of trafficking. FCM in SCID increased relative to normal females as pregnancy progressed. This suggested that SCID mice were not able to clear fetal cells as efficiently as wild-type mice, which implied involvement of the maternal immune system. In the same study, Bonney and Matzinger (1997) demonstrated higher levels of FCM in pregnant females following allogenic matings. In another study, our laboratory showed that there was a non-statistically significant trend toward higher levels of cell-free fetal DNA in maternal plasma from allogenic compared with congenic females (Khosrotehrani et al. 2004). We suggested that this was due to a more robust maternal immune response to fetal allo-antigens, which resulted in increased placental apoptosis and levels of fetal DNA in the maternal circulation. In a follow-up investigation, we also demonstrated that there was a statistically significantly higher level of FCM in maternal solid tissues from congenic than from allogenic matings, particularly in the spleen and liver, although only five allogenic pregnancies were analyzed (Khosrotehrani et al. 2005). In an investigation that specifically examined the effect of histocompatibility between mother and fetus on cell migration through the placenta, FCM was higher in allogenic than syngenic gestations (Vernochet et al. 2007).

Taken together, these studies suggest that genetic differences between the mother and fetus, as well as the maternal immune system, can influence FCM. A consistent limitation of these previous studies, however, was that PCR amplification was used to measure fetal DNA sequences. PCR has a sensitivity of detection of 1 fetal in 10^5 maternal cells. In our experience, flow cytometry is a more robust method for the detection of fetal cells, as it has significantly higher sensitivity (5×10^7), and allows for the simultaneous assessment of cell surface phenotype (Fujiki et al. 2008b; 2009).

The purpose of the current study was to determine if FCM is affected by maternal background strain and/or immune competence using the most sensitive technique available, flow cytometry. We examined maternal organs that we have previously shown to contain the highest frequency of fetal cells (lung, liver, spleen, bone marrow, and blood) from immunocompetent and immunodeficient females that were allogenic and congenic with their breeding partners. We further sought to characterize the fetal cells through analysis of specific cell surface markers, including antigens expressed by hematopoietic cells (CD34), myeloid cells (CD11b), and mesenchymal cells (CD29, CD44, and CD105) (Fujiki et al. 2009).

2. Materials and Methods

2.1. Animals

All protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of the Tufts University School of Medicine Division of Laboratory Animal Medicine (DLAM). All institutional guidelines regarding the ethical use of experimental animals were followed.

Wild-type C57BL/6J (congenic, immunocompetent; n=18), FVB/NJ (allogenic, immunocompetent; n=15), or B6129S7-Rag1^{tm1Mom}/J (with a C57BL/6J background; from here on in referred to as “RAG”; congenic, immunodeficient; n=16) females, all purchased from Jackson Laboratory (Bar Harbor, ME, USA), were mated to male C57BL/6-Tg(CAG-EGFP)C14-Y01-FM131OSb (Riken BioResource Center, Ibaraki, Japan) mice that were homozygous for the enhanced green fluorescent protein (GFP) transgene. These mice have a copy of the *Gfp* transgene on each homolog of chromosome 14 under the control of a ubiquitous chicken beta-actin promoter and a cytomegalovirus enhancer (Nakanishi et al. 2002).

Eight- to ten-week-old virgin female mice were used for the matings. They were weighed prior to being placed with a male. They remained in their cages with males during the entire gestation period, and were considered to be pregnant when they had gained 10% of their baseline body weight (Johnson et al. 2010). Gestational age of the pups was determined at the time of sacrifice based on Theiler staging (Theiler 1989). For each mouse, the total litter size, as well as the average crown–rump length of the litter, was recorded. Only females between late gestation (day e16–18) and one day post-partum were studied.

2.2. Tissue collection and single cell suspension

Blood was obtained by cardiac puncture. Erythrocytes were removed from 600 μ L of whole blood by lysis (0.15M NH₄Cl, 1.0 mM KHCO₃, 0.1 mM Na₂EDTA). Mononuclear cells from the blood samples were washed twice with flow cytometry buffer (phosphate buffered saline [PBS] with 2% fetal bovine serum and 0.1% sodium azide). Lung, liver, spleen, and bone marrow were obtained after gross dissection, and bone marrow cells were isolated from bilateral femoral bones. Solid tissues were dissected into small pieces in flow cytometry buffer and homogenized using a GentleMacs[®] homogenizer instrument (Miltenyi, Bergisch Gladbach, Germany). Bone marrow cells and homogenates from solid organs were filtered through a 30- μ m nylon filter (Miltenyi) to remove debris and cell clumps. Single cell suspensions from homogenized organs and bone marrow were washed once in flow cytometry buffer.

2.3. Fetal cell phenotyping

Cells were stained for 60 to 90 min with monoclonal antibody. The antibodies used were allophycocyanin-conjugated anti-mouse CD44 (eBioscience, San Diego, CA, USA), and

phycoerythrin-conjugated anti-mouse CD11b (BD Pharmingen, San Diego, CA, USA), CD29, CD34, and CD105 (eBioscience). All antibody concentrations were optimized by flow cytometry using serial dilutions of each antibody with a constant number of cells from each target organ prior to analysis. After antibody staining, cells were washed twice and resuspended in flow cytometry buffer. High-speed flow cytometric analysis was conducted using a MoFlo flow cytometry instrument (Dako, Fort Collins, CO, USA). GFP was excited at 488 nm and measured at 510 nm (530/30 filter). Allophycocyanin was excited at 633 nm and measured at 660 nm (680/30 filter) and phycoerythrin was excited at 488 nm and measured at 575 nm (585/42 filter). Abnormally shaped cells were excluded by forward-angle (FSC) and side-angle (SSC) scattered light gating. Autofluorescent noise, which distributes in a linear pattern through 570/40 and 670/40 filters, was gated out. An example of our gating strategy is shown in Fig. 1. High-speed flow sorting (2 to 8×10^4 cells per second) was conducted for each sample. These numbers do not include all of the GFP-positive cells owing to the gating strategy shown in Fig. 1. Summit V4.3 software (Dako) was used for data analysis. Fetal GFP-positive cell counts were recorded as the number per 10^7 maternal cells. Antibody-positive fetal cell counts were recorded as the number of cells among the total number of GFP-positive cells in each organ. Three to four mice from each strain were analyzed for each antibody.

2.4. Statistical Analyses

The median number of GFP-positive fetal cells as a proportion of 10^7 maternal cells in each maternal organ among the three female strains was compared using the Kruskal–Wallis test. Subsequently, the Mann–Whitney test was performed as a post-hoc test of pair-wise comparisons. The effect of maternal strain on the percentage of antibody-positive fetal cells was tested using linear regression analysis with the adjustment for antibody class for each organ separately. The cell counts were logarithmically transformed to account for skewed data distribution. Statistical significance was assigned when $p < 0.05$. All statistical tests were performed using SAS/STAT software (SAS Institute, Cary, NC, USA).

3. Results

The raw numbers of GFP-positive cells relative to 10^7 maternal cells and the proportion of fetal cells that were antibody-positive from all three female strains are shown in Table 1. In C57BL/6J and FVB/NJ females, the median number of fetal cells in solid organs was consistently highest in the lung, followed by the liver and spleen. In RAG females, the median numbers of GFP+ fetal cells were roughly equivalent in the lung and liver. The number of fetal cells was lowest in bone marrow and blood in all three female strains (Table 1).

Between female strains, the highest median number of fetal cells as a proportion of maternal cells in solid organs (lung, liver, and spleen) was in immunocompetent allogenic matings compared with both strains of congenic females, although this difference reached statistical significance only in lung and liver (overall $p=0.045$ and 0.005 respectively; Fig. 2). In the lung, pair-wise comparison showed that this difference was solely driven by that between allogenic immunocompetent and congenic immunocompetent females ($p=0.01$). In the liver, this difference was also driven by that between allogenic immunocompetent and congenic immunocompetent females ($p=0.003$), although there was also a statistically significant difference between congenic immunocompetent and congenic immunocompromised females ($p=0.007$). The number of fetal cells in bone marrow was also statistically significantly different between strains (overall $p=0.005$), although the numbers were much lower than in lung or liver. Nevertheless, as with lung and liver, pair-wise comparison showed that this difference was driven by that between allogenic immunocompetent and congenic immunocompetent females ($p=0.001$). There was also a marginal yet statistically significant

difference between allogenic immunocompetent and congenic immunocompromised females ($p=0.045$; Fig. 2).

Within the GFP-positive population, the distribution of the five cell surface markers that were analyzed in the three female strains is shown in Table 2. For all organs, maternal strain is a statistically significant predictor of the proportions of antibody-positive fetal cells: lung, $p < 0.0001$; liver, $p < 0.0001$; spleen, $p=0.0003$; bone marrow, $p=0.0004$; and blood, $p=0.004$ with the C57BL/6J mice having the largest number of cells (Table 2). After adjustment for maternal strain, antibody class was associated with the proportion of antibody-positive fetal cells in lung ($p=0.0004$) and possibly in liver ($p=0.066$), where fetal cells expressed CD29 more than any other antigen. CD44-positive fetal cells were predominantly found in the immunocompetent congenic matings. Although the immunocompetent allogenic matings had the highest total median numbers of fetal GFP-positive cells in maternal organs, the fetal cells expressed the overall lowest percentages of all antibodies analyzed here (Table 2).

4. Discussion

In this study, maternal background strain was a more important determinant of FCM than maternal immune competence. The results showed higher proportions of fetal cells in lung, liver and spleen from allogenic females compared with both congenic immunocompetent and immunodeficient females. However, while allogenic females had the highest proportion of GFP-positive fetal cells, the relative proportions of fetal cells that expressed the markers analyzed here (CD 11b, 29, 34, 44 and 105) were lower than those found in congenic females. These results suggest that maternal strain plays a role in the distribution of fetal cell types within maternal organs.

These results are in contrast with previous studies that suggested that congenic females have more fetal cell trafficking (or less fetal cell clearance) than allogenic females (Bonney and Matzinger, 1997; Khosrotehrani et al. 2004, 2005). These differences could largely be due to the fact that the earlier studies used real-time PCR amplification to detect fetal cells, with either the Y chromosome (Bonney and Matzinger, 1997) or the *Gfp* transgene (Khosrotehrani et al., 2004, 2005) sequences as the fetal cell identifier. In addition, Khosrotehrani et al. (2004, 2005) used a paternal mouse that was hemizygous for *Gfp*, so not all fetal cells were detected. In the present study, we used flow cytometry to detect fetal cells, which, as shown in previous studies, is a more sensitive technique (Fujiki et al. 2008a). In addition, we have focused on FACS analysis for the measurement of microchimerism, not only because of the improved sensitivity over PCR, but also because FACS allows for simultaneous detection of GFP and cell surface antibody signals. There were also differences in the timing of gestation of the previous studies. For example, Bonney and Matzinger's 1997 study did not go beyond day e14. We have previously shown that fetal-maternal trafficking peaks at day e18, so the earlier study may have missed a large fetal-maternal transfusion of cells (Fujiki et al. 2008b). Similarly, there were differences between the current and previous studies in some of the maternal organs analyzed. Most importantly, Bonney and Matzinger (1997) did not assay maternal lung, which is the organ that contains the most fetal cells (Fujiki et al. 2008b). However, our results do agree with Bonney and Matzinger (1997) in that the data from congenic females showed more fetal cells in the organs of the immunodeficient females compared with the immunocompetent ones.

It is important to note that we cannot definitively conclude that background strain is the primary contributor to differences in microchimerism. For example, to fully assess for the effect of background strain, the immunocompetency of the different strains must be

comparable. This may not be the case in this study, as FVB/NJ mice have been shown to have elevated IgE levels and are higher Th2 responders than C57BL/6J mice (Zhu and Gilmour 2009). To substantiate our conclusions, a GFP-positive FVB/NJ strain, which at the present is not commercially available, could be incorporated into a future study design. In addition, the use of a method to generate immunodeficiency (e.g., pharmacological immunosuppression) could also be incorporated to create immunocompromised mice of the identical genetic background to those that are immunocompetent. The effect of the degree of allogenicity among “congenic” animals must also be controlled. The extent of congenicity between wild-type C57BL/6J and GFP transgenic mice on a C57/BL6J background are likely to be different, as they were derived and are maintained in different ways (e.g., derivation directly from an embryonic stem cell line, backcrossing). Thus, future studies should also incorporate efforts to control for this variable, such as including wild-type littermates from a transgenic mating for study, as well as regularly backcrossing transgenic animals with original background stock to minimize genetic drift. Finally, the analysis of larger numbers of samples would allow for greater statistical power to assess for the distribution of specific cell types among maternal organs.

Nevertheless, this study validated our previous data that showed that the lung, liver, and spleen are the organs that contain the most fetal cells (Fujiki et al. 2008b). However, although there were more fetal cells detected in the matings with allogenic females, the fetal cells present expressed different cell surface antigens in the organs studied than those in matings from congenic females. Thus, the maternal strain and strain differences between the mother and father appear to affect the numbers and the types of cells found in the maternal organs. In the congenic matings, independent of the mother's immune competence, a wide distribution of cell types was observed. This is similar to that shown in a previous study (Fujiki et al. 2009). In the allogenic matings, however, there was a relative paucity of fetal cells that expressed certain markers, such as CD34, 44 or 105. These data imply that in pregnant allogenic females fetal cells express antigens other than those studied here. Indeed, there were considerable differences among the three maternal strains in the proportion of fetal cells that were negative for the antigens studied here; overall, a large percentage of cells were of an unidentified type. Therefore, additional antibodies representing different cell types must be incorporated into future study design.

In summary, these data suggest that fetal-maternal immune differences affect the types of cells that enter or remain in maternal organs. These differences could contribute to the varying hypothesized consequences of microchimerism for long-term maternal health (Johnson and Bianchi 2004). Since allogenic matings are more similar to those that occur in humans, future studies of FCM using animal models should consider incorporating maternal strain differences into experimental design.

Acknowledgments

The authors wish to thank Stephen Kwok and Allen Parmelee for flow cytometry expertise and Stephanie Pritchard for manuscript preparation. This study was supported by NIH grant R01 HD049469-05 to DWB. Homozygous transgenic mice were provided by Dr. Masaru Okabe, Research Institute for Microbial Disease, Osaka University, Japan through Riken BioResource Center, Ibaraki, Japan.

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Figure 1.

Flow cytometry profiles of maternal spleen cells stained with CD11b antibody from control (C57BL/6J females $\times$ C57BL/6J males; left panel) and congenic immunocompromised (B6129S7-Rag1^{tm1Mom}/J females $\times$ GFP transgenic males; right panel) matings. The R1 gate represents the GFP+ cell population and the R2/R4 gates represent the GFP+/CD11b+ cell population. The R2 and R4 gates are discontinuous to avoid inclusion of autofluorescent cells.

Figure 2.

Boxplots showing the numbers of total GFP-positive fetal cells in maternal lung, liver, spleen, bone marrow and blood among three maternal strains. The Y axes indicate the number of fetal cells among 10 million maternal cells. Note the differences in scale on the Y axes. The box represents the 25th and 50th percentiles; the line within the box is the median; the whiskers represent the 10th and 90th percentiles. Asterisks indicate those organs with statistically significant differences across the three maternal strains (lung: $p=0.045$, liver: $p=0.005$, bone marrow: $p=0.005$). Statistically significant pair-wise comparisons are represented by bars above the corresponding box plots, with associated p values shown.

Table 1
Number of GFP⁺ and GFP/antibody⁺ cells between maternal strains among maternal organs

Animal ID	Strain ^a	CRL	#pups	Antibody	Lung		Liver		Spleen		BM		Blood	
					GFP ⁺	Ab ⁺	GFP ⁺	Ab ⁺	GFP ⁺	Ab ⁺	GFP ⁺	Ab ⁺	GFP ⁺	Ab ⁺
1	C57BL/6J	2	7	CD11b	197	32	69	20	70	12	177	174	76	51
2	C57BL/6J	1.7	9	CD11b	222	64	165	113	103	43	260	252		
3	C57BL/6J	1.7	7	CD11b	464	237	252	98	148	36	83	73	69	53
4	C57BL/6J	2	8	CD29	683	435	509	494	51	18	320	226		
5	C57BL/6J	0.9	7	CD29	140	137	10	9	0	0	4	4	0	0
6	C57BL/6J	1.1	11	CD29	451	403	114	67	17	6	20	19	2	1
7	C57BL/6J	2	7	CD29									41	24
8	C57BL/6J	1.5	10	CD34			152	82	121	19	61	50	20	2
9	C57BL/6J	1.5	9	CD34	99	32	104	60	20	7	19	17	9	5
10	C57BL/6J	2.1	3	CD34	80	50	25	13	7	3	7	6	22	21
11	C57BL/6J	1.9	7	CD34	1444	1152								
12	C57BL/6J	1.9	10	CD44	109	58	114	101	37	9	33	29		
13	C57BL/6J	1.2	7	CD44									0	0
14	C57BL/6J	1.9	5	CD44	161	52	84	39	34	1	12	9		
15	C57BL/6J	2	6	CD44	61	30	27	12	8	1	5	5	2	2
16	C57BL/6J	1.2	8	CD105	72	63	86	58	20	12	31	27	9	5
17	C57BL/6J	1.3	8	CD105	337	167	102	42	69	1	14	10	20	4
18	C57BL/6J	1.5	10	CD105	129	41	135	46	60	0	20	11	17	13
Median	C57BL/6J				161		104		37		20		17	
19	RAG	1.7	7	CD11b	127	13	95	82	20	16	14	14	2	2
20	RAG	1.7	9	CD11b	404	38	297	84	93	4	17	8	12	4
21	RAG	1.4	8	CD11b	310	4	292	13	79	1	15	0	14	0
22	RAG	1.2	8	CD29	551	535	97	58	41	30	72	71	8	4
23	RAG	1.3	8	CD29	874	426	365	188	29	3	4	0	7	0
24	RAG	1	9	CD29	105	75	148	90	1	0	5	2	5	1
25	RAG	1.6	7	CD34	101	38	73	62	34	24	22	20	45	35
26	RAG	1.3	9	CD34	64	7								

Animal ID	Strain ^a	CRL	#pups	Antibody	Lung		Liver		Spleen		BM		Blood	
					GFP+	Ab+	GFP+	Ab+	GFP+	Ab+	GFP+	Ab+	GFP+	Ab+
27	RAG	1.1	8	CD34	340	50	2926	26	45	4	28	12	209	21
28	RAG	1.2	7	CD34	220	10	329	17	95	1	14	2	1	0
29	RAG	1.3	10	CD44	193	118	146	97	21	14	63	10	1	0
30	RAG	1.3	6	CD44	422	21	275	13	21	0	4	0	2	0
31	RAG	1.5	8	CD44	802	56	547	4	19	0	3	0	1	0
32	RAG	1.4	11	CD105	5267	361	11474	531	259	24	164	24		
33	RAG	1.4	10	CD105	197	35	172	45	35	4	11	5	2	1
34	RAG	1.3	9	CD105	104	10	141	33	48	2	9	6	5	0
Median	RAG				265		275		35		14		5	
35	FVB/NJ	1.3	11	CD11b	1216	100	1421	0	182	0	10	0	12	0
36	FVB/NJ	1.3	10	CD11b	1413	56	265	15	168	0	11	4	787	3
37	FVB/NJ	1.4	9	CD11b	745	16	275	3	450	0	7	4	45	3
38	FVB/NJ	2	11	CD29	328	303	310	236	39	24	3	2	0	0
39	FVB/NJ	1.8	10	CD29	652	164	284	36	141	7	22	17	0	0
40	FVB/NJ	1.5	8	CD29	569	113	342	15	89	0	1	0	20	0
41	FVB/NJ	1.4	8	CD34	172	3	95	15	24	2	4	1	1	1
42	FVB/NJ	1.4	9	CD34	286	2	1021	15	149	0	18	2	24	2
43	FVB/NJ	1.4	9	CD34	1000	76	589	5	191	0	15	1	10	0
44	FVB/NJ	1.6	9	CD44	149	10	43	0	3	0	0	0	0	0
45	FVB/NJ	1.1	10	CD44	208	13	61	4	2	0	1	0	0	0
46	FVB/NJ	1.1	6	CD44	258	3	1348	1	84	0	19	7	4	0
47	FVB/NJ	1.5	10	CD105	859	539	402	205	32	1	8	1	139	116
48	FVB/NJ	2	8	CD105	161	2	142	0	494	0	1	0	0	0
49	FVB/NJ	1.6	11	CD105	879	8	786	8	169	0	6	0	22	0
Median	FVB/NJ				569		310		141		7		10	

All data represented as number of cells per 10 million maternal cells
 All females mated to males homozygous for the *GFP* transgene (strain: C57BL/6-Tg[CAG-EGFP]C14-Y01-FM1310sb)
 CRL: crown-rump length of pups, BM: bone marrow, nd: not determined
 RAG: B6.129S7-Rag1^{tm1Mom}/J

Table 2

Antibody positive fetal cell trafficking: comparison of maternal background strains across organs^a

	Female strain ^b															p value ^c
	Immunocompetent Congenic (C57BL/6J)					Immunodeficient Congenic (RAG)					Immunocompetent Allogenic (FVB/NJ)					
Organ	11b	29	34	44	105	11b	29	34	44	105	11b	29	34	44	105	
Lung	28.8	89.4	62.5	49.2	49.6	9.4	71.4	12.8	7.0	9.6	4.0	25.2	1.7	6.3	1.2	
Liver	38.9	90.0	53.9	46.4	41.2	28.3	59.8	5.2	4.7	23.4	1.1	12.7	1.5	0.1	1.0	
Spleen	24.3	35.3	35.0	12.5	1.5	4.3	10.3	8.9	0	9.3	0	5.0	0	0	0	
Bone marrow	96.9	95.0	85.7	87.9	71.4	47.1	40.0	42.9	0	45.5	36.4	66.7	11	0	0	
Blood	72.0	50.0	55.6	50.0	55.6	33.3	20.0	10.0	0	25.0	0.4	0	8.3	0	0	

^aData presented as median percentage of antibody positive cells among total GFP positive cells

^bStrain indicated by immune status of female and genetic relatedness of female compared to male

^cp values represent the overall effect of strain on the proportion of antibody-positive fetal cells after adjustment for antibody class RAG: B6.129S7-Rag1^{tm1}Mom/J