Published in final edited form as:

Methods Mol Biol. 2018 ; 1784: 243–258. doi:10.1007/978-1-4939-7837-3_22.

Genetic Models of Macrophage Depletion

Li Hua, Jiayuan Shi, Leonard D. Shultz, and Guangwen Ren*

The Jackson Laboratory, Bar Harbor, ME 04609, USA.

Abstract

Macrophages are a heterogeneous population of innate immune cells and are distributed in most adult tissues. Certain tissue resident macrophages with a prenatal origin, together with postnatal monocyte-derived macrophages, serve as the host scavenger system to eliminate invading pathogens, malignant cells, senescent cells, dead cells, cellular debris and other foreign substances. As a key member of the mononuclear phagocyte system, macrophages play essential roles in regulation of prenatal development, tissue homeostasis and disease progression. Over the past two decades, considerable efforts have been made to generate genetic models of macrophage ablation in mice. These models support investigations of the precise functions of tissue specific macrophages under physiological and pathological conditions. Herein, we overview the currently available mouse strains for in vivo genetic ablation of macrophages and discuss their respective advantages and limitations.

Keywords

Macrophages; Gene deficiency models; Genetic depletion models; Conditional ablation

1. Introduction

In this chapter, we focus on mouse macrophages because the currently available tools for genetic ablation of macrophages have been mostly developed in mice (1). Although species variations have been demonstrated in the biology of macrophages (2,3), the murine models of in vivo macrophage manipulation indeed provide critical insights for characterizing how endogenous macrophages contribute to development, tissue homeostasis and disease in the host (4). Accelerated determination of the heterogeneity of endogenous macrophages as well as their precise biological functions with these macrophage ablation models, will support testing of specific therapeutic strategies targeting macrophage subsets for treatment of fibrosis, cancer, chronic inflammation and other macrophage-related disorders.

Based on their anatomical locations, tissue resident macrophages have their respective names, such as Kupffer cells in the liver, alveolar macrophages in the lung, osteoblasts in the bone, microglia in the central nervous system, intraglomerular mesangial cells in the kidney, etc (4). Certain mouse tissue macrophage populations such as microglia and Kupffer cells, develop at the very early yolk sac stage, whereas other populations are generated at later

*Address correspondence to: Guangwen Ren, Ph.D., The Jackson Laboratory, 600 Main Street, Bar Harbor, Maine, USA, Gary.Ren@jax.org, Phone: 207.288.6783; Fax: 207.288.6077.

embryonic stages or during postnatal life (5). In the adult bone marrow (BM), monocytes, which serve as macrophage precursors, are differentiated from hematopoietic stem/progenitor cells (HSPCs). These BM monocytes regularly emigrate to various tissues or organs where they give rise to resident macrophages, maintaining tissue homeostasis (1). Under infection, tissue injury and other inflammatory conditions, numbers of monocytes can rapidly expand in hematopoietic organs and then migrate towards the inflamed tissues guided by chemotactic factors (6). At the inflamed sites, monocytes, their differentiated macrophages, together with other resident and recruited innate immune cells, build the first defense line against foreign substances. It is now recognized that macrophages may have two functionally distinct forms—"M1" (classically activated) and "M2" (alternatively activated) macrophages (7,8). "M1" macrophages are usually present at early stages of inflammation and function in the phagocytosis of invading pathogens, dead cells and mutated cells. With the resolution of inflammation, "M1" macrophages can be reprogrammed into an "M2" phenotype which serves to facilitate injured tissue repair and tissue remodeling via releasing cytokines, chemokines and other trophic factors (9). Therefore, "M1 means kill; M2 means heal" has been proposed to describe the biology of macrophages in a wound healing process (10).

Development of tools for genetic manipulation of macrophages relies on understanding lineage regulating factors and phenotypic markers specific for macrophages. The predominant signaling pathway controlling lineage differentiation of macrophages from HSPCs is macrophage colony-stimulating factor (M-CSF) (or colony stimulating factor 1, CSF1) and its receptor-CSF1R in mice. The CSF1R (CD115), is expressed on mononuclear phagocytic cell populations in most tissue types and therefore the CSF1R promoter has been extensively used to make reporter mice as well as CSF1R deficient mice to support studies of the effects of macrophage deficiency in vivo. In addition to CSF1, granulocyte–macrophage colony-stimulating factor (GM-CSF) and IL-3 have also been shown to regulate macrophage differentiation (4). In mice, the most widely used phenotypic markers of macrophages include CD11b, F4/80, CSF1R and CD68 (4). For some tissue specific macrophage subsets, unique markers are expressed such as CD169 which identifies splenic macrophages in the marginal metallophilic and marginal zone, as well as macrophages in the lymph node and BM (11). Moreover, distinct surface markers have been reported to be associated with M1 versus M2 macrophages. Among these markers, CD206 has been suggested to be specific for M2 macrophages in mice and CD206 expression has been leveraged to create a conditional M2 macrophage depletion model (12).

On the basis of these macrophage-specific factors and markers, a series of genetic models have been developed for macrophage ablation in mice in the past two decades. These models encompass spontaneous gene deficiency and genetic targeting models, which are discussed below respectively.

2. Gene deficiency models

Macrophage-specific gene knockout (KO) models are valuable tools for a comprehensive understanding of the functional importance of this population of mononuclear phagocytes in host tissue development and homeostasis, as well as under pathological conditions. The

currently available gene deficiency models are mostly related to the crucial macrophage lineage differentiation factor CSF1 and its receptor CSF1R.

2.1 *Csf1^{op}*

Mice homozygous for the spontaneous “osteopetrosis” (*op*) mutation in the coding region of the *Csf1* gene (13,14), were initially recognized by absence of teeth and by skeletal abnormalities (15). Accordingly, this new null mutation was named osteopetrosis (*op*). Mice homozygous for the *Csf1^{op}* mutation, abbreviated as *op*, are viable but show multiple bone developmental deficiencies such as impaired tooth growth, defective bone remodeling, and aberrant calcium regulation (16). Young *op/op* mice exhibit a systemic reduction of macrophage cellularity throughout the host tissues, and particularly in the BM. The BM cellular density of *op/op* mice is only about one-tenth of that in the wild type controls. Such deficiencies in *op/op* mice are corrected with age, and by 22 week of age, the BM cellularity can recover to normal levels (17,18).

In the 1990s, several groups detailed the macrophage deficiency of *op/op* mice (16,18–21). They found that the macrophage deficiency in *op/op* BM occurred early in life and macrophage numbers gradually recovered to normal levels by 8–9 months of age (18). These findings suggested there are alternative mechanisms to compensate for the CSF1 deficiency in the hematopoietic system. Furthermore, there is a tissue-specific variation in macrophage deficiency in *op/op* mice. Using F4/80 protein expression to mark tissue macrophages by immunohistochemistry, Cecchini et al. listed four different tissue-specific patterns for macrophage deficiency in vivo: 1) macrophages were absent throughout postnatal life in the following tissues: striated muscle, tendon, dermis, periosteum, synovium, retina and kidney; 2) macrophage numbers were reduced in postnatal life in the following tissues: adrenal glands, bladder, submandibular salivary glands and BM; 3) macrophage numbers were initially normal but changed with age in the following tissues: liver, stomach, small and large intestines, and spleen (red pulp); and 4) macrophage numbers were normal through life in the following tissues: epidermis (Langerhans cells), thymus, lymph nodes, and monocyte numbers were normal in the BM (19).

In addition to the tissues investigated above, macrophages in the peritoneal cavity, splenic marginal zone (metallophils) and lymph node subcapsular sinus were shown to be rare or absent, whereas lung alveolar macrophage numbers reached substantial levels in *op/op* mice at 4–6 week of age (20). Although CSF1 stimulation and CSF1R expression was revealed to be essential for macrophage development from as early as the yolk sac stage in the prenatal life, microglia in the adult brain were not significantly affected in morphology and frequency (20,21). Later on it was shown that microglia were however absent in *Csf1r* knockout mice (22,23) and the microglia development may be dependent on the second CSF1R ligand, interleukin 34 (IL-34) (24,25). Therefore, the macrophage defective phenotype is age- and tissue-dependent in *op/op* mice and special attention should be accordingly paid when using this strain.

2.2 *Csf1r*-KO

CSF1R, the receptor for CSF1, also called CD115, is a tyrosine kinase which is encoded by the proto-oncogene *c-fms*. *Csf1r*-KO mice were generated by inserting a neomycin resistance (PGKneo) cassette containing stop codons into exon 3 of both *Csf1r* alleles (26). *Csf1r*-KO mice showed a very similar phenotype to the *op/op* mice: they are toothless and osteopetrotic, and have similarly reduced BM cellularity with the ability to recover to normal levels by 35 week of age. The growth curves and postnatal survival are also indistinguishable between *Csf1r*-KO and *op/op* mice.

With regards to macrophage deficiency, a similar depletion pattern was also demonstrated in these two strains. In *Csf1r*-KO mice, frequencies of monocytes and lymphocytes decreased by 70–75% and 30–40%, respectively, whereas a significant increase (by 100–135%) was found for granulocytes in the circulation as measured by flow cytometry analysis (26). Macrophages were substantially defective in the peritoneal and pleural cavities, BM, liver, kidney, thymus, dermis and testes of the *Csf1r*-KO mice. Levels of macrophage depletion in *Csf1r*-KO mice were similar but in general more severe when compared to *op/op* mice (26).

In contrast to the above similarities, two lineages of the monocyte phagocytic system, Langerhans cells and microglia, were found to be largely absent in *Csf1r*-KO mice but remained normal in *op/op* mice (20–22,27). An alternative ligand for CSF1R, later discovered to be IL-34 (28), was thought to support the development of Langerhans cells and microglia in mice.

2.3 *Csf1r*^{dex5}

The *Csf1r*^{dex5} strain was generated from crossing *Csf1r*^{fl/fl} floxed mutant mice possessing loxP sites flanking exon 5 of the *Csf1r* gene, with Zp3-cre mice in which cre-recombinase is expressed from the oocyte specific zona pellucida 3 (Zp3) (29). Such a deletion of exon 5 of the *Csf1r* gene resulted in a null allele whose phenotype in the homozygous state is indistinguishable from the complete *Csf1r*-KO strain.

Similar to *op/op* and *Csf1r*-KO mice, *Csf1r*^{dex5} mice also have osteopetrosis, toothlessness and a low body weight. In the tissues examined, including kidney and liver in *Csf1r*^{dex5} mice, macrophages were reduced to the comparable levels to *op/op* or *Csf1r*-KO mice (29).

The *Csf1r*^{fl/fl} floxed strain is suitable for studying macrophage biology in adult mice which have already developed normal macrophages and other immune cells before crossing with various cre driver mice. This partially solved the issues caused by some developmental defects in various tissues and organs found in *op/op* and *Csf1r*-KO mice.

Overall, the gene deficiency models facilitate our comprehensive understanding of the role of macrophages in development and homeostasis, as well as in disease progression. However, the overbroad effects caused by the key lineage gene deficiency of macrophages may limit our exploration of the temporal and spatial functions of macrophages in vivo. The recently developed genetic depletion models described below provide us conditional ablation approaches to overcome these shortcomings.

3. Macrophage depletion Models

To precisely investigate how macrophages exert temporal and spatial effects in various physiological and pathological conditions, conditional ablation approaches, on the basis of “toxin receptor-mediated cell knockout” (30), were accordingly developed over the last decade. The currently available models for conditional ablation of macrophages include MAFIA (macrophage Fas-induced apoptosis) strain and several strains expressing human diphtheria toxin receptor (DTR).

3.1 MAFIA transgenic mice

Macrophage Fas-induced apoptosis (MAFIA) transgenic mice were constructed via an inducible Fas-suicide system driven by the mouse *Csf1r* gene. The suicide system encompasses cytoplasmic Fas which is membrane-tethered by two copies of the covalently linked FK506-binding protein (FKBP). Administration of the dimerization drug AP20187 results in preferential binding to the FKBP which in turn activates the cytoplasmic Fas fragments and induces apoptosis of the *Csf1r* transgene-expressing cells (31). Allowing a systemic and reversible depletion of macrophages in vivo, this strain has been widely utilized in studying the roles of macrophages in tissue development, fibrosis, inflammation, cancer and other diseases.

Prior to AP20187 treatment, MAFIA mice have a normal phenotype. A single high dose (50mg/kg) injection of AP20187 did not lead to a significant macrophage depletion in MAFIA mice, whereas multiple low dose (10mg/kg) injection for 3–5 consecutive days was sufficient to deplete macrophages in BM, spleen, blood, lymph nodes, thymus, peritoneal cavity, lung and brain, without impacts on numbers of CD3⁺ T cells and B220⁺ B cells (31). Levels of depletion of CD11b⁺F4/80⁺ macrophages can reach as high as 70–95% as measured by flow cytometry and immunohistochemistry. With regards to the injection routes, intravenous injection of AP20187 is recommended since the drug can result in peritoneal adhesions when giving via the intraperitoneal route (32).

Long-term depletion of macrophages in MAFIA mice can cause several abnormalities, including reduced body weight, splenomegaly, lymphadenopathy, thymic atrophy and extramedullary hematopoiesis (31). Such extramedullary hematopoiesis might be a result of the reduction of CXCL12 in the bone marrow and subsequent HSPC mobilization from the BM to the periphery in the absence of BM macrophages in AP20187-treated MAFIA mice (11). It should also be noted that CD11c⁺ cells, including certain subsets of dendritic cells (DCs), could also be depleted in MAFIA mice with a comparable efficacy to macrophages (31).

In addition to studying macrophage depletion in otherwise unmanipulated MAFIA mice, effective macrophage depletion in these mice was also demonstrated in various pathological conditions. As macrophages play dominant roles in multiple diseases, depletion of macrophages was speculated to interfere with disease progression. In a bacterial infection model, depletion of macrophages reduced the host’s capability to clear bacteria from the circulation leading to increased bacterial growth in the liver (31). In a dextran sulfate sodium (DSS)-induced colitis model, intravenous injection with AP20187 for 5 consecutive days

caused an 80–90% reduction of colonic macrophages (and DCs) in MAFIA mice. The macrophage depletion and subsequent increased influx of neutrophils was found to aggravate DSS-induced colitis severity. The results therefore indicated a protective role of macrophages during development of acute colitis through a down-regulation of neutrophil infiltration in the colon (33). In mouse models of pulmonary fibrosis and liver fibrosis, AP20187-induced macrophage depletion in the lung (CD11c⁺F4/80⁺ macrophages, but not alveolar macrophages) and liver (Kupffer cells) in MAFIA mice, accelerated resolution of established pulmonary fibrosis (34) but impeded resolution of liver fibrosis (35). These results suggested multifaceted effects of tissue-specific macrophages in pathological processes.

Besides the above inflammatory disease models, effective macrophage depletion was also shown in malignant tissues implanted in MAFIA mice in certain mouse tumor models such as melanoma, breast carcinoma and GL261 glioma (36–38). Intriguingly, in the GL261 glioma orthotopic injection model, only the “pro-tumor” M2 macrophages were ablated which was associated with an increase of “anti-tumor” M1 macrophages in MAFIA mice upon administering AP20187 (37). Thus, there is a selective depletion of macrophages in the tumor microenvironment using the MAFIA mice leading to a significant reduction of glioma burden. A preferential CD206⁺ M2 macrophage depletion was also revealed in cardiac tissue of MAFIA mice in myocardial infarction model. In this study, ablation of M2 macrophages in MAFIA mice retarded the resolution of inflammation associated with post myocardial infarction (39).

3.2 CD11b-DTR

CD11b-DTR transgenic mice have a diphtheria toxin (DT) inducible system encompassing a fusion product of the human DT receptor under control of the human CD11b promoter. Since the human DTR has a much higher sensitivity to DT than the mouse DTR, relatively low dose DT administration will result in macrophage ablation without killing other mouse cells (30). Although CD11b is expressed on multiple myeloid lineage cell populations, including monocytes, macrophages and neutrophils, DT injection depletes monocytes and macrophages, but not neutrophils (40).

CD11b-DTR homozygous mice are viable, and phenotypically normal prior to DT treatment. F4/80⁺ macrophage populations are also unaffected in the transgenic mice prior to treatment. Upon DT treatment by intraperitoneal injection, macrophage depletion resulted in tissue-specific macrophage depletion efficacies. F4/80⁺ macrophages in the peritoneal cavity, peripheral blood, BM, spleen, lung, liver, ovary, kidney, lymph nodes, brain and pancreas (11,40–44), can be efficiently depleted in CD11b-DTR mice upon DT injection, while DT is incapable of ablating hepatic sinusoidal macrophages and alveolar macrophages (41). Of note, elevation of the neutrophil frequency in various tissues has been reported to be associated with macrophage depletion in CD11b-DTR mice (40,41).

In addition to treatment of otherwise unmanipulated CD11b-DTR mice, macrophage depletion was also studied in association with various disease models to determine the functional involvement of endogenous macrophages in disease progression. In multiple inflammatory disease models such as glomerulonephritis, peritonitis, insulinitis, arthritis,

traumatic injury-caused brain inflammation, liver fibrosis and atherosclerosis, DT treatment efficiently depleted macrophages in various types of lesions. Functionally, macrophage depletion either positively or negatively altered disease progression suggesting versatile functions of tissue resident macrophages under pathological conditions (40,42,45–48). In mouse models of lymphoma, melanoma, colorectal cancer and breast cancer, injection of CD11b-DTR mice with DT substantially depleted tumor-infiltrating macrophages at the site of the primary tumors and at the sites of metastases, as well as depleting monocytes and macrophages in the circulation (49–52). Ablation of tumor-associated macrophages generally reduced tumor growth and metastases.

In spite of some controversies regarding efficacies of tissue-specific macrophage depletion using CD11b-DTR mice, this strain was shown to be highly valuable for gaining a better understanding of macrophage biology in tissue homeostasis and various disease processes. The DT administration schedule, dose and route should be optimized in individual experiments to achieve the optimal depletion efficacy.

3.3 CD169-DTR

Similar to the CD11b-DTR model, the CD169-DTR strain was also constructed based on the DT-inducible system containing the human DTR under the control of the CD169 sialoadhesin gene promoter (53). CD169, also known as Siglec1 or Sialoadhesin, is a sialic acid- and glycoprotein-binding adhesion molecule expressed on macrophages of certain tissues such as spleen marginal metallophilic zone, lymph node, and BM, therefore this strain is quite specific for conditional ablation of macrophages in these tissues (11,53).

In the original study of CD169-DTR mice, 10 µg/kg of DT was sufficient to completely deplete spleen marginal zone macrophages and marginal metallophilic macrophages, and the depletion lasted as long as 7 days after DT injection (53). As the spleen marginal macrophages are mainly responsible for eliminating apoptotic cells, CD169⁺ macrophage depletion led to a delayed clearance of injected apoptotic cells (53). In further studies, conditional ablation of CD169⁺ macrophages in the sinuses of draining lymph nodes, caused a failure of presentation of non-viable tumor cell antigens by lymph node macrophages for priming an effective CD8⁺ T cell-mediated anti-tumor immunity (54). Moreover, depletion of BM CD169⁺ macrophages elicited a rapid emigration of HSPCs from the BM to the circulation suggesting a critical role of CD169⁺ BM macrophages in retention of HSPCs in the steady state (11). Other than above hematopoietic organs/tissues, efficient CD169⁺ macrophage depletion was also manifested in gut, kidney and lung using CD169-DTR mice in models of colitis, ischemia-reperfusion injury and influenza virus infection (55–57).

Overall, the CD169-DTR strain provides a conditional ablation model for a more specified macrophage subset. As it does not deplete other myeloid lineage cells including monocytes, DCs and neutrophils (1,11,53), CD169-DTR mice may facilitate exploration of more precise functions of CD169⁺ macrophages in homeostasis and diseases. The CD169-DTR strain may also enlighten the development of other similar models towards understanding the heterogeneity of macrophages in vivo.

3.4 CD206-DTR

CD206, also known as mannose receptor, is a pattern recognition receptor (PRR) belonging to the C-type lectin superfamily. It is expressed on macrophages, dendritic cells and endothelial cells (58), and it was also recently suggested as a marker for M2 macrophages (59). The CD206-DTR strain was therefore constructed for defining the endogenous functions of M2 macrophages in mice (12). In an endotoxin-induced acute lung injury model, the efficacy of CD206⁺ macrophage depletion was measured in DT-injected CD206-DTR mice. DT treatment of CD206-DTR mice caused a significant reduction of the mRNA expression of M2 macrophage markers, Ym1 and Arginase 1, in lungs. Ablation of CD206⁺ macrophages in vivo aggravated endotoxin-induced lung inflammation suggesting a role of M2 macrophages in antagonizing pulmonary inflammatory responses (12).

In an obesity model using CD206-DTR mice, DT administration led to a specific reduction of M2-like macrophages, but not M1 macrophages in adipose tissues. Depletion of CD206⁺ macrophages resulted in an increased number of smaller adipocytes, as well as an amelioration of systemic insulin sensitivity. The results indicated that CD206⁺ macrophages may contribute to a special niche in adipose tissues which functions to suppress the growth and differentiation of adipocyte progenitors (60).

This strain may be useful for further elaborating the distinct functions of M1 and M2 macrophages in various disease contexts. However, due to the non-specific expression of CD206 in mouse tissue cells and the uncertainty of M1/M2 macrophage definition, more experimental validations are still needed for this strain.

The DTR system provides an excellent framework for conditional depletion of specific populations of mouse macrophages, as mentioned above. In 2005, a Cre-inducible DTR transgenic strain was generated in which expression of DTR is blocked by an upstream loxP-flanked STOP sequence (61). When crossed to Cre-driver mice, the STOP sequence is deleted in Cre-expressing tissues which in turn allows DTR expression. Such an inducible DTR technique has been recently applied to create the *Lysozyme M* (*LysM*)-cre/iTDR mice for inducible macrophage depletion (62–64). With this iDTR strain, a more precise screening for appropriate targeting of specified tissue resident macrophages will advance our understanding of the macrophage lineage development and their functional diversity in vivo.

4 Conclusions

A comparison of macrophage depletion efficacy in different tissues/organs of above gene deficiency and genetic depletion models is listed in Table 1. As indicated from this summary, macrophages are a highly heterogeneous innate immune cell population crucial for development, homeostasis and various diseases. There are huge variations in surface markers and regulatory genes for macrophages present in different tissues and at different stages of development and pathological processes. As well, a tremendous difference was also revealed between infiltrating macrophages and resident macrophages. Such multiple layers of heterogeneity of macrophages lead to a lack of perfect genetic depletion model specific for macrophages until now. A deeper understanding of macrophage biology especially the identification of transcriptional factors and surface markers specific for

distinct tissue macrophage subsets will help development of more precise genetic depletion tools for macrophages.

Acknowledgements

This work was supported by grants from the National Institutes of Health (R00 CA188093 to G.R. and P30 CA034196 to E.L.) of the United States. We apologize to our colleagues whose works are not cited in this manuscript due to space limitations.

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Table 1

Macrophage depletion efficacy in various mouse strains

Tissues/Mouse strains	<i>Csf1^{op/op}</i>	<i>Csf1r-KO</i>	MAFIA	CD11b-DTR	CD169-DTR	LysM-Cre/DTR
Bone marrow	Yes(16,18,19)	Yes(26,65)	Yes(11,31,66)	Yes(11)	Yes(11,67,68)	
Spleen red pulp	Yes/No(16,19,20)		Yes(31)	Yes(42,43)	Yes(1)	Yes(64)
Spleen marginal zone	Yes(19,20)				Yes(53,69,70)	
Lymph node	No(19,20)		Yes(31)	Yes(42)	Yes(53,54,71)	
Peritoneal cavity	Yes(16,20)	Yes(26)	Yes(31)	Yes(40,41)	No(1)	Yes(64)
Lung	Yes/No(16,20)		Yes(31,34,72,73)	Yes/No(41,74)	Yes(57,68,75)	
Liver	Yes/No(16,19,20)	Yes(26)	Yes(35)	Yes/No(40,41,76)	No(53)	Yes(64,77)
Intestine	Yes/No(19,20)				Yes(55)	
Central nervous system	No(20,21)	Yes(22,23)	Yes/No(31,78,79)	Yes/No(44,47,80)	No(1)	
Skin	Yes(19)	Yes(26)		Yes(81,82)	No(1)	Yes(63,64)
Kidney	Yes(19)	Yes(26)		Yes(41,45,83)	Yes(56)	Yes(84)
Thymus	Yes/No(16,19,20)	Yes(26)	Yes(31)			
Muscle	Yes(19)			Yes(85,86)		Yes(77)
Synovium	Yes(19)			Yes(48)		
Circulating monocytes	Yes(16)		Yes(11,31)	Yes(40,41)	No(1)	Yes(62,64)
Testes		Yes(26)				
Colon			Yes(33)			
Heart			Yes(39)	Yes(87)		
Cornea			Yes(88,89)			
Ovary				Yes(41,90)		
Pancreas				Yes(42)		