

## Pluripotent stem cell-derived dendritic cells for immunotherapy

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## 1. ABSTRACT

Dendritic cell (DC) is regarded as a powerful means for anti-cancer immunotherapy. Clinical trials of cancer therapy with DC loaded with cancer antigens, such as tumor cell-lysates or HLA class I-binding antigenic peptides, have been conducted. Antigen-specific negative manipulation of the immune response by DC is a potential treatment for autoimmune diseases and also for control of allo-reactive immune responses in transplantation medicine. Currently, DC for clinical use are generated from peripheral blood monocytes of the patients. However, the number of monocytes obtained from the patients is limited and the potential of monocytes to differentiate into DC varies depending on the blood donor. Thus, the issue of limited cells is a serious obstacle for DC therapy. ES cells and iPS cells have pluripotency and unlimited propagation capacity and may be an ideal cell source for DC-therapy. Several groups, including us, have developed methods to generate DC from ES cells or iPS cells. This review introduces the studies on generation, characterization, and genetic modification of DC derived from ES cells or iPS cells.

## 2. PHYSIOLOGICAL FUNCTIONS AND MEDICAL APPLICATION OF DENDRITIC CELLS

Dendritic cells (DC) are the most potent antigen presenting cells and they are known to play a pivotal role in the development of immune responses. DC, a type of hematopoietic cell, exist in various tissues in the body. Upon invasion of exogenous antigens to the tissues, tissue-localized DC capture the antigen through phagocytosis, receptor-mediated endocytosis, or pinocytosis. DC digest the ingested protein antigen to produce oligo peptides. In the steady state, DC are efficient in capturing antigens and similar to macrophages. However, after exposure to the constituents of exogenous micro-organisms, such as bacteria and fungi, that transduce danger signals, DC become potent T cell-stimulators. Accompanying this functional change termed “maturation”, their morphology changes and DC begin to form dendritic protrusions. At the molecular level, they up-regulate the cell surface expression of MHC class I and class II, CD80, CD86, and CD40, molecules involved in the T-cell stimulation or DC-T cell interaction, and they produce cytokines such as TNF- $\alpha$  and IL-12.

After antigen capture, DC migrate to the draining lymphoid tissues such as the lymph nodes. In the lymphoid tissues, DC present the antigenic peptides in the context of MHC class I and MHC class II. Circulating T cells migrate from the blood stream into the T-cell area of the lymph nodes and recognize the complex of MHC plus peptide on the cell surface of DC. Importantly, mature DC are very potent in stimulating T cells, and can prime naive T cells that have not yet experienced antigenic stimulation. Antigen-specific T cells stimulated by DC proliferate vigorously and produce various kinds of cytokines that stimulate immune cells, consequently resulting in the development of an antigen-specific immune response. Therefore, the stimulation of naive antigen-specific T cells by antigen-presenting DC is a crucial step for the initiation of immune responses.

In addition, several studies have indicated that DC are involved also in the induction and maintenance of immunological self-tolerance (1). The mechanisms of negative regulation of immune response by DC include expansion of CD4<sup>+</sup> CD25<sup>+</sup> regulatory T cells (2) (3) and catabolism of tryptophan (4). Collectively, DC physiologically regulate immunity both positively and negatively.

Because DC are immune-regulating cells, the application of DC for clinical immunotherapy is an attractive option. Studies in mice have demonstrated that cellular vaccination with antigen-bearing DC is efficient in stimulating antigen-specific T cell responses (5). DC are now regarded as a powerful tool for anti-cancer immunotherapy. Genetically engineered DC with enhanced T cell-stimulating capacity should yield an even more powerful anti-cancer therapy. To treat patients who suffer from autoimmune or inflammatory diseases, it is desirable to down-modulate immune responses in an antigen-specific manner without causing systemic immune suppression. GVHD and graft rejection are the most serious problems in transplantation medicine, and control of allo-reactive immune response is the key to overcome them. Antigen-specific negative regulation by DC is considered to be a promising means also in the field of transplantation medicine (6).

Within the past decade, various clinical anti-cancer therapy trials using DC have been conducted. Cancer antigens such as tumor cell-lysates or HLA class I-binding antigenic peptides are loaded onto DC and transferred to the patients, where a cancer antigen-specific T cell response is expected, in particular a cytotoxic T lymphocyte (CTL) response. DC used for clinical anti-cancer therapy are generated from peripheral blood monocytes of cancer patients. In order to obtain sufficient number of monocytes, apheresis procedure is used to isolate white blood cells from a large volume of patient blood, and subsequently monocytes are isolated from the white blood cell population. However, the number of monocytes obtained from the peripheral blood is limited even with apheresis. In addition, the DC-differentiation potential of monocytes varies depending on the blood donor. Therefore, this continuing cell source limitation remains one of the major obstacles for DC therapy.

### 3. GENERATION OF DENDRITIC CELLS FROM MOUSE ES CELLS

ES cells are characterized by pluripotency and infinite propagation capacity. Therefore, if we can use ES cells as a source of DC, the cell source limitation for DC therapy can be overcome. We and others developed methods to generate DC from mouse ES cells, and the functions of ES cell-derived DC (esDC or ES-DC), including the stimulation of allogeneic T cells, processing and presentation of antigenic proteins, and migration upon *in vivo* transfer, were comparable to those of DC generated *in vitro* from bone marrow cells (7) (8).

For hematopoietic differentiation of ES cells *in vitro*, Embryoid Body (EB)-mediated methods and the feeder-coculture method have been established. OP9 is a bone marrow stromal cell line that originated from a Macrophage Colony Stimulating Factor (M-CSF)-defective op/op mouse, and generation of various hematopoietic cells from ES cells using OP9 cells as feeder cells has been reported, with ES cells yielding granulocytes, erythrocytes, and B lymphocytes (9). The method has been applied to a number of molecular and cellular analyses for investigations of hematopoiesis.

In 2000, Fairchild and colleagues reported the first study on the generation of functional dendritic cells from mouse ES cells (7). Their method is based on the formation of EB. At the initiation of the differentiation, ES cells were grown in suspension to allow EB formation. After culturing for 14 days, EB were cultured in medium supplemented with Granulocyte/Macrophage Colony Stimulating Factor (GM-CSF) and Interleukin (IL)-3. The combination of these 2 cytokines uniquely supported the development of cells with an appearance suggestive of DC within 4 days of culture. These cells expanded rapidly over the following 7–10 days to form lightly adherent clusters, reminiscent of those observed in cultures of immature DC derived from bone marrow cells. The cells released from these clusters seeded uncolonised areas of the dish and displayed typical dendritic morphology, and they were designated as esDC.

A flowcytometry analysis showed that esDC expressed CD11c, MHC class II, CD40, CD54, CD80, and CD86. On the other hand, expression of CD8 and Dec-205 (CD205) was not observed, thus suggesting that esDC were derived from a myeloid lineage. RT-PCR analysis revealed that esDC expressed mRNAs for IL-20, IL-18, and IL-1 $\beta$  converting enzyme (ICE). They possessed a potent capacity to process a protein antigen, Hen Egg Lysozyme (HEL), and present the resultant epitope in the context of H-2E<sup>k</sup>, as demonstrated by the response of co-cultured antigen-specific T cell hybridoma. Upon co-culture with allogeneic T cells, esDC stimulated the T cells to vigorously proliferate, indicating that esDC had a strong T cell-stimulating capacity. Collectively, the ES cell-derived cells with DC-like morphology were fully functional in antigen-presentation and T cell-stimulation.

Hematopoietic differentiation of mouse ES cells by co-culture with M-CSF-deficient mouse stromal

cells, OP9, was developed by Nakano and colleagues in 1994 (9). Similarly, we established a method to generate dendritic cells from mouse ES cells by aid of OP9 stromal cells (8).

In our method, mouse ES cells were transferred onto the OP9 cell layers and cultured in  $\alpha$ MEM medium containing fetal calf serum without exogenous cytokines. ES cell colonies changed their morphology from undifferentiated dome-like structure to a differentiated flattened morphology in 4 to 5 days. To induce DC differentiation, the mesodermally differentiated ES cell-derived cells harvested from a 5 or 6 -day culture on OP9 feeder layers were cultured on fresh OP9 cell layers in the presence of exogenous GM-CSF. In comparison to the culture without exogenous GM-CSF, addition of this cytokine resulted in appearance of a larger number of floating cells in a few days, indicating that cells grew in response to GM-CSF.

At day 3 following transfer, we observed many round and relatively homogenous floating cells, most of which expressed CD11b, thus suggesting their commitment to the myeloid cell lineage. On day 10 or 11, we recovered the cells floating or loosely adherent to feeder cells and transferred them to bacteriologic Petri dishes without feeder cells. After this passage, approximately half of the transferred cells adhered to the dish surface and resembled macrophages. In addition, clusters of floating cells were observed after days 17 to 19, and the cell clusters gradually increased. The cells were of irregular shape and possessed protrusions. We designated them ES-DC. Addition of maturation stimuli, IL-4, TNF- $\alpha$ , plus anti-CD40 antibody or LPS, to the cells resulted in typical morphology of mature DC with long protrusions.

ES-DC were positive for MHC class I, MHC class II, CD80, CD86, CD40, DEC205, and CD11c. They had the capacity to process and present protein antigens to T cells. After exposure to maturation stimuli, the expression of MHC class I, MHC class II, CD80, CD86 and CD40 were increased. Mature ES-DC were highly competent in stimulating T cells, based on the proliferative response of the allogeneic T cells co-cultured with ES-DC. ES-DC were positive for F4/80 and CD11b and negative for CD8, thereby suggesting that they were of myeloid lineage.

#### 4. MOUSE STUDIES ON THE INDUCTION OF ANTI-CANCER IMMUNITY BY GENETICALLY ENGINEERED ES-DC

Non-virus-mediated methods for gene transfer, including targeted gene integration and procedures for isolation of appropriate transfectant cell clones, have been established for ES cells. We developed a strategy for the genetic modification of mouse ES-DC. Expression vectors were introduced into ES cells by electroporation and subsequently the transfected ES cell clones were induced to differentiate into ES-DC. The capacity of ES-DC introduced with an ovalbumin (OVA)-expression vector to prime OVA-specific T cells *in vivo* was demonstrated (8).

In anti-cancer immunotherapy with administration of DC, the efficiency of T cell-priming *in vivo* by transferred DC is critical. T cell-priming *in vivo* by injected DC should depend on their encounter with T cells. When exogenous antigen was injected intracutaneously, about 25% of the DC capturing the antigen migrated to the T cell area of draining lymph nodes (10), where they presented the antigen to prime naive T cells specific to the antigen. On the contrary, when BM-DC or splenic DC were transferred exogenously by s.c. or i.p. injection, the absolute number of the DC found within the draining lymph nodes represented only a small proportion (0.1-1%) (11) (12). It has been also reported that almost all the transferred DC remained at the s.c. immunization site up to 24 hours after transfer in the patients who received DC therapy (13). The inefficient migration of the exogenous DC to lymphoid organs may lower the frequency of their encounter with T cells. It may be possible to improve the efficacy of exogenously transferred DC to prime immune responses by augmenting their encounter with T cells. We attempted to improve the capacity of DC to prime T cells *in vivo* by genetic modification to express a chemokine with T cell-attracting properties. We generated double-transfectant DC expressing a chemokine along with OVA by sequential transfection of ES cells with 2 kinds of expression vectors and then induction of DC differentiation (14).

Several kinds of chemokines with the capacity to attract T cells are produced by different cell types. CCL21 (Secondary lymphoid tissue chemokine, SLC) is produced in T cell regions of lymph nodes and spleen and also by high endothelial venules in lymph nodes. SLC chemoattracts T cells, NK cells, B cells, and DC. CXCL9 (Monokine induced by IFN- $\gamma$ , Mig) is produced by macrophages and binds to the chemokine receptor CXCR3, which mediates the recruitment of predominantly Th1 cells and activated NK cells. XCL1 (Lymphotactin, Lptn), produced by activated T cells, has chemoattractive properties on CD4<sup>+</sup> and CD8<sup>+</sup> T cells and on NK cells. We comparatively evaluated the effect of these 3 kinds of chemokines.

All three types of double-transfected ES-DC primed OVA-specific CTL *in vivo* more efficiently than did ES-DC expressing only OVA, and co-expression of SLC or Lptn was more effective than that of Mig. Immunization with DC expressing OVA plus SLC or Mig provided protection from OVA-expressing tumor cells more potently than that with OVA only, and SLC was more effective than Mig. Conversely, the co-expression of Lptn gave no additive effect on protection from the tumor. Collectively, among the three chemokines, expression of SLC was the most effective in enhancing anti-tumor immunity by transferred ES-DC *in vivo*.

NKT cells are a group of T cells expressing invariant T cell receptors and recognize lipid ligands, for example  $\alpha$ -galactosylceramide ( $\alpha$ GalCer), in the context of CD1d, a nonclassical MHC class I molecule. On stimulation with  $\alpha$ GalCer, NKT cells rapidly produce large amount of cytokines, resulting in activation of conventional T cells and NK cells. ES-DC loaded with  $\alpha$ GalCer potently

stimulated NKT cells, and  $\alpha$ GalCer-loaded ES-DC expressing OVA elicited anti-tumor activity against peritoneally injected tumor cells expressing the OVA antigen (15). Expression of SLC with OVA further enhanced the anti-tumor activity of  $\alpha$ GalCer-loaded ES-DC expressing OVA. These results suggest that  $\alpha$ GalCer-loaded ES-DC expressing tumor-associated antigen along with SLC can stimulate multiple subsets of effector cells to induce a potent therapeutic effect against peritoneally disseminated tumor cells.

The anti-tumor immunity obtained by ES-DC directed to natural tumor antigens was examined. Glypican-3 is a tumor antigen expressed in hepatocellular carcinoma and melanoma (16) (17). ES-DC expressing Glypican-3 could induce anti-tumor activity against Glypican3-transfected tumor cells and also against the mouse melanoma B16-F10, which endogenously expresses Glupican3 (18). Immunization of mice with a mixture of ES-DC expressing GP100, SPARC, and Tyrosinase-related protein-2 induced potent immunity against B16-BL6 melanoma in the peritoneal dissemination model and also lung metastasis models (19). Collectively, we have demonstrated that genetically modified ES-DC are highly potent in induction of anti-tumor immunity in mouse models.

## 5. STUDIES ON THE TREATMENT OF EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS BY ES-DC

We investigated whether we could negatively modulate immune responses with genetically modified ES-DC expressing specific antigen along with immune-suppressive molecules. Experimental Autoimmune Encephalomyelitis (EAE), characterized by neurological impairment resulting from de-myelination in the central nervous system caused by activated myelin antigen-reactive  $CD4^+$  T cells, is induced by immunization with myelin antigens such as Myelin Oligodendrocyte Glycoprotein (MOG) or Myelin Basic Protein (MBP). We attempted to prevent MOG-induced EAE by pre-treating mice with genetically modified DC presenting a MOG peptide in the context of MHC class II molecules and simultaneously expressing molecules with T cell-suppressive property (20).

For efficient presentation of the MOG peptide in the context of MHC class II molecules, we used a previously devised expression vector in which cDNA for human MHC class II-associated invariant chain (Ii) was mutated to contain antigenic peptide in the CLIP (class II-associated invariant chain peptide) region. An epitope inserted in this vector is efficiently presented in the context of co-expressed MHC class II molecules (21).

The T cell-suppressive molecules we tested were TNF-Related Apoptosis-Inducing Ligand (TRAIL) and Programmed Death-1 Ligand (PD-L1). TRAIL is a member of the TNF superfamily and is constitutively expressed in a variety of cell types including lymphocytes, NK cells, and neural cells. TRAIL<sup>-/-</sup> mice are hypersensitive to collagen-induced arthritis and streptozotocin-induced diabetes. PD-L1, a ligand for PD-1

and member of the CD28/CTLA-4 family, is expressed on DC, IFN- $\gamma$ -treated monocytes, activated T cells, placental trophoblasts, myocardial endothelium, and cortical thymic epithelial cells. PD-1<sup>-/-</sup> mice spontaneously develop a lymphoproliferative / autoimmune disease, a lupus-like disease, arthritis, and cardiomyopathy (22). Thus, abrogation of either of these two molecules make mice autoimmune-prone, suggesting that these molecules play significant roles in maintaining immunological self-tolerance in physiological situations.

ES cells were sequentially transfected with an expression vector for TRAIL or PD-L1 and an MHC class II-associated invariant chain-based MOG epitope-presenting vector. Subsequently, double-transfectant ES cell clones were induced to differentiate into ES-DC expressing the introduced genes, ES-DC-TRAIL/MOG and PDL1/MOG.

The severity of MOG peptide-induced EAE was significantly reduced in mice pre- or post-treated with ES-DC-TRAIL/MOG or ES-DC-PDL1/MOG. The severity of myelin basic protein-induced EAE was also reduced by ES-DC-TRAIL/MOG but not by PD-L1/MOG. In addition, adoptive transfer of  $CD4^+$  T cells from ES-DC-TRAIL/MOG-treated mice protected the recipient mice from subsequent induction of EAE, thus suggesting the involvement of  $CD4^+$  T cells with immune-regulatory function in the disease-preventative effect of ES-DC-TRAIL-MOG (23). These results demonstrate the prevention of autoimmune disease by treatment with ES-DC expressing target antigen along with immune-suppressive molecules, and also suggest the potential benefits of future clinical application of this technology.

Currently, corticosteroids and other immune suppressants are commonly used for treatment of subjects with autoimmune diseases. The medication with these drugs often leads to systemic immune suppression and consequent opportunistic infections. Thus, it is desirable to develop a therapeutic means to down-modulate immune responses in an antigen-specific manner without causing systemic immune suppression. Our results demonstrated that genetically modified ES-DC expressing antigenic protein along with immune-regulatory molecules control autoimmunity without causing general immune suppression.

In the clinical settings, a larger number of immune cells may be necessary to control autoimmunity by administration of immune regulatory cells. Although *in vitro* expanded antigen-specific T cells with regulatory function (Treg cells) may be useful for such purpose, it is currently difficult to expand human Treg cells specific to a certain antigen. On the other hand, unlimited number of DC with immune regulatory functions can be generated by using pluripotent stem cells as cell source.

## 6. GENERATION OF DC FROM HUMAN ES CELLS

In 2004, Zhan and colleagues reported the generation of functional antigen presenting cells from

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human ES cells (24). Similar to the method of generating dendritic cells from mouse ES cells as described by Fairchild and colleagues, Zhan and colleagues induced the formation of EB by suspension cultures of human ES cells, and then cultured the EB in the presence of hematopoietic cytokines to generate leukocytes with antigen presenting functions.

In their method, human ES cells were cultured at a high density on Matrigel before induction of differentiation. After reaching confluency, undifferentiated ES cell colonies were harvested with dispase. Cell colonies were cultured in medium for induction of differentiation with fetal calf serum and without basic Fibroblast Growth Factor (bFGF). To inhibit adherence to the plastic surface of the culture plates, ES cell colonies were plated in low-attachment plates. Cystic embryonic bodies emerged after 5-20 days in the suspension culture. When harvested at days 10-20, 50-80% of EB were cystic, each EB containing approximately 10,000 cells.

Whole EB formed in suspension were transferred onto tissue culture plates and allowed to differentiate into hematopoietic and other cell types. To stimulate production of hematopoietic progenitor cells and also dendritic cells, they added Stem Cell Factor (SCF), FLT3-ligand, and Thrombopoietin (TPO), cytokines widely used to maintain human postnatal hematopoietic stem cells. To expand dendritic cell-committed progenitor cells, IL-3, GM-CSF, and IL-4 were added. A part of the resulting leukocytes expressed MHC class II, CD80, CD86, CD40, and CD83. The MHC class II<sup>+</sup> leucocytes resembled dendritic cells and macrophages. They functioned as stimulator cells capable of eliciting allogeneic T-cell responses in culture.

After the above study was published, Su and colleagues and Tseng and colleagues reported similar methods of EB-mediated generation of DC from human ES cells (25) (26).

We generated ES-DC from human ES cells by using OP9 feeder layers (27). The protocol for the differentiation culture to generate ES-DC from human ES cells developed in our study is composed of 3 steps. At the beginning of the differentiation culture, undifferentiated ES cells were plated on OP9 feeder cell layers. Thereafter, the ES cells grew and formed clusters composed mainly of epithelial cell-like large flat cells. Clusters of round, cobble stone-like cells also appeared at about day 8. The size and number of round cell clusters gradually increased, and, by around day 15, covered 20-30 % of the surface area.

On days 15-18 of step 1, cells were recovered from the dishes using trypsin/EDTA and non-adherent cells were isolated, and then the cells were seeded onto freshly prepared OP9 cell layers, beginning step 2. The culture medium containing GM-CSF and M-CSF was used in this step. Thereafter, small round cells, floating or loosely adhering to the feeder layer, appeared and gradually increased in number. The growth of the round cells depended primarily upon GM-CSF, thus suggesting that

they grew in response to that factor. These cells expressed CD31, CD43, and CD11b, thus collectively indicating a commitment to a myeloid cell lineage.

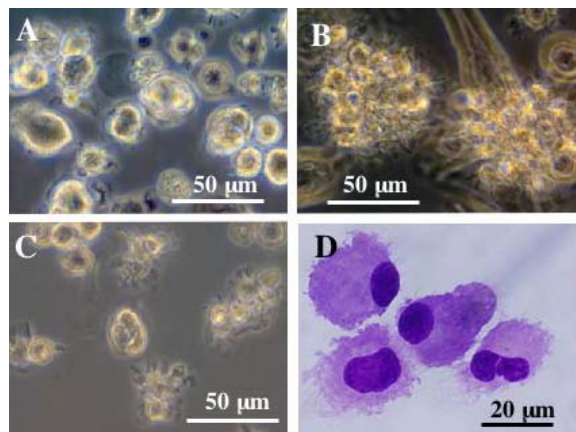
On days 7-10 of step 2, the floating or loosely adherent cells were harvested by pipetting and they were transferred to Petri dishes without feeder cells and cultured in the presence of GM-CSF and IL-4 (step 3). Following this passage, the cells changed their morphology from round to irregular shape, and some cells displayed protrusions. Cells with protrusions gradually increased and more than 50% of the cells exhibited a DC-like irregular shape after 2-3 days. The floating cells expressed CD86 and CD40, but scarcely expressed CD80 and CD83.

The simultaneous addition of TNF- $\alpha$ , LPS, soluble CD40-ligand, and IL-4 to the cells induced maturation. Flow cytometric analysis revealed increased expression of CD86 and the expression of CD80, CD83, and HLA-DR. Collectively, the cells exhibited the characteristics of DC in morphology and expression of surface molecules, and thus they were designated as human ES-DC. OK-432 is a *Streptococcus*-derived immunotherapeutic agent and known to be very potent in stimulating dendritic cells to produce IL-12. The production of TNF- $\alpha$  was profoundly induced by either LPS or OK432. OK432 but not LPS induced the production of IL-12, consistent with reports that OK432 is an efficient inducer of IL-12 (28) (29). Generation of ES-DC from the non-human primate, cynomolgus monkey, ES cells was also achieved by this method (27).

Slukvin and colleagues preceded us in reporting a method for generation of human ES cell-derived DC using OP9 feeder layers (30). Slukvin's method is also composed of 3 steps and is different from ours in that OP9 feeders were used only in the first step.

The capacity of the human ES-DC to stimulate T cells was examined based on the proliferative response of allogeneic T cells co-cultured with ES-DC. ES cell-derived floating cells recovered from the 2nd step (pre-ES-DC) showed little capacity to induce a response of T cells. In contrast, ES-DC following the 3rd step before the addition of maturation stimuli (immature ES-DC) showed a weak but definite stimulation, and following exposure to maturation stimuli (mature ES-DC) showed a strong capacity to stimulate allogeneic T cells to proliferate. The antigen presenting capacity of ES-DC was confirmed by their ability to present Glutamic Acid Decarboxylase 65 (GAD65) antigen to GAD65-specific HLA-DR53-restricted human T cell clone, SA32.5 (31).

Su and colleagues introduced mRNA for green fluorescence protein (GFP) or prostate-specific antigen (PSA) into DC generated from human ES cells (25). They showed induction of CTL specific to the antigens by the *in vitro* stimulation of semi-allogeneic HLA-A\*0201<sup>+</sup> donor-derived T cells with genetically modified DC.



**Figure 1.** Morphology of mouse iPS-DC. Phase-contrast images of immature iPS-DC (A) and mature iPS-DC (B, C) are shown. (D) Mature iPS-DC on a glass slide stained with May-Grunwald-Giemsa are shown.

### 7. GENERATION OF DC FROM IPS CELLS

As described so far, we can generate ES-DC from both mouse and human ES cells. Mouse systems have demonstrated the induction of anti-cancer immunity and the prevention of autoimmune disease by *in vivo* administration of genetically engineered ES-DC.

Considering the future clinical application of ES-DC technology, however, the unavailability of human ES cells genetically identical to the patients to be treated is a problem. Alloreactivity caused by differences in the genetic background between human ES cell lines and the recipients is a critical problem in medical application of ES-DC. In addition, ethical concerns related to the use of human ES cells are anticipated to be serious obstacles, which will hinder the realization of the medical use of human ES-DC.

It was recently revealed that ES cell-like pluripotent stem cells, designated as induced pluripotent stem (iPS) cells, can be generated by the simultaneous introduction of several factors into somatic cells, for both mice and humans (32) (33) (34). The issue of histoincompatibility between patients to be treated and ES cells may be overcome by the generation of iPS cells from somatic cells of the patients such as fibroblasts or blood cells. The major ethical issues related to human ES cells may be avoided by the use of iPS cell-technology, because the use of human embryos is not necessary for the generation of iPS cells.

We recently reported the generation and characterization of DC derived from mouse iPS cells (35). The iPS cell-derived DC (iPS-DC) possessed the characteristics of DC including morphology (Figure 1), the capacity for T cell-stimulation, antigen-processing and presentation, and cytokine production. There was some delay in the kinetics of differentiation process of iPS cells, in comparison to the mouse ES cell lines that we have tested. On the other hand, the yield of differentiated cells

was higher than that in the cases of most mouse ES cell lines. Genetically modified iPS-DC expressing antigenic protein primed T cells specific to the antigen *in vivo* and elicited efficient antigen-specific anti-tumor immunity.

Using the OP9-co-culture-based method, Choi and colleagues generated various myelomonocytic cells including DC, macrophages, osteoclasts, neutrophils, and eosinophils from human iPS cells (36). We have also recently generated iPS-DC from human iPS cells. Human iPS-DC exhibited characteristics of DC, as human ES-DC do, in morphology, surface molecules, and T cell-stimulating capacity. Theoretically, we can now generate an infinite number of DC genetically identical to the patients by using iPS cells generated from relatively small numbers of somatic cells of the patients, such as dermal fibroblasts or blood cells.

The iPS cell technology provides unlimited cell source of DC for immune therapy. However, safety issues related to tumor formation need to be clearly resolved before clinical applications. Currently, we are using mouse-derived OP9 feeder cells and culture media containing fetal calf serum in the differentiation culture to generate DC from human iPS cells or ES cells. For the clinical application of iPS-DC, it is also desirable to develop a fully xeno-free culture procedure to generate iPS-DC.

We will circumvent the issue of histocompatibility between patients and DC to be used for the treatment, if iPS cell lines are generated from somatic cells of the patients. However, generation of clinical grade iPS cells for individual patients may be too costly, time consuming, and labor-intensive to be broadly applied. This may be resolved by the establishment of banks of iPS cells covering major HLA-haplotypes in each ethnic group (37). A bank of iPS cells with specific HLA-A, HLA-B, and HLA-DR haplotypes is feasible and may be more realistic resolution of the issue of histocompatibility than “fully personalized” iPS cells. According to the calculation by Nakatsuji and colleagues, a cell bank size of 30 iPS cell lines homozygous for the HLA haplotype would be able to find a three-locus match in 82.2 % of the Japanese population. Their calculation also indicates that at least one homozygote for each of 30 different haplotypes can be identified by examining 15,000 individuals in an HLA-type database.

### 8. SUMMARY

Because of the activity of DC as immune-regulatory cells, immune-modulation therapy by transfer of DC is regarded as promising to treat various diseases. In particular, DC-based cellular vaccination is considered to be a powerful means for active immunization therapy for cancer. ES cells possess a potential to differentiate into various kinds of cells and also an unlimited propagation capacity. Several groups including us have established methods to generate DC from mouse or human ES cells. Using mouse models, we have demonstrated the usefulness of genetically modified ES cell-derived DC in the induction of anti-cancer immunity and also in the control of

autoimmune disease. Recently, it has been discovered that ES cell-like pluripotent stem cells can be generated by introduction of several reprogramming factors into somatic cells, yielding iPS cells. DC can be generated from mouse and human iPS cells by the methods developed for ES cells. By the use of iPS cells instead of ES cells as the cell source of DC, we can overcome the issues of histoincompatibility and resolve ethical concerns related to human ES cells. For the clinical application of iPS cell-derived DC, it is necessary to develop a xeno-free culture procedure to generate iPS-DC.

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**Key Words:** Dendritic Cells, Embryonic Stem Cells, iPS Cells, Cell Therapy, Review

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