Neural progenitor cells from human induced pluripotent stem cells generated less autogenous immune response

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The breakthrough development of induced pluripotent stem cells (iPSCs) raises the prospect of patient-specific treatment for many diseases through the replacement of affected cells. However, whether iPSC-derived functional cell lineages generate a deleterious immune response upon auto-transplantation remains unclear. In this study, we differentiated five human iPSC lines from skin fibroblasts and urine cells into neural progenitor cells (NPCs) and analyzed their immunogenicity. Through co-culture with autogenous peripheral blood mononuclear cells (PBMCs), we showed that both somatic cells and iPSC-derived NPCs do not stimulate significant autogenous PBMC proliferation. However, a significant immune reaction was detected when these cells were co-cultured with allogeneous PBMCs. Furthermore, no significant expression of perforin or granzyme B was detected following stimulation of autogenous immune effector cells (CD3⁺CD8⁺ T cells, CD3⁺CD8⁺ T cells or CD3⁺CD56⁺ NK cells) by NPCs in both PBMC and T cell co-culture systems. These results suggest that human iPSC-derived NPCs may not initiate an immune response in autogenous transplants, and thus set a base for further preclinical evaluation of human iPSCs.

induced pluripotent stem cells, immunogenicity, iPSC-derived neural progenitor cells

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The ability of human embryonic stem cells (ESCs) to differentiate into all three embryonic germ layers represents a great potential avenue for cell-based transplantation medicine [1,2]. Recently, therapeutics derived from human ESCs have been used in animal models of diseases, such as Par-

kinson's disease [3,4], ischemic-reperfusion injury [5], and spinal cord injury [6]. However, several challenges to human ESC-based therapy have arisen. Aside from ethical concerns and a low efficacy in establishment, immune rejection of allogeneous transplantation currently limits the serious application of ESCs in clinical studies. These issues have been resolved by the development of induced pluripotent stem cells (iPSCs). iPSCs are reprogrammed from so-

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matic cells by the use of four defined factors (Oct4, Sox2, Klf4, and c-Myc) [7,8]. iPSCs have similar characteristics to those of ESCs, including unlimited growth capacity, multi-lineage *in vitro* differentiation, germline transmission, and even contribution to create entire animals [9–11]. However, the overwhelming advantage of iPSCs technology over ESCs is that patient-specific iPSCs are easily generated [12–14]. Moreover, iPSC-derived functional cells have been reported to successfully treat animal models of sickle-cell anemia and Parkinson's disease, amongst others [15–18]. Thus, iPSC technology offers the unprecedented opportunity to use human cells to study human diseases with further application to clinical therapies free of the immune rejection.

Despite these promises, whether cells derived from iPSCs are tolerated by the immune system remains uncertain. Fairchild expressed doubts regarding the lack of immunogenicity of iPSCs and iPSC-derived cell types as early as 2010 [19]. Shortly thereafter, Zhao et al. [20] reported that the transplantation of undifferentiated mouse iPSCs induced a T-cell-dependent immune response even in a syngeneic mouse. In this study, teratomas formed by B6 iPSCs were mostly immune-rejected by B6 recipients, while B6 ESCs were not. Several genes, including Zg16 and Hormad1, were found to directly affect the immunogenicity of iPSC derivatives. However, undifferentiated iPSCs would never be used in a treatment application because of their ability to randomly differentiate into teratomas. Therefore, it is possible that T-cell infiltration appears in the developing teratomas [21]. Recently, Araki et al. [22] demonstrated a limited or absent immune response, including T-cell infiltration, to differentiated skin and bone marrow tissues derived from mouse iPSCs. Additionally, no significant difference in the rate of transplantation success of iPSC-derived versus ESC-derived tissues was identified. Moreover, expression of Zg16 and Hormad1 genes was not increased in regressing skin and teratoma tissues. However, in this study the differentiation process was performed *in vivo*, which varies from the course of differentiation *in vitro*. Thus, the model used in this study does not exactly represent the approach to clinical therapies using human iPSCs. More recently, Guha et al. [23] found no increase in T cell proliferation *in vitro*, no antigen-specific secondary immune response and no rejection of iPSC-derived embryoid bodies (EBs) and tissue-specific cells after their transplantation into syngeneic mice. They further observed little evidence of an immune response to undifferentiated syngeneic mouse iPSCs following transplantation. Therefore, they concluded that differentiated cells derived from syngeneic mouse iPSC transplants were not rejected. However, all of the above studies were performed in mouse models, and the immunogenicity of human iPSCs and iPSC derivatives has not yet been explored intensively.

In the current study, we investigated the immunogenicity of human iPSCs derived from adult skin fibroblasts (SFs)

and urine cells (UCs). Following differentiation of iPSCs into neural precursor cells (NPCs), autogenous and allogeneous immune responses to NPC stimulation were compared. NPCs derived from human iPSCs did not induce an obvious immune response from autogenous immune cells, while a significant response from allogeneous immune cells was observed. These findings indicate that human iPSC derivatives could potentially be applied for auto-transplantation without inducing immune rejection.

1 Materials and methods

All samples were collected following principles approved by the Guangzhou Institute of Biomedicine and Health Ethical Committee. All volunteers who donated skin, urine or blood samples have signed consent forms upon request.

1.1 Cell culture and iPSC generation

Human adult skin fibroblasts (SFs) and urine cells (UCs) were obtained from healthy volunteers 20–40 years old. Human SFs were cultured in Dulbecco's Modified Eagle's Medium (DMEM; Hyclone, Logan City, Utah, USA) supplemented with penicillin/streptomycin (Hyclone), L-glutamine (Gibco, Carlsbad, California, USA), non-essential amino acids (NEAA; Gibco) and 10% fetal bovine serum (FBS; PAA, Queensland, Australia). Human UCs were cultured in primary medium containing DMEM/F12 1:1 (Hyclone), 10% FBS, SingleQuot Kit CC-4127 Renal Epithelial Growth Medium (REGM; Lonza, Basel, Switzerland), amphotericin B and penicillin/streptomycin for 2 d and then changed to Renal Epithelial Basal Medium (Lonza) containing SingleQuot Kit CC-4127 REGM for subsequent days [24,25]. Both SF-derived iPSCs (SF-iPSCs) and UC-derived iPSCs (UC-iPSCs) were obtained from South Stem Cell Bank in Guangzhou, China. SF-iPSCs were generated by transduction using pMX-based retroviruses by supplementation with Vitamin C and valproic acid as described [26]. UC-iPSCs were induced by oriP/EBNA episomal vectors carrying a combination of the reprogramming factors OCT4, SOX2, SV40LT, KLF4 and miR302/367 through electroporation [27,28]. H1 human ESCs were purchased from WiCell (WA01/H1; WiCell, USA). Both H1 ESCs and other human iPSCs were cultured on matrigel (BD Biosciences, Franklin, New Jersey, USA) using mTeSR1 medium (Stemcell, Vancouver, Canada). For teratoma formation, three million hESCs or hiPSCs were injected subcutaneously into severe combined immunodeficiency mice. Teratomas were sectioned after eight weeks, and further examined for ZG16 and HORMAD1 expression.

1.2 NPC differentiation *in vitro*

H1 ESC and iPSC colonies were detached with 0.2 mg mL^{-1}

Disperse (Gibco) and grown in suspension for 4 d in EB medium (DMEM/F12, 20% knockout serum replacement (Gibco), NEAA, penicillin/streptomycin, L-glutamine and beta-mercaptoethanol (Gibco)) supplemented with 5 $\mu\text{mol L}^{-1}$ SB431542 (Calbiochem, Darmstadt, Germany) and 5 $\mu\text{mol L}^{-1}$ dorsomorphin (Sigma, St. Louis, Missouri, USA). ESC- or iPSC-derived EBs were treated with neural induction medium containing DMEM/F12, N2 supplement (Gibco), heparin (2 $\mu\text{g mL}^{-1}$, PeproTech, Crescent AveRocky Hill, USA), and NEAA for a further two days followed by adhesion to a matrigel-coated plate. After two weeks' culture, neural rosettes were blown off and cultured in suspension for a further 2–4 d in N2B27 medium (1:1 DMEM/F12 and Neurobasal medium (Gibco), N2 supplement, B27 supplement (Gibco), 20 ng mL^{-1} epidermal growth factor (PeproTech) and 10 ng mL^{-1} b-fibroblast growth factor (Sigma)). Neural spheres (suspensions of NPCs) were formed, purified by several passages, and harvested to examine the expression of neuronal markers NESTIN and PAX6 via flow cytometry and immunofluorescence staining.

1.3 Isolation of PBMCs and T cells and proliferation assay

Human peripheral blood mononuclear cells (PBMCs) were isolated from the blood of healthy volunteers with density-gradient centrifugation as described [29–32]. Briefly, OptiPrep™ density gradient solution (Axis-shield, Oslo, Norway) was added to peripheral blood samples at a 1:8 ratio followed by centrifugation at 900 $\times g$ for 30 min. PBMCs were collected from the interphase and washed twice with RPMI 1640 medium (Gibco). T cells within PBMCs were isolated by incubation with CD3 MicroBeads (Miltenyi, Cologne, Germany) and separated using a MiniMACS™ Separator with an MS Column (Miltenyi). Both PBMCs and T cells were maintained in RPMI 1640 medium containing 10% FBS. Cell viability was examined by trypan blue exclusion staining.

Co-culture experiments were carried out as described previously [30,33,34]. Culture volumes of 100 μL per well in a 96-well plate were used, with the ratio of antigen cells to PBMCs kept at 1:100. Before co-culture, antigen cells were pre-treated with mitomycin C. Detailed information

for all groups is displayed in Table 1. Following a three-day co-culture, PBMC proliferation was examined using a cell proliferation BrdU ELISA kit (Roche Diagnostics, Basel, Switzerland) according to the manufacturer's instructions. The reaction was quantified by measuring the optical density at a wavelength of 370 nm with a reference wavelength of 492 nm. The degree of proliferation was calculated as $(A_{370}-A_{492})-(A'_{370}-A'_{492})$, where A =mean absorbance value of experimental group and A' =mean absorbance value of the blank group. Analysis was performed with Graphpad Prism 5.0. Three allogeneous PBMC samples and one autogenous PBMC were used in the co-culture system. The final proliferation level of the negative group was regarded as one, and the proliferation level of experimental group was analyzed in relation to this.

1.4 Assessment of cytotoxic molecule expression in the co-culture system

1×10^6 PBMCs or T cells were added to each well of a 96-well plate in a 200 μL volume. The ratio of antigen cells to PBMCs/T cells was 1:100. The PBMC co-culture system had both positive and negative control groups, while the T cell co-culture system negative control group contained lymphocytes only and T cells in the positive control group were activated with Human T-Activator CD3/CD28 Dynabeads® (Life Technologies, Carlsbad, California, USA) as per the manufacturer's instructions. Following overnight co-culture, the expression levels of cytotoxic molecules (perforin and granzyme B) in CD3⁺CD8⁺ T cells, CD3⁺CD8⁺ T cells and CD3⁺CD56⁺ NK cells were examined by flow cytometry.

1.5 Real-time quantitative PCR (qPCR)

Total RNA was extracted with Trizol (Invitrogen, Carlsbad, California, USA) and reverse-transcribed using an RT-PCR kit (TaKaRa, Tokyo, Japan). qPCR was performed using a Thermal Cycler Dice™ Real Time System and SYBR Green Premix EX Taq™ (TaKaRa). Relative quantification of target gene expression was determined relative to that of β -actin and all measurements were in triplicate. Primer sequences (5'→3') were as follows: ZG16 (forward: TCCGG-

Table 1 Design of each group in the PBMC co-culture system^{a)}

Groups	Design (volum =100 μL)
Somatic cells group	2×10^5 PBMCs + 2×10^3 somatic cells
NPCs group	2×10^5 PBMCs + 2×10^3 NPCs
Positive group	2×10^5 PBMCs + PHA(4 ng mL^{-1})
Negative group	2×10^5 PBMCs only
Blank group for antigen cell stimulated group	2×10^3 somatic cells or NPCs
Blank group for positive or negative group	Medium only

a) The ratio of antigen cells to PBMCs was kept at 1:100, and 100 μL was set up as a volume system per well in a 96-well plate.

GTCCGAGTCAACA; reverse: TCCTCCAGGTCTCCGTTGC); *HORMAD1* (forward: GCCCAGTTGCAGAGGACTC; reverse: TCTTGTTCATAAGCGCATTCT); β -*ACTIN* (forward: CCCAGAGCAAGAGAGG; reverse: GTCCAGACGCAGGATG).

1.6 Immunofluorescence staining

Cells were fixed for 20 min in 4% paraformaldehyde dissolved in 0.1 mol L⁻¹ phosphate buffer solution (PBS) and washed thrice with 0.01 mol L⁻¹ PBS. Cells were further incubated with primary antibodies (Rabbit anti-NESTIN, 1:1000 (Millipore, Billerica, MA, USA); Mouse anti-PAX6, 1:1000 (Developmental Studies Hybridoma Bank, Iowa city, Iowa, USA)) in PBS with 10% goat serum and 0.3% Triton X-100 overnight at 4°C. Species-specific secondary antibodies conjugated to Alexa Fluor[®] 448 or 568 (1:400, Invitrogen) were used to visualize primary antibodies. Nuclei were counterstained with 4',6-diamidino-2-phenylindole (DAPI; Sigma). Samples were mounted in anti-fade medium (Invitrogen) and preserved at 4°C.

1.7 Flow cytometry

NPCs derived from iPSCs (SF-NPCs and UC-NPCs) were

prepared at a concentration of 1×10^5 cells in 100 μ L PBS containing 2% FBS, while PBMCs and T lymphocytes were prepared at 1×10^6 cells in 100 μ L PBS containing 2% FBS. Antibodies used were NESTIN-PerCP-Cy[™] 5.5, PAX6-Alexa Fluor[®] 488, CD3-PerCP, CD8-FITC, CD56-APC, perforin-PE and granzyme B-PE (all purchased from BD Biosciences). Antibodies were added and incubated for 30 min at 4°C. After two washes in PBS, cells were acquired and analyzed on a FACSCalibur (BD Biosciences).

1.8 Statistical analysis

Values for qPCR and PBMC proliferation were expressed as mean $\pm$ SEM. Differences between groups were analyzed by one-way analysis of variance (ANOVA) followed by *t*-tests. The *P*-value was set at 0.05. All statistical analyses were performed with SPSS 17.0.

2 Results

2.1 NPC differentiation from hESCs/hiPSCs

NPC differentiation from hESCs and hiPSCs derived from both SFs and UCs was performed as described previously [26,30,35,36]. The typical neural rosettes were blown off and picked out to produce a neural sphere consisting of

Figure 1 Characterization of human ESC/iPSC-derived NPCs. A, Phase contrast micrographs of somatic skin fibroblasts (SFs) and urine cells (UCs)), somatic cell-derived iPSCs, iPSC-derived neural rosettes and neural spheres. B, Flow cytometry analysis of neural markers (NESTIN and PAX6) in H1-ESC/iPSC-derived NPCs (H1-NPCs, SF-NPCs, UC-NPCs). C, Immunofluorescence microscopy depicting NESTIN (green) and PAX6 (red) in NPCs. DAPI is shown in blue. Scale bar, 100 μ m.

NPCs (Figure 1A). The differentiated cells from both cell origins were indeed NPCs confirmed with NPC markers (NESTIN and PAX6). Both SF-NPCs and UC-NPCs expressed the NPC marker NESTIN at a similar level to that detected in H1 hESC-derived NPCs (Figure 1B). The NPC marker PAX6 was also expressed in a similar manner from both cell types (Figure 1B). After attachment to culture plates, NPCs displayed the polarity typical of such cells. Positive expression of NESTIN (green) and PAX6 (red) was detected by immunofluorescence (Figure 1C). Therefore, the iPSC-derived NPCs used here retain the characteristics of NPC imprinting.

2.2 PBMC proliferation in a co-culture system

We next examined the immunogenicity of iPSC-derived NPCs. NPCs served as antigens and were co-cultured with autogenous or allogeneous PBMCs. For the measurement of PBMC proliferation in each sample, the degree of prolifera-

tion of PBMCs stimulated with NPCs was analyzed relative to a negative group with slight modification to the method previously described [33]. Reactions of the somatic cell lines (SF3, UC5) and the relative NPCs (SF3-NPCs, UC5-NPCs) are displayed as examples (Figure 2A). No significant proliferation was observed when PBMCs were stimulated with either autogenous SF3 or SF3-NPCs (Figure 2A). Meanwhile, significant immune reactions were detected when SF3 or SF3-NPCs were co-cultured with three different allogeneous PBMCs (Figure 2A). UC5 and UC5-NPC co-culture did not induce significant activation of autogenous PBMC proliferation, while obvious proliferation was observed in three different allogeneous PBMC co-culture systems (Figure 2B). When compared with autogenous PBMCs, the relative proliferation level of each allogeneous PBMC co-culture condition was significantly higher ($P < 0.05$). The relative proliferation levels stimulated by each cell line are summarized in Figure 2C. These data revealed that human iPSC-derived NPCs do not induce im-

Figure 2 Proliferation of PBMCs stimulated by somatic cells and NPCs derived from SF and UC origins in a co-culture system. A, Relative PBMC proliferation in the co-culture system when stimulated with SF3 (left) and SF3-NPCs (right). B, Relative PBMC proliferation in the co-culture system when stimulated with UC5 (left) and UC5-NPCs (right). C, Summary of the relative autogenous or allogeneous PBMC proliferation level following stimulation by somatic cells or NPCs from two SF and three UC donors in the co-culture experiment. Results are expressed as mean $\pm$ SEM, and differences among groups were analyzed by one-way analysis of variance (ANOVA) followed by the single sample *t*-tests. “—” indicates a relative proliferation level of less than 1.5 when compared with the negative group; “*” indicates a relative proliferation level of 1.5–2; “**” indicates a relative proliferation level of 2–3; “***” indicates a relative proliferation level greater than 3.

mune response in autogenous cells.

2.3 Distinct expression of the cytotoxic molecules perforin and granzyme B in co-culture system

We further investigated whether the distinct kinetics profiles of perforin and granzyme B existed in autogenous PBMCs or T lymphocytes when co-cultured with iPSC-derived NPCs. The percentage of perforin and granzyme B expression in $CD3^+CD8^-$ T cells, $CD3^+CD8^+$ T cells or $CD3^+CD56^+$ NK cells was analyzed in PBMCs or T lymphocyte co-cultures with iPSC-derived NPCs via flow cytometry (Figures 3A and 4A). No activation of expression of perforin or granzyme B was detected in any of the cell groups within autogenous PBMC populations when stimulated by both NPC cell types (Figure 3B and C; Supplemental Files 1 and 2 in Supporting Information). However, significant activation of perforin expression by all three immune cell groups was observed in allogeneous PBMCs following co-culture with both NPCs (Figure 3B and C).

Similar activation of perforin expression was detected in

the T cell co-culture system (Figure 4B; Supplemental File 3 in Supporting Information). With the exception of the one allogeneous T cell group stimulated by SF1-NPCs that showed a modest increase in relative expression of granzyme B, all other groups showed no significant activation, including autogenous T cell groups (Figure 4C; Supplemental File 4 in Supporting Information). The results also raised the consistent opinion that human iPSC-derived NPCs do not activate immune cells and initiate immune response in autogenous cells.

3 Discussion

iPSCs are considered potentially more useful than ESCs for studying and treating human diseases, with functional iPSC derivatives displaying promising progress towards clinical applications [12,13,15,16]. The immunogenicity of iPSC derivatives is one of the most important issues that needs to be addressed before clinical therapies using iPSC techniques can proceed [19,20,22]. Currently, this issue is high-

Figure 3 Perforin and granzyme B expressions in a PBMC co-culture system with hiPSC-derived NPC stimulation. A, Analysis of surface phenotypic markers (CD3, CD8, CD56) present on T cells and NK cells. For each cell origin, one strain of autogenous PBMCs and three strains of allogeneous PBMCs were individually co-cultured with the relevant NPCs and labeled with appropriate fluorescence-conjugated antibodies. Three obvious cell groups ($CD3^+CD8^-$ T cells, $CD3^+CD8^+$ T cells and $CD3^+CD56^+$ NK cells) were identified via flow cytometry analysis. B and C, Analysis of perforin and granzyme B expression in T cells and NK cells stimulated with SF-NPCs or UC-NPCs.

Figure 4 Perforin and granzyme B expressions in a T cell co-culture system with NPC stimulation. A, Analysis of surface phenotypic markers (CD3, CD8) of T cells. For each cell origin, one strain of autogenous T cells and three strains of allogeneous T cells were individually co-cultured with the relevant NPCs and labeled with appropriate fluorescence-conjugated antibodies. Two obvious cell groups (CD3⁺CD8⁻ T cells and CD3⁺CD8⁺ T cells) were identified by flow cytometry analysis. B and C, Analysis of perforin and granzyme B expression in T cells stimulated with SF1-NPCs or UC5-NPCs.

ly controversial with the majority of studies based on rodent models [20,22,23]. Here, we have explored the immunogenicity of human iPSC-derived NPCs using a PBMC and T cell co-culture system.

PBMCs include immune effector cells (T cells, B cells and NK cells) and antigen presenting cells (monocytes/macrophages and dendritic cells), which perform a range of different functions during the immune rejection process [37,38]. In this study, we examined the proliferation and cytotoxic molecule expression of this mixed cell population following stimulation by somatic cells and NPCs. Relative levels of cellular proliferation and cytotoxic molecule expression were much higher in most allogeneous immune cell samples than in autogenous immune cells when stimulated with NPCs. However, the allogeneic T cells present in peripheral blood are mostly in the naive stage, and stimulation by professional antigen presenting cells (APCs) is required for their proliferation. The proliferation observed in allogeneous groups (Figure 2) was likely caused by cross-presentation of NPC-derived allogeneic antigens (minor antigens) by APCs such as B cells and monocytes/

macrophages that are included within PBMCs. In other words, the observed proliferation reflects an indirect rather than direct T cell response. However, to examine direct T cell stimulation, pre-stimulated allo-reactive T cells rather than PBMCs should be used for the T cell response assay, and a cytokine production assay may prove more sensitive than the proliferation assay employed here. We further investigated the expression of the cytotoxic molecules perforin and granzyme B by several kinds of immune effector cells in a T cell co-culture system (Figure 4). Even though the PBMC or T cell co-culture system imperfectly mimics the *in vivo* immune response, it is the most reliable human model currently available. Moreover, individual difference is likely to present more difficulties for investigation. Nevertheless, our data showed no large difference in the immunogenicity of NPCs derived from iPSCs created from two different cell sources. SF-iPSCs were generated by transduction with pMX-based retroviruses, while UC-iPSCs were induced with electroporation of oriP/EBNA episomal vectors. The UC-iPSCs in particular are regarded as virus-free and integration-free human iPSC lines, and repre-

sent a step forward in clinical study of iPSCs. Taken together, our data indicate that these iPSC derivatives are not recognized as allogeneous factors by autogenous immune cells and do not initiate a self immune reaction.

Additionally, ZG16 and HORMAD1 have been demonstrated to directly affect the immunogenicity of iPSC derivatives in syngeneic mice [20]. We therefore investigated the expression of ZG16 and HORMAD1 in somatic cells, iPSCs, iPSC-derived teratomas and NPCs of five different cell origins. Limited or no expression of both markers above normal was observed in cells of all origins. ZG16 expression level in each iPSC-derived teratoma was significantly higher, while HORMAD1 was found to remain silenced in the teratomas (Supplemental File 5 in Supporting Information). Therefore, the mechanisms by which ZG16 and HORMAD1 affect immunogenicity may differ. Furthermore, these results suggest that the immunogenicity of iPSC-derived teratomas may be affected in the development process, and this may have caused the autogenous T-cell infiltration observed previously [20,21]. However, the silenced expression of ZG16 and HORMAD1 in iPSC-derived NPCs indicates that the differentiation process does not change the immunogenicity of these NPCs and could not activate autogenous lymphocytes. Our current study supports this conclusion (Figures 2–4).

In summary, we have demonstrated that NPCs derived from human iPSCs *in vitro* are not recognized by autogenous immune cells, while allogeneous immune cells were activated by NPCs. These findings suggest that human iPSC-derived NPCs hold potential value for development of further clinical therapies. However, it is impossible to investigate the immunogenicity of human iPSCs and iPSC derivatives in human bodies. Therefore, in addition to *in vitro* studies, evaluation of the immunogenicity of iPSCs and their derivatives should be further explored in functional humanized animals prior to clinical application.

The authors declare no conflict of interest.

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Supporting Information

Supplemental File 1 Percentage of perforin expression in various immune effector cells in the PBMC co-culture system.

Supplemental File 2 Percentage of granzyme B expression in various immune effector cells in the PBMC co-culture system.

Supplemental File 3 Percentage of perforin expression in the immune effector cells in the T cell co-culture system.

Supplemental File 4 Percentage of granzyme B expression in the immune effector cells in the T cell co-culture system.

Supplemental File 5 Expression of ZG16 and HORMAD1 determined via qPCR. Similar results were obtained in three independent experiments. Results are expressed as mean $\pm$ SEM.

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