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Role of Regulatory T Cells in Noninherited Maternal Antigen-Related Tolerance in Cord Blood: An in Vitro Study

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A B S T R A C T

Cord blood (CB) is an alternative stem cell source for allogeneic hematopoietic stem cell transplantation (HSCT). The unique advantages of using CB as a stem cell source are a degree of permissibility for HLA mismatch, rapid availability, and relatively risk-free cell collection. Because HLA is highly polymorphic and population-specific, optimal HLA-matched unrelated donors or cord blood units (CBUs) might not be available. In view of the possibility that matched CBUs that include noninherited maternal antigens (NIMAs) might contain acceptable HLA mismatches, we attempted to determine the degree of alloreactivity of CB mononuclear cells (MNCs) on stimulation by the maternal, paternal, and unrelated stimulator cells. Suppression of T cell proliferation, cytotoxicity, and a cytokine profile indicating suppressed Th1 and elevated IL-10 and TGF-β1 responses were observed in the mixed lymphocyte reaction in response to NIMAs. The increases in IL-10 and TGF-β1 production may be due to the Th2 response and/or regulatory T cells (Tregs). The reduced IL-10 and TGF-β1 production after CD25 depletion could have been due to removal of Tregs from the CB cells. Thus, Tregs appear to play an important role in the CB MNC response to NIMAs, possibly due to the induction of IL-10 and TGF-β1. We hope that our work can provide some evidence of the beneficial effect of NIMAs.

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) is the treatment of choice for many hematologic malignancies and immunodeficiency disorders in pediatric patients [1]. According to the Hong Kong Cancer Registry of the Hospital Authority, the average age-standardized incidence rate of hematologic malignancies is .54 per 1 million population [2]. Despite more than 30 million volunteer donors registered at Bone Marrow Donors Worldwide [3], finding an HLA-matched unrelated donor remains very difficult. Along with unrelated volunteer bone marrow (BM) donors, cord blood (CB) is another important source for HSCT, accounting for approximately 22% of all unrelated transplantations worldwide in 2011 [4]. CB offers many advantages over BM, including easy procurement, immediate access, and no risk to the donor. Most importantly, it has less stringent requirements for HLA matching and higher permissibility for HLA mismatch [5].

In Hong Kong, there has been an increasing use of CB units (CBUs) over the past decade, particularly for pediatric patients with hematologic malignancies who lack HLA-matched volunteer adult donors. Currently, almost one-half of all allogeneic HSCTs performed in young patients use CB as the stem cell source. In 2016, a total of 217 Hong Kong and 424 overseas patients requested a search of the register, but only 60 (10%) of these patients located a suitable donor (annual report of Hong Kong Bone Marrow Donor Registry). Eight of these patients underwent CB transplantation (CBT), including 5 from the Hong Kong Catherine Chow Cord Blood Bank and 3 from overseas CB banks. Thus, the chance of finding a suitable donor is low, and consequently, there is a pressing need to develop effective strategies to increase the donor pool.

Transplantation tolerance was first described in recipients who had been exposed to alloantigens. In 1945, Owen et al. [6] reported that fetal exposure to alloantigens via vascular anastomoses led to indefinite allogeneic HSCT survival between bovine twins. Later, Billingham et al. [7] described the first animal model of neonatal tolerance induction. Maternal cells and

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microorganisms traffic bidirectionally between the mother and fetus during pregnancy and between the mother and neonate during breast-feeding in a mouse model [8]. This phenomenon has been implicated in the offspring's ability to accept allogeneic transplants [9]. The most compelling evidence for the maternal influence on the offspring's immune system has been shown in studies on the role of noninherited maternal antigens (NIMAs) in transplantation; Van Rood et al. [9] reported the influence of NIMAs on both humoral and cellular alloimmunity in humans. Most patients who produced donor- specific antibodies after blood transfusion did not form anti-NIMA antibodies but mounted humoral responses to noninherited paternal antigens (NIPAs) [10]. Later, Bean et al. [11] also found increased responses in mixed lymphocyte reactions (MLRs) after blood transfusions from paternal donors compared with maternal donors. Other studies have shown that NIMAs in the donor might improve patient survival in kidney transplantations and reduce the incidence of graft-versus-host disease (GVHD) in HSCT, supporting the tolerogenic effect of NIMAs [12,13].

NIMAs are HLA haplotypes from the mother not inherited by the offspring. It has been suggested that the induction of tolerance mediated by NIMAs is due to maternal microchimerism [14,15]. The maternal cells in fetuses have been found to be persistently engrafted in the offspring [16]. The induction of NIMA-mediated tolerance by exposure of the fetus and

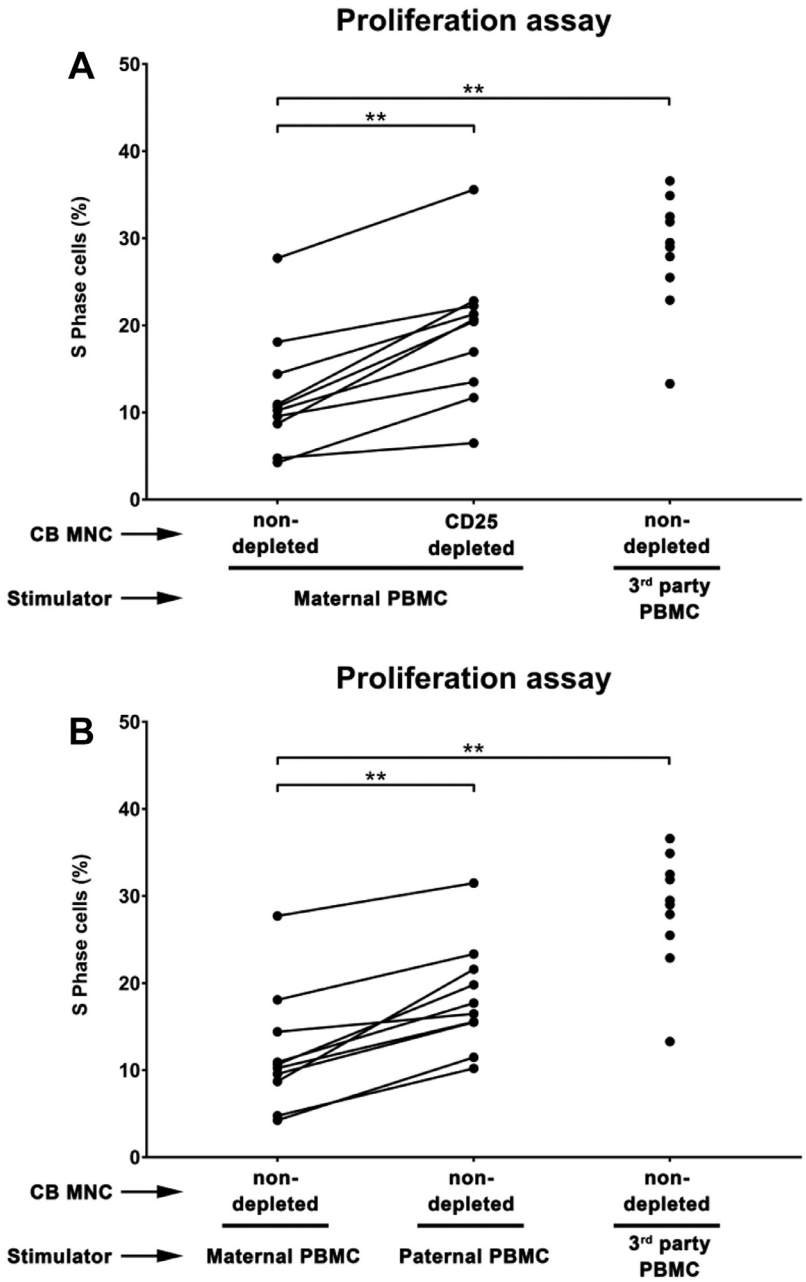

Figure 1. Proliferation response of CB MNC to different PBMC stimulators before depletion of $CD25^+$ Tregs. (A) Responses to depleted and nonepleted maternal PBMC stimulators. (B) Responses to maternal, paternal, and third-party stimulators of nondepleted CB MNC. n = 10. $^{**}P < .01$.

newborn to maternal cells has been found in mouse models and human transplants [13,17–19]. Van Rood et al. [20] reported improved patient survival after NIMA-matched CBT, and Rocha et al. [21] reported similar findings in a larger cohort. These studies demonstrated that the presence of NIMAs could be an important criterion for the selection of volunteer donors or CBUs for HLA-mismatched transplantation. The reduced GVHD and better transplantation outcomes increase the possibility of matching based on the increased likelihood of matching 4 to 5/6 out of 6 matched.

In a retrospective study of 41 pediatric CBTs performed for hematologic malignancies between January 2008 and December 2016 in Hong Kong, we observed better outcomes in patients who underwent NIMA-matched CBT compared with those who underwent NIMA-mismatched CBT [22]. Our results echo the findings of Rocha et al. [21].

Several previous studies have shown the tolerogenic effects of NIMAs in allotransplantation [17,23,24], but conflicting results have been reported by Molitor-Dart et al. [25]. The mechanisms by which NIMA drive the immune system toward transplant tolerance or rejection remain unclear; further characterization is needed and could lead to novel tolerance induction profiles in clinical transplantation. In the present study, we addressed this important issue using standard technology with some modifications. Our aims were to elucidate the roles of NIMAs and the mechanism of NIMA-mediated tolerance in

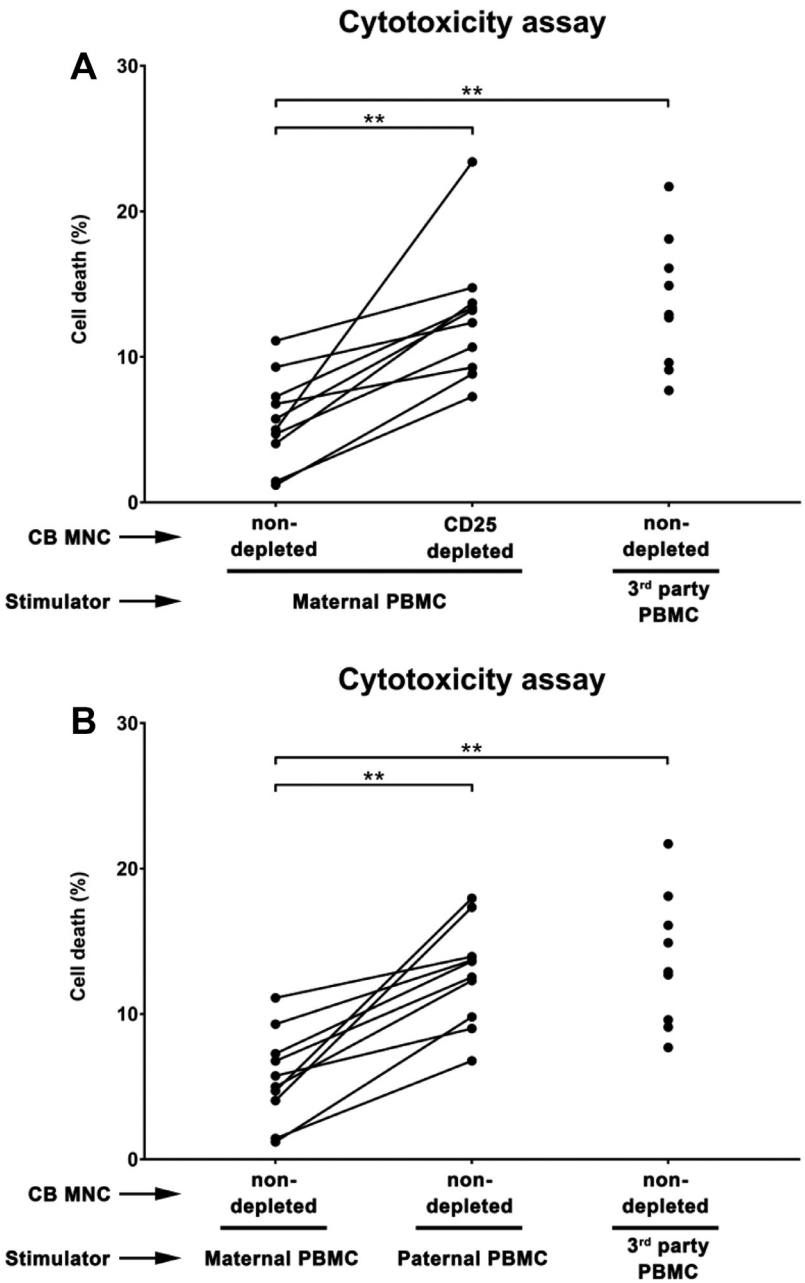

Figure 2. Percent killing of maternal, paternal, and third-party PBMC by CB MNC. (A) CB MNC showed significantly higher killing of maternal PBMC after $CD25^+$ Treg depletion, approaching the level of those for third-party target cells ($P < .01$). (B) Cytotoxic responses of CB MNC were higher against paternal than maternal target cells ($P < .01$). n = 10. $^{**}P < .01$.

CBT and to study the involvement of NIMA as an additional criterion for choosing the CBU for transplantation.

T cells respond to different major histocompatibility complex (MHC) molecules, minor histocompatibility antigens, and other tissue antigens following cell or organ transplantation [26]. The immune response is initiated when the T cell receptor (TCR) recognizes nominal peptide antigens bound to the MHC and T cell alloreactivity in the MLR is similar [27]. Thus, the MLR provides a simple in vitro model for the study of T cell activation and proliferation. Donor T cells recognizing allogeneic HLA molecules on host tissue are mediators of graft-versus-host disease (GVHD) [28]. Donor-specific T cell alloreactivity can be analyzed; for example, the frequency of donor-specific IFN-γ-producing T cells is recognized as a predictive indicator of GVHD [29,30]. In the present study, we investigated the proliferation, cytotoxic response, and cytokine profiles of CB mononuclear cells (MNCs) on stimulation with self, maternal, paternal, and third-party cells in the MLR model.

METHODS

Study Subjects

CB and related maternal and paternal blood specimens were collected after written consent. Unrelated stimulator cells were collected from healthy individuals. MNCs were isolated by Ficoll-Hypaque gradient configuration and cryopreserved for the subsequent experiments.

NIMA-Tolerance Assays

MLR, cell proliferation assay, cytotoxicity assay, cytokine production, and enzyme-linked immunosorbent assay (ELISA) were used to determine the degree of alloreactivity of CB MNCs on stimulation by maternal, paternal, and unrelated third-party stimulator cells. The presence of NIMA-mediated

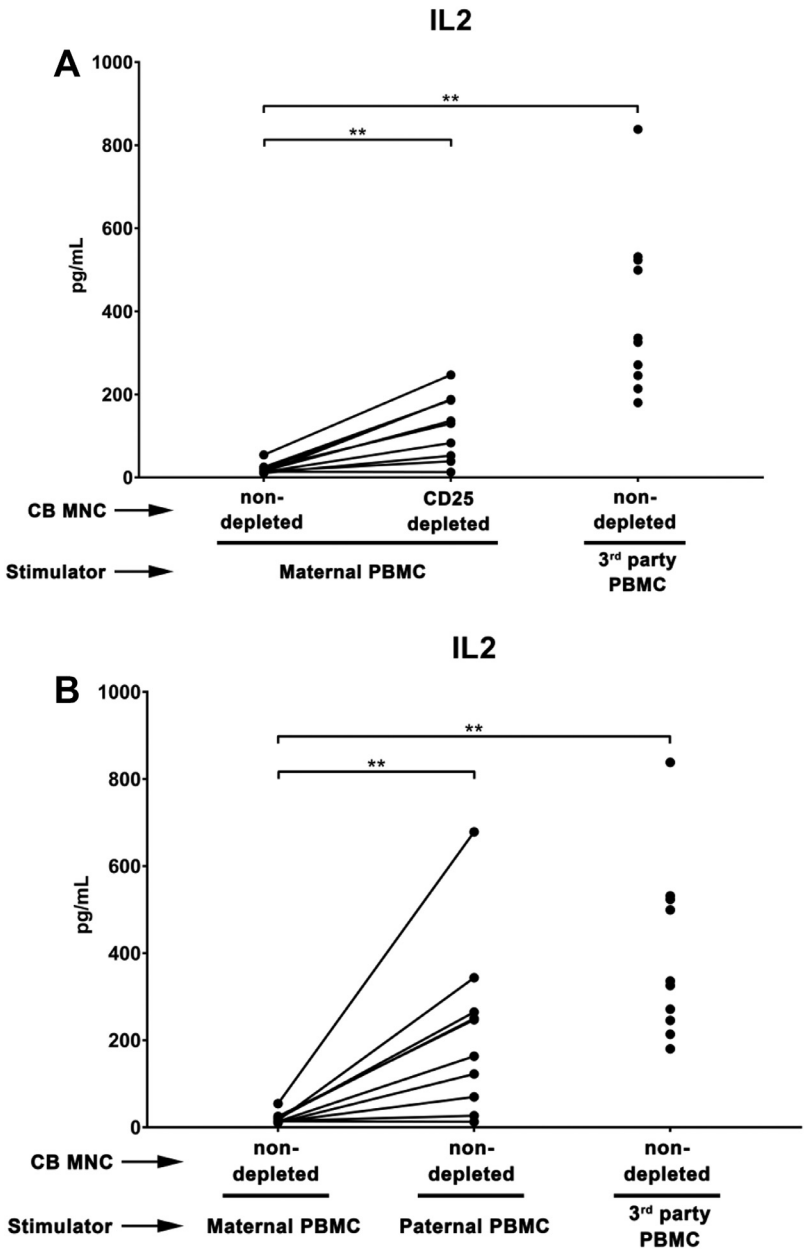

Figure 3. Effect of CD25$^+$ cell depletion on secretion of immunostimulatory cytokine IL-2 in cultures stimulated with maternal, paternal, or third-party PBMCs. n = 10. $^*P < .05$; $^{**}P < .01$. Panel (A) compares results to depleted and non-depleted maternal PBMC stimulators whereas Panel (B) compares results to maternal, paternal and third-party stimulators of non-depleted CB MNC.

tolerance between donor and recipient was investigated in vitro using MLR. In brief, thawed CB MNCs (responder cells) were cultured in RPMI 1640 medium supplemented with 10% FBS (Gemini Bio-Products, West Sacramento, CA) and human recombinant IL-2 (rIL-2) (20 U/mL; Miltenyi Biotec, San Diego, CA) overnight at 37°C in 5% CO_2. Thawed maternal, paternal, and unrelated third-party cells were irradiated and used as stimulator cells. Responder and irradiated stimulator cells were mixed at a 1:1 ratio and cultured in pairs of 96-well plates at 37°C for 6 days for proliferation assays and for 10 days for cytotoxicity assays. Responder cells were cocultured with irradiated responder cells as controls.

Depletion of CD25$^+$ Tregs

Depletion of CD25$^+$ responder cells was done using the EasySep Human Pan-CD25 Positive Selection and Depletion Kit (STEMCELL Technologies, Cambridge, MA) following the manufacturer's instructions. The cell mixture was pooled with Magnetic RapidSpheres (STEMCELL Technologies) and then incubated at room temperature. Then separation medium was added to the cell mixture, gently pipetted up and down, and then placed into the magnet (EasySep Magnet; STEMCELL Technologies), followed by incubation at room temperature. The purity of the isolation was checked by flow cytometry (99 ± 3%). An example flow cytometry analysis of CB cells before and after CD25$^+$ Treg depletion is shown in Supplementary Figure 1.

Proliferation Assays

T cell proliferation was determined by a flow cytometry-based assay. The cell cycle can be subdivided into two main stages: interphase and mitosis. G1, S, and G2 phases are the 3 distinct and successive stages within the interphase. During S phase, DNA synthesis and replication of chromosomes is completed. Bromodeoxyuridine (BrdU) is incorporated into newly synthesized DNA by cells entering and progressing through the S phase. The incorporated BrdU is then stained with anti-BrdU fluorescent antibodies.

In brief, responder cells and irradiated stimulator cells were mixed at a 1:1 ratio and cultured in 96-well plates at 37°C for 6 days. Responder cells were labeled with BrdU at 18 hours before the end of culturing and with CD3 antibodies and anti-BrdU antibodies after the end of culturing, and the cultures were analyzed using the BD FACSVerse system (BD Biosciences, San

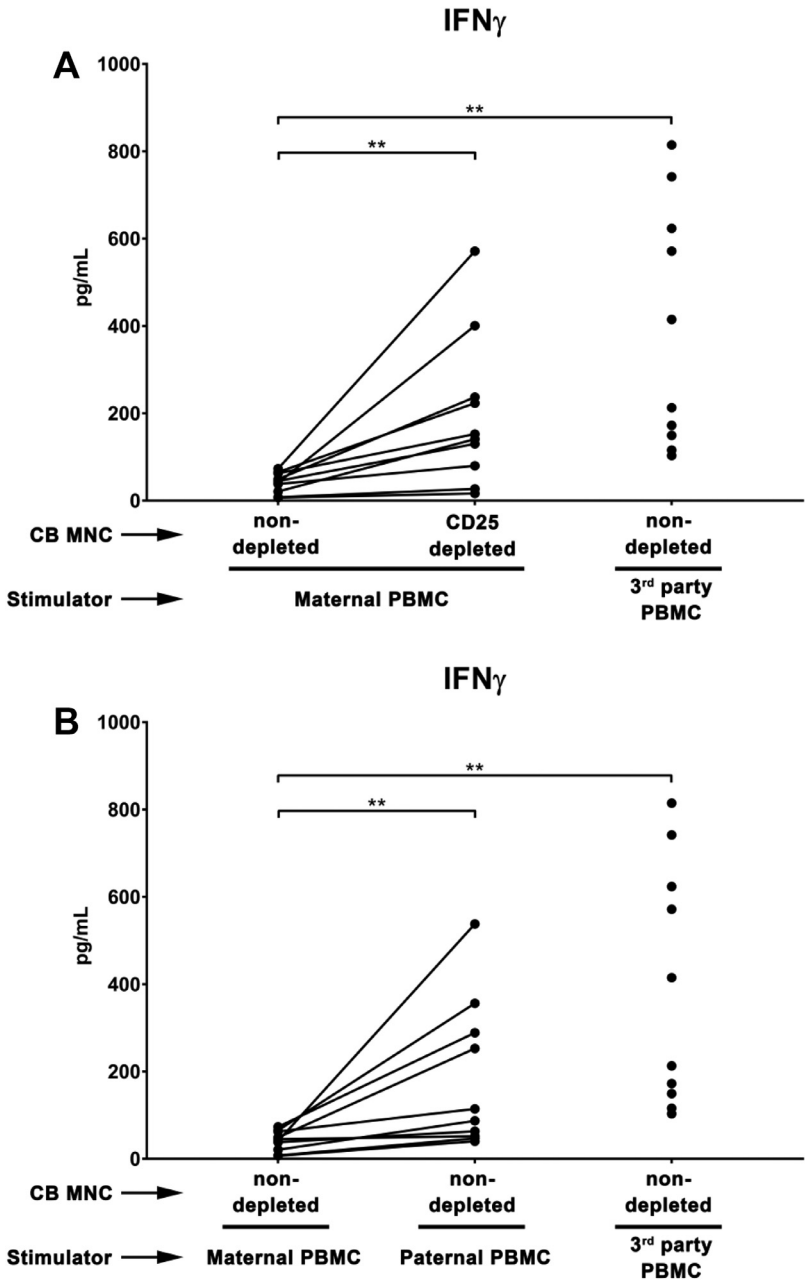

Figure 4. Effect of CD25$^+$ cell depletion on secretion of type 1 proinflammatory cytokine IFN-γ. in cultures stimulated with maternal, paternal, or third-party PBMCs. n = 10. $^*P < .05$; $^{**}P < .01$. Panel (A) compares results to depleted and non-depleted maternal PBMC stimulators whereas Panel (B) compares results to maternal, paternal and third-party stimulators of non-depleted CB MNC.

Jose, CA). Results of a representative flow cytometry analysis on the gating of BrdU-positive population are shown in Supplementary Figure 2.

Cytotoxicity Assay

The cytotoxicity assay was set up by culturing responder cells with irradiated (50 Gy) stimulator cells at a 1:1 ratio in 96-well plates at 37°C with 5% CO_2 for 10 days (Supplementary Figure 3). Human rIL-2 (20 U/mL) was added on days +3 and +6. The target cells were generated from the original stimulator or third-party peripheral blood mononuclear cells (PBMCs) by incubating the cells with purified phytohemagglutinin-P (5 mg/mL; Difco, Detroit, MI) from days 0 to +6 and with rIL-2 (20 U/mL) from days +3 to +10. On day +10, stimulator cells as target cells were separated from responder cells and labeled with Vybrant DiO cell-labeling solution (Thermo Fisher Scientific, Waltham, MA) at 37°C for 5 minutes. Labeled target cells were washed twice with RPMI 1610 medium with 10% FBS and added to the corresponding MLR cultures, followed by further incubation with 5% CO_2 at 37°C for 4 hours. After incubation, 7-aminoactinomycin D solution (eBioScience; Thermo Fisher Scientific) was added, followed by incubation at room temperature for another 15 minutes. The percentage of dead target cells was assayed by flow cytometry. The value of net killing of target cells by responder cells was calculated by subtracting the percentage of dead target cells in the autologous control culture. The data are expressed as percentage of dead cells. An example of flow cytometry analysis of live and dead cells in the cytotoxicity assays is shown in Supplementary Figure 4.

Cytokine Assay Detection

Supernatants from MLR cultures of CB MNCs and allogeneic stimulator cells were collected on days +3 and +6, and the levels of cytokines IFN-γ, IL-2, IL-4, IL-10, IL-17, TNF-α, and TGF-β were measured with the DuoSet ELISA Development System (R&D Systems, Minneapolis, MN). IFN-γ, TNF-α, TGF-β, and IL-17 production was assayed after 6 days of MLR, whereas IL-2, IL-4, and IL-10 production was measured after 3 days. To study the effect of Tregs on allogeneic stimulation, all T cell stimulations were conducted in the presence or absence of $CD25^+$ Tregs.

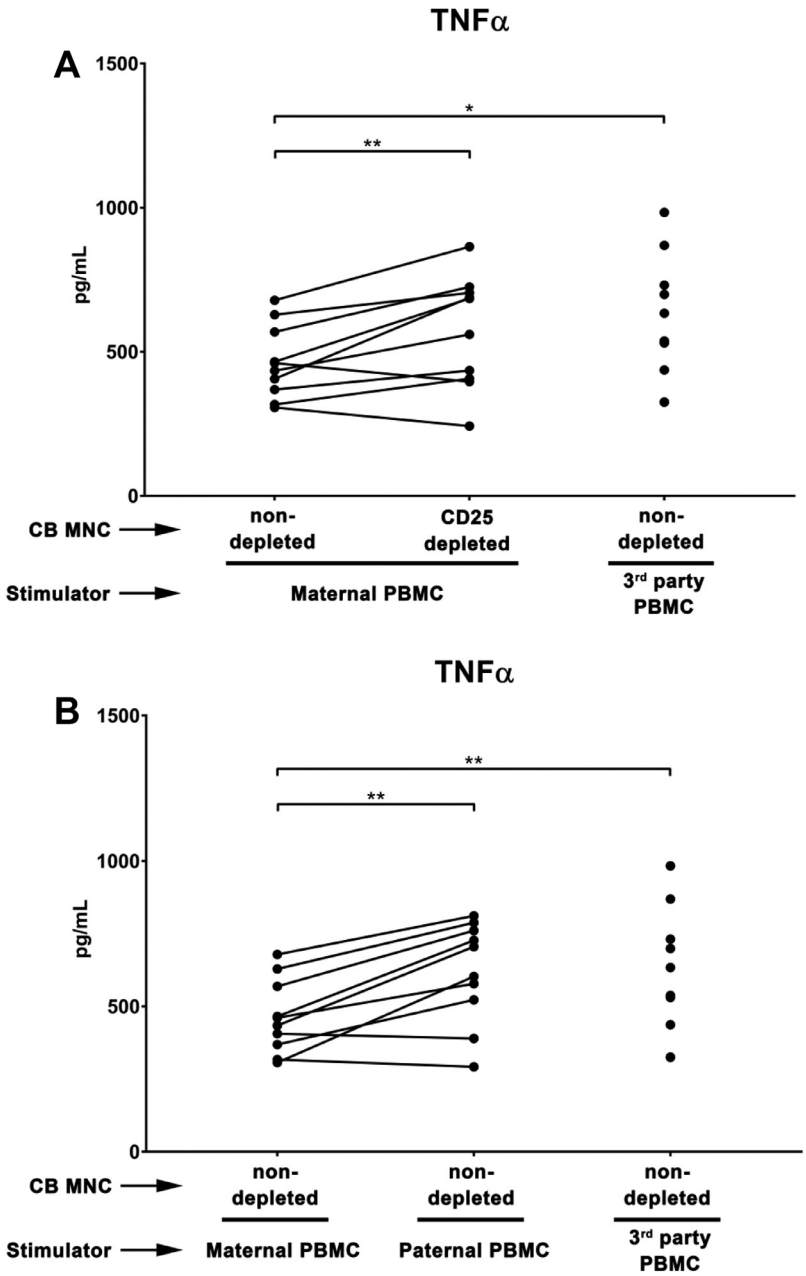

Figure 5. Effect of $CD25^+$ cell depletion on secretion of type 1 proinflammatory cytokine TNF-α in cultures stimulated with maternal, paternal, or third-party PBMCs. n = 10. $^*P < .05$; $^{**}P < .01$. Panel (A) compares results to depleted and non-depleted maternal PBMC stimulators whereas Panel (B) compares results to maternal, paternal and third-party stimulators of non-depleted CB MNC.

Statistical Analysis

Data were analyzed with GraphPad Prism 7 (GraphPad Software, La Jolla, CA). Using the Wilcoxon paired signed-rank test, differences were considered statistically significant at $P < .05$. In the figures, $^{*}P < .05$, $^{**}P < .01$, and $^{***}P < .001$.

RESULTS

Proliferation of CD25-Depleted CB MNCs Stimulated by Maternal PBMCs

Preliminary experiments identified day +6 as the optimum day for the proliferation assay (Supplementary Figure 45). The proliferative response of CB MNCs to related maternal and paternal and unrelated third-party PBMC stimulator cells before and after depletion of $CD25^{+}$ Tregs (n = 10) is shown in Figure 1. The data are expressed as percentage of S phase cells. CB MNCs stimulated with maternal cells after $CD25^{+}$ Treg depletion showed higher proliferative responses compared with nondepleted cultures ($P < .05$; Figure 1A). CB MNCs stimulated with paternal cells showed a higher proliferative response compared with those stimulated with maternal cell stimulator cells before $CD25^{+}$ Treg depletion ($P < .05$; Figure 1B). CB MNCs stimulated with paternal cells showed a similar proliferative response before and after CD 25^{+} Treg depletion ($P > .05$) but an increased proliferative response to third-party stimulator cells compared with paternal cells before $CD25^{+}$ Treg depletion (Supplementary Figure 6).

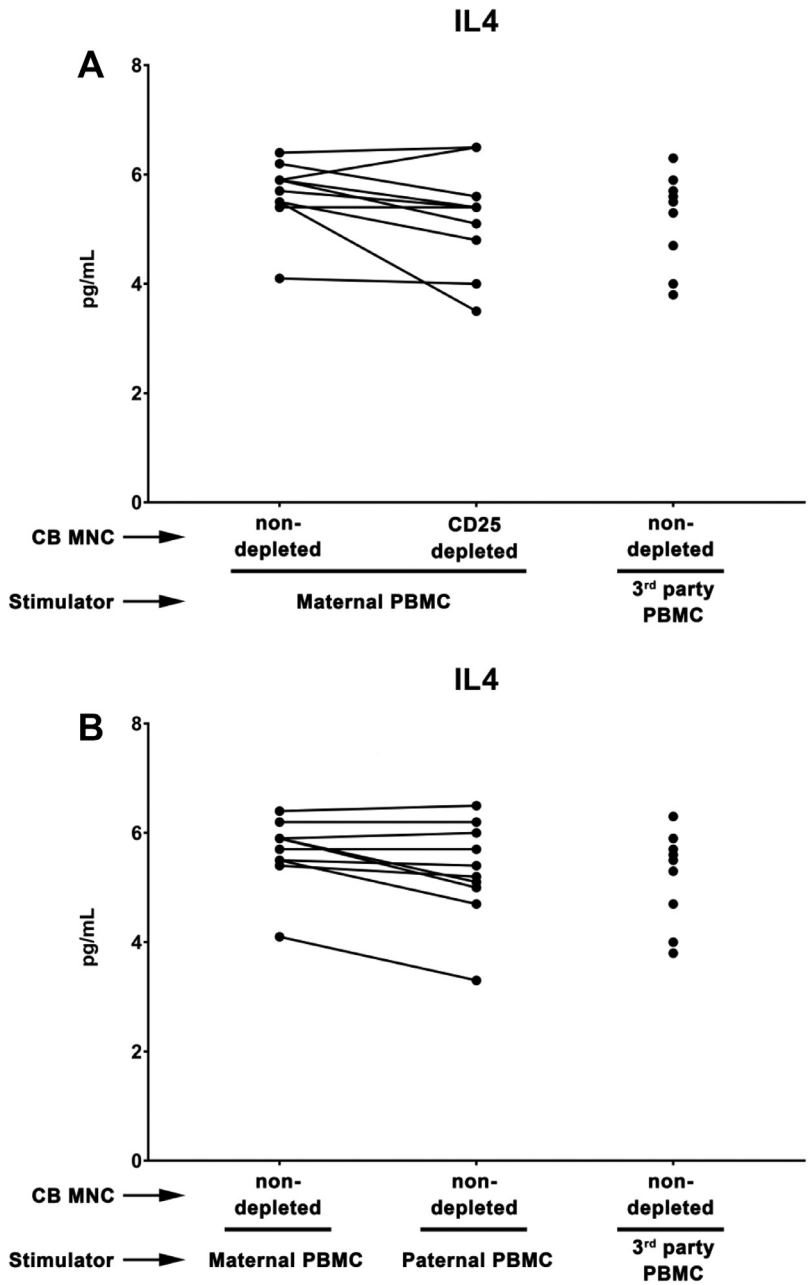

Figure 6. Effect of $CD25^{+}$ cell depletion on secretion of type 2 cytokine IL-4 in cultures stimulated with maternal, paternal, or third-party PBMCs. n = 10. $^{*}P < .05$; $^{**}P < .01$. Panel (A) compares results to depleted and non-depleted maternal PBMC stimulators whereas Panel (B) compares results to maternal, paternal and third-party stimulators of non-depleted CB MNC.

Depletion of CD25⁺ Tregs from CB MNCs Increases Cytotoxicity Against Maternal PBMCs

Alloantigen-specific cytotoxicity against maternal, paternal, third-party, and autologous PBMCs was examined before and after Treg depletion. CB MNCs showed stronger cytotoxic responses to paternal and third-party PBMCs in vitro than to maternal PBMCs, and CB MNCs showed stronger cytotoxic responses against maternal PBMCs after CD25⁺ Treg depletion ($P < .01$; Figure 2A). CB MNCs stimulated with paternal cells also showed stronger cytotoxic responses compared with those stimulated with maternal cells before CD25⁺ Treg depletion ($P < .01$; Figure 2B). CB MNCs stimulated with paternal cells before CD25⁺ Treg depletion showed an increased cytotoxic response compared with those after CD25⁺ Treg depletion ($P < .01$), but no difference in response compared with third-party cell stimulation (Supplementary Figure 7).

Comparison of Cytokines Produced by CB MNCs and CD25-Depleted CB MNCs in MLR

After depletion of CD25⁺ cells, IL-2 and IFN-γ were significantly increased in culture supernatants of CB MNCs stimulated with maternal PBMCs compared with nondepleted CB MNCs ($P < .01$; Figures 3A and 4A, respectively). CB MNCs stimulated with paternal or third-party PBMCs produced equal levels of IL-2 and INF-γ but higher levels compared with CB MNCs stimulated with maternal cells ($P < .01$; Figures 3B and 4B, respectively).

TNF-α was significantly increased by removal of CD25⁺ cells in CB MNCs stimulated by maternal PBMCs, to virtually the level

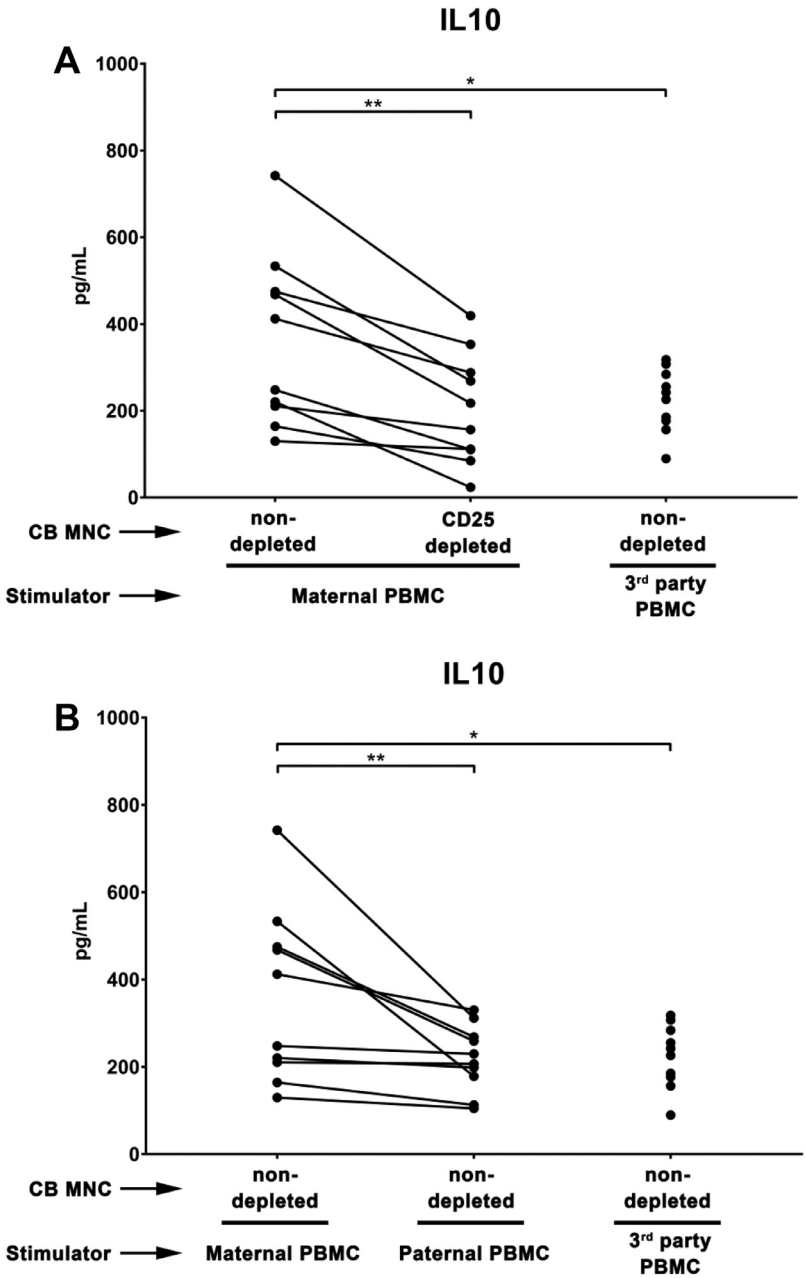

Figure 7. Effect of CD25+ cell depletion on secretion of Treg cytokine IL-10 in cultures stimulated with maternal, paternal, or third-party PBMCs. n = 10. *$P < .05$; **$P < .01$. Panel (A) compares results to depleted and non-depleted maternal PBMC stimulators whereas Panel (B) compares results to maternal, paternal and third-party stimulators of non-depleted CB MNC.

seen with paternal and third-party PBMCs (Figure 5). IL-4 production was not significantly affected by removal of $CD25^+$ cells, and levels were similar when CB MNCs were stimulated with maternal, paternal, or third-party PMNCs (Figure 6). IL-10 and TGF-β1 were significantly reduced to levels seen with paternal or fully allogeneic PBMCs by $CD25^+$ cell depletion in response to maternal PBMCs (Figures 7 and 8). Th 17 was significantly reduced by $CD25^+$ cell removal when CB MNCs were stimulated with maternal PBMCs. Stimulation with maternal or paternal PBMCs induced significantly lower levels of IL-17 compared with stimulation with third-party PBMCs (Figure 9A and B).

Depletion of $CD25^+$ cells from CB MNCs did not significantly affect the production of IL-2, TNF-α, IL-4, or TGF-β1 when stimulated with paternal PBMCs, whereas increased INF-γ and IL-10 and decreased IL-17 were observed in paternal PBMC-stimulated cultures. IL-2, TGF-β1, and IL-17 were increased when using third-party stimulator PBMCs compared with paternal stimulator cells ($P < .05$; Supplementary Figures 8–14).

DISCUSSION

MLR is a useful in vitro model for assessing alloantigen recognition, activation, and proliferation of T lymphocytes. Thus, examining the cellular parameters of MLR may provide evidence of the mechanisms responsible for T cell activation in vivo. In this study, cells with maternal, paternal, and third-party HLA phenotypes were used in the MLR as stimulators to observe the response of CB PBMC T lymphocytes reactive to these antigens.

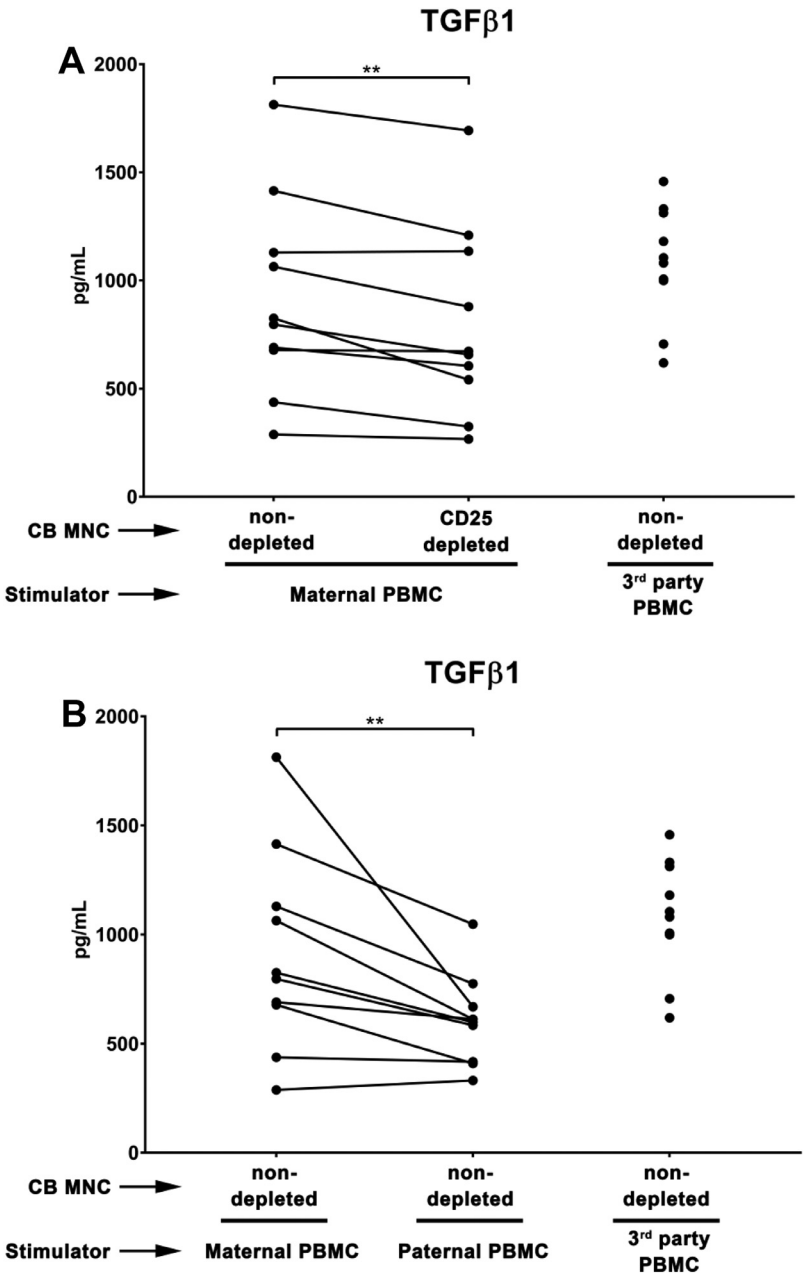

Figure 8. Effect of CD25+ cell depletion on secretion of Treg cytokine TGF-β1 in cultures stimulated with maternal, paternal, or third-party PBMCs. n = 10. *$P < .05$; **$P < .01$. Panel (A) compares results to depleted and non-depleted maternal PBMC stimulators whereas Panel (B) compares results to maternal, paternal and third-party stimulators of non-depleted CB MNC.

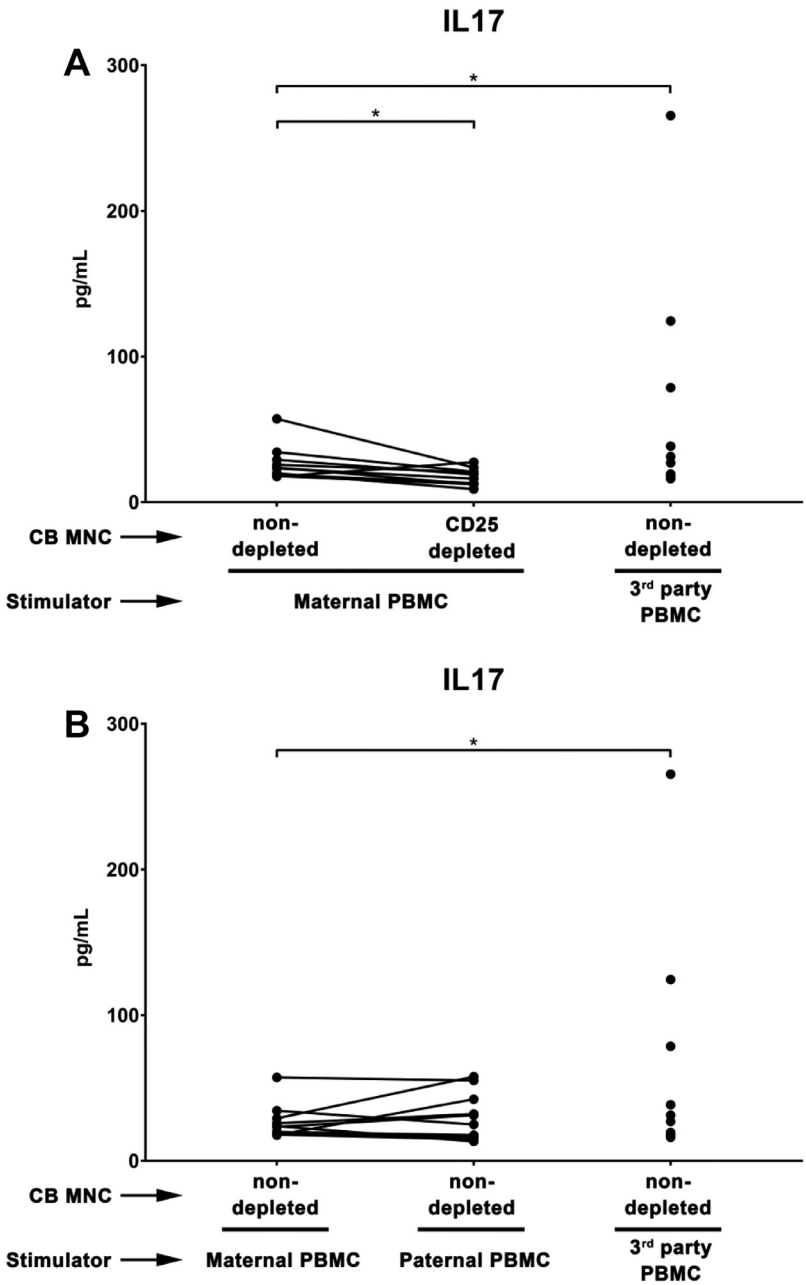

Figure 9. Effect of CD25$^+$ cell depletion on secretion of Th 17 cytokine IL-17 in cultures stimulated with maternal, paternal, or third-party PBMCs. n = 10. $^*P < .05$; $^{**}P < .01$. Panel (A) compares results to depleted and non-depleted maternal PBMC stimulators whereas Panel (B) compares results to maternal, paternal and third-party stimulators of non-depleted CB MNC.

This is the first study conducted in Hong Kong of individuals of Chinese ethnicity examining the cellular responses in CB MNC responder cells and maternal, parental, and third-party stimulator PBMC mismatched for NIMAs. An apparent influence of NIMAs on the in vitro alloreactive T cell repertoire in CB MNCs was demonstrated by MLR and FACS analysis. Our results echo the findings of Tsafrir et al. [31] showing a weaker cellular response to NIMAs compared with NIPAs with the use of CB MNCs as responder cells. However, Harris et al. [32] failed to show that CB MNCs can detect differences between stimulation with maternal and paternal cells. It has been shown that immature T cell subsets of CB MNCs exert suppressive activity on Th and cytotoxic T lymphocyte functions [33]. Moreover, cells derived from an immature immune system can be tolerant of maternal cells.

We observed stronger cytotoxic responses to paternal or third-party alloantigens compared with maternal and control autologous cells, and these responses were associated with the development of Th 1 responses. When CB MNCs were stimulated with autologous or maternal PBMCs, proliferation, cytotoxicity, and IL-2 and IFN-γ production were lower than the responses to paternal and third-party cells. The NIMA effect was influenced by CD25$^+$ Tregs, as demonstrated by IL-10 and TGF-β production. After stimulation with maternal PBMCs, IL-10 and TGF-β levels were lower after depletion of CD25$^+$ Tregs. These findings are consistent with the hypothesis

that the depleted CD25$^+$ Tregs constituted a T regulatory population [34].

It is significant that the IL-2 response of CB MNCs to NIMAs is under T regulation, given that it has been shown that calcineurin inhibitors, which suppress IL-2 production, are often effective in the prophylaxis of acute GVHD in CBT. Thus, the in vitro effect of NIMA tolerance may be obscured in the clinical transplantation setting. It will be informative to measure IL-2 production in patients who underwent NIMA-matched and -mismatched HSCT and correlate the level with clinical outcomes.

In the present study, we found an effect of NIMAs in CB MNCs. However, in vivo reexposure to NIMAs also might be essential for the induction of NIMA-specific tolerance [11]. On the other hand, it should be kept in mind that the NIMA effect might not be present in every CBU. Van Rood et al. [35] reported that anti-NIMA antibodies were not formed in approximately one-half of their highly sensitized patients.

Because Tregs have been hypothesized to play an important role in the induction of donor-specific tolerance, their presence might indicate a favorable NIMA effect in patients who undergo NIMA haplotype-mismatched HSCT or CBT. Several types of Treg cells have been described in humans, including naturally occurring CD25$^+$ cells [36]. Matsuoka et al. [37] found that the tolerogenic NIMA effect disappeared when the donor inocula were depleted of CD25$^+$ Tregs in a murine model of BM transplantation. In the present study, we observed similar disappearance of the tolerogenic effect with increases in proliferation, cytotoxic responses, and IL-2 and IFN-γ levels after depletion of CD25$^+$ Tregs in CB MNCs stimulated with maternal cells compared with those stimulated with parental cells. The decreased IL-10 and TGF-β1 levels also suggest that CD25$^+$ Tregs play a significant role in the tolerance effect of NIMAs. There appear to be no effects on IL-4 in our system, but the effects of CD25$^+$ Tregs on NIMA-induced IL-4 and Th 2 merit further study.

A beneficial effect of NIMA-expressing grafts on transplantation survival has been demonstrated in many retrospective studies of both solid organ and BM transplantations [13,19,38,39]. Although maternal and paternal renal allografts have shown similar graft survival [38], a significantly better graft survival of NIMA haplotype-mismatched sibling grafts compared with NIPA haplotype- mismatched sibling grafts has been reported [19]. Therefore, we analyzed the response in CB MNCs to both parental and third-party cells. In both situations, significant differences in the immune response toward NIMAs versus NIPAs were observed. Moreover, when CD25$^+$ Tregs were depleted in CB MNCs, differences in the immune response were lost.

In the present study, immune responses to NIMAs was measured, but the differential alloreactivity of memory T cells and naïve T cells was not, and thus the possibility that memory T cells might have a role in the NIMA effect cannot be excluded. Analysis of T cell subsets in future work could lead to a better understanding of this issue, and a more detailed analysis of donor T cell reactivity that takes into account the frequency of alloantigen-specific memory cells could be appropriate for identifying immunologic predictors of GVHD.

Despite being limited by its small sample size, this study provides some evidence that demonstrating the influence of NIMAs on the alloreactive T cell repertoire. Nonetheless, this project may be extended to larger cohort studies involving more subjects with different haplotypes. Studies in patients undergoing transplantation with NIMA haplotype-mismatched stem cells also might help elucidate the immunologic mechanism involved.

In conclusion, suppression of T cell proliferation, cytotoxicity, and changes in cytokine profiles suggesting suppressed Th1 and elevated Treg-associated IL-10 and TGF-β1 have been shown in the MLR responses of CB MNCs to NIMAs. The increased IL-10 production might be due to Th2 response. The responses were reversed after CD 25$^+$ Treg depletion, suggesting that Tregs might play a role in the response of CB MNCs to NIMAs and might be related to the induction of IL-10 and TGF-β secretion. In contrast to the practice in Western countries, HLA typing for the donor's mother is not a common practice in Hong Kong, and thus the donor's NIMA profile is not known. As clinical studies report reductions in GVHD and better outcomes of CBT, we hope that our work can provide solid evidence of the beneficial effect of NIMAs by increasing the likelihood of matching. Including analysis of NIMAs in donor selection will significantly expand the donor pool size, increase the chance of finding a matched donor, shorten waiting times, and finally improve patient survival.

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Conflict of interest statement: There are no conflicts of interest to report.

SUPPLEMENTARY DATA

Supplementary data related to this article can be found online at doi:10.1016/j.bbmt.2018.10.021.

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