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Particulate poly(methyl methacrylate) could stimulate proinflammatory CD4 T cell responses in a monocyte-dependent manner, and directly mediate cell death

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ABSTRACT

Poly(methyl methacrylate) (PMMA) is a synthetic polymer that has been widely used in various medical implants. Traditionally considered a biologically inert material, it is now understood that PMMA may have proinflammatory properties. Here, we present a proof-of-concept study of the effect of PMMA on CD4 T cells. Using particulate PMMA, a material that resembled wear debris in orthopedic implants, to stimulate whole peripheral blood mononuclear cells, we found that the expression of IFNgamma, IL-4, IL-17, and TGFbeta could all be upregulated in CD4 T cells in a manner that was dependent on the dose of particulate PMMA. Furthermore, compared to direct anti-CD3/CD28 stimulation, PMMA preferentially stimulated the expression of IFNgamma and IL-17 but not the expression of IL-4 or TGFbeta. Interestingly, the presence of autologous monocytes was required, since PMMA had no stimulatory effect on isolated CD4 T cells. We further demonstrated that direct monocyte-CD4 T cell contact was required, and the costimulatory molecules CD80 and CD86 were involved for the optimal stimulation of CD4 T cells. PMMA also directly mediated the death of CD4 T cells in a manner that was dependent on dose but independent of the presence of monocytes. Overall, our study revealed that PMMA could induce CD4 T cell death, and also could result in CD4 T cell activation with a preference toward proinflammatory responses in a monocyte-dependent manner.

1. Introduction

Poly(methyl methacrylate), abbreviated as PMMA, is a synthetic polymer with a variety of biomedical applications [1,2]. As an optically clear thermoplastic with light weight and high impact strength, PMMA has been used to make intraocular lenses to replace original lenses in cataract treatment. PMMA is also considered biologically inactive and compatible with human tissues, and has been used as bone cement in orthopedic surgeries and as soft tissue fillers in cosmetic surgeries. PMMA may also be used to construct porous particles that can be used to mediate controlled drug release [3].

In recent years, it is increasingly recognized that PMMA may interact with surrounding tissues and cells and cause physiological and immunological changes in the host. A candidate DNA vaccine for *Leishmania major* has contained PMMA nanoparticles as adjuvants in its formulation, and when used in *L. major*-infected mice, this vaccine was shown to reduce the parasite burden in the spleen and induce Th1/IFNgamma responses in mice [4,5]. Aseptic loosening, a major long-term complication in total joint arthroplasty, is associated with wear debris from prosthetic implants. These wear debris are composed of metals and/or polymers that include PMMA, and can interact with surrounding tissues and immune cells to activate proinflammatory

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signaling events that alter the expression of RANK/RANKL and OPG [6,7]. When injected into mice, particulate PMMA was shown to stimulate the monocytes and promote IL-1 and TNF expression [8,9]. Compared to injected particulate polymers of other materials, such as polystyrene (PS) and polyethylene (PE), PMMA was less phagocytosed by macrophages [9]. However, while the PS- and PE-containing macrophages tended to infiltrate the peritoneal fatty tissues around the pancreas and the spleen, the PMMA-containing macrophages preferentially infiltrated and enlarged the spleen. PE and PMMA injections also induced the production of reactive oxygen species [9]. In another investigation, PMMA particles with varying sizes were added at different amounts to in vitro cultures of human monocyte-derived macrophages. The cell viability decreased with increasing PMMA particle size, and the proinflammatory cytokine production elevated with increasing PMMA amount [10].

Overall, the above studies demonstrated that PMMA debris might contribute to aseptic loosening and other inflammation-associated symptoms by interaction with monocytes/macrophages. Of note, lymphocytes, especially T cells, can play a crucial role in modulating the immune responses around wear debris and implant tissues [11]. T helper (Th)1 cells with signature IL-2 and IFNgamma production are the most commonly observed T cells in the peri-implant region, followed by IL-17-producing Th17 cells [12–14]. Whether PMMA has direct effects on T cells is unclear. In this study, we investigated the effect of PMMA on CD4 T cells.

2. Methods

2.1. Cell collection

The collection of human samples was approved by the Ethics Review Board of Peking University Third Hospital. Seven healthy adult (18 to 65 years of age) volunteers were recruited. Peripheral blood samples were collected via venipuncture and mixed with citrate. Under strictly sterile conditions, the blood samples were layered on top of Ficoll (GE Healthcare) at a 2/1 blood/Ficoll ratio, and centrifuged at 300*g* for 30 min. When the rotor reached a natural stop, peripheral blood mononuclear cells (PBMCs) were collected from the murky cell layer of the blood-containing tubes, and washed in sterile PBS. The PBMCs were then placed in freezing medium (90% heat-inactivated fetal bovine serum [Gibco]/10% DMSO [Sigma]) and stored at −150 °C.

2.2. In vitro cell culture

PBMCs were thawed briefly in 37 °C and washed in sterile culture medium (RPMI 1640 supplemented with 1% L-glutamine, 1% penicillin-streptomycin, and 10% heat-inactivated fecal bovine serum [All from Gibco]). PBMCs were then rested overnight at 37 °C and 5% CO_2 before use. Untouched CD4 T cells and monocytes were isolated using Human CD4 T cell Enrichment Kit and Human Monocyte Enrichment Kit (both from Stemcell Technologies), respectively. Purity was consistently above 95% by flow cytometry staining.

For stimulation of whole PBMCs or purified CD4 T cells, cells were resuspended at 2×10^5 cells per mL, and plated at 200 μL per well in a 96-well plate. For CD4 T cell-monocyte coculture, each cell type was resuspended at 2×10^5 cells per mL, and combined at 100 μL each in the same well of the 96-well plate, or in separate compartments of the same well of a 96-well Transwell plate with 0.1 μm-pore membrane (Corning). Endotoxin-free particulate PMMA with diameters between 0.1 and 10 μm (mean 4.0 μm) were purchased from Polyscience, washed in sterile PBS, and added to the cell cultures at concentrations indicated in the experiments. Monoclonal antibodies, including anti-CD3, CD28, CD80, and CD86, were purchased from BioLegend, and added at concentrations indicated in the experiments.

2.3. ELISpot

After incubation, the CD4 T cells were harvested and purified using Human CD4 T cell Enrichment Kit. The viability of the cells was determined using Trypan Blue (Thermo Fisher) counting, and was calculated by the formula (the number of dark blue [dead] cells)/(the number of dark blue cells + the number of light blue [live] cells) × 100%. ELISpot was then performed using commercial kits (R& D Systems) for human IFNgamma, IL-4, IL-17, and TGFbeta, following the manufacturer's instructions. Specifically, 2×10^4 live CD4 T cells were aliquoted into each well of the 96-well PVDF-backed, capture antibody-coated microplates, provided by the kits' manufacturer. The plates were then placed in a 37 °C 5% CO_2 incubator for 18 h undisturbed. The cells were removed by washing, and the plates were treated in sequence with detection antibody mixture, streptavidin-alkaline phosphatase, and BCIP/NBT substrate, provided by the kits' manufacturer. The number of spots was counted using ImmunoSpot system single-color counting program (Cellular Technology Limited). The frequency of cytokine-expressing cells was calculated by the formula (the number of spots)/(the number of live cells in each well, i.e., 2×10^4) × 100%. The average of three independent triplicates was shown.

2.4. Cytokine detection

Whole PBMCs or isolated CD4 T cells were stimulated using 100 μg/mL PMMA or using anti-CD3 (2 μg/mL) and anti-CD28 (4 μg/mL) antibodies as described above for 12 days. After stimulation, CD4 T cells were isolated using Human CD4 T cell Enrichment Kit, resuspended at 1×10^6 cells/mL, and incubated in a 96-well plate with IFNgamma, IL-4, IL-17, and TGFbeta magnetic beads for multiplex assay (R&D Systems). After 18 h, the beads were collected using a special magnetic device, and were treated in sequence with biotin-antibody cocktail and steptavidin-PE as instructed by the manufacturer (R&D Systems). The level of cytokines was examined using a Luminex 200 instrument (Luminex Corp).

2.5. Flow cytometry

The following anti-human antibodies, including PE-conjugated anti-CD4, APC-conjugated CD3, PE-conjugated CD14, FITC-conjugated CD16, PE-conjugated CD80, and APC-conjugated CD86, were purchased from BioLegend. For staining, cells were incubated with various combinations of the antibodies for 30 min in sterile PBS supplemented with 2% heat-inactivated fetal bovine serum. LIVE/DEAD Violet Dead Cell Stain (Invitrogen) in sterile PBS was used to evaluate viability of the cells. The samples were acquired in FACSCanto cytometer and analyzed using FlowJo (Tree Star).

2.6. Statistical analysis

Data were represented as scatterplots, with mean ± SD when applicable. Ordinary or repeated-measures one-way ANOVA followed by Tukey's multiple comparisons were used for tests between more than two normally distributed groups. Kruskal-Wallis ANOVA followed by Dunn's test was used for nonparametric comparisons. Paired *t* test was used for tests between two matched groups. P lower than 0.05 was considered significant.

3. Results

3.1. Effect of PMMA on CD4 T cells in total PBMCs

To simulate PMMA wear debris, we utilized particulate PMMA with diameters between 0.1 and 10 μm (mean 4.0 μm), and incubated PMMA particles at various concentrations with human PBMCs from a healthy

individual. For comparison, anti-human CD3/CD28 was added to directly stimulate CD4 T cells from the T cell receptor (TCR). The CD4 T cells were then isolated using negative magnetic sorting, and ELISpot assays were performed to examine the frequencies of IFNgamma, IL-4, IL-17, and TGFbeta-expressing CD4 T cells. To optimize the assay condition, the stimulation was performed for a total of 15 days, and the frequency of cytokine-expressing CD4 T cells was examined at 12 h, 24 h, 3 days, 6 days, 9 days, 12 days, and 15 days. While PMMA could stimulate IFNgamma, IL-4, IL-17, and TGFbeta expression in CD4 T cells, those cytokines only became detectable at 6 to 9 days, while anti-CD3/CD28 stimulation resulted in detectable cytokine-expressing cells as early as at 12 h (Fig. 1A to 1D). PMMA stimulation required 12 days for the levels of IFNgamma, IL-4, IL-17-expressing CD4 T cells to peak (Fig. 1A–C). At 15 days post-stimulation, the levels of IFNgamma, IL-4, IL-17-expressing CD4 T cells presented a small decrease (Fig. 1A–C), while the level of TGFbeta presented a small increase (Fig. 1D). In anti-CD3/CD28 stimulation, the peak levels of IFNgamma, IL-17-expressing CD4 T cells were at 3 days (Fig. 1A and B), and the peak levels of IL-4-expressing and TGFbeta-expressing CD4 T cells were at 6 days and 9 days, respectively (Fig. 1C and D).

Subsequently, the stimulation experiment was performed in seven healthy individuals for 12 days, with varying levels of PMMA concentration during stimulation. In pure medium (PMMA concentration = 0) control, little IFNgamma-expressing CD4 T cells could be detected (Fig. 2A). 10 μg/mL PMMA increased the frequency of IFNgamma-expressing cells, but the result was not statistically significant from the 0 PMMA control. 25, 50, and 100 μg/mL PMMA significantly elevated the frequencies of IFNgamma-expressing cells. PMMA also significantly promoted IL-4 expression at 50 and 100 μg/mL (Fig. 2B), IL-17 expression at 25, 50, and 100 μg/mL (Fig. 2C), and TGFbeta at 50 and 100 μg/mL (Fig. 2D). For all cytokines investigated in this experiment, 100 μg/mL PMMA did not induce additional expression compared to 50 μg/mL PMMA ($P > 0.05$). Also, anti-CD3/CD28 stimulation was significantly more effective than PMMA, regardless of the concentration of PMMA.

It appeared that compared to anti-CD3/CD28 stimulation, PMMA stimulation was better at promoting proinflammatory IFNgamma and IL-17 responses, than antiinflammatory IL-4 and TGFbeta responses. For verification, we calculated the ratio of proinflammatory to antiinflammatory CD4 T cells, by using the formula (IFNgamma-expressing frequency + IL-17-expressing frequency)/(IL-4-expressing frequency + TGFbeta-expressing frequency). Anti-CD3/CD28 stimulation produced a ratio of between 0.9 and 1.6 (median 1.3), while PMMA stimulation consistently produced ratios above 1.8, with high variations among different subjects (Fig. 3A). The ratios produced by 25 μg/mL, 50 μg/mL, and 100 μg/mL PMMA were significantly higher than the ratios produced by anti-CD3/CD28 stimulation.

We also assessed the viability of the CD4 T cells after various forms

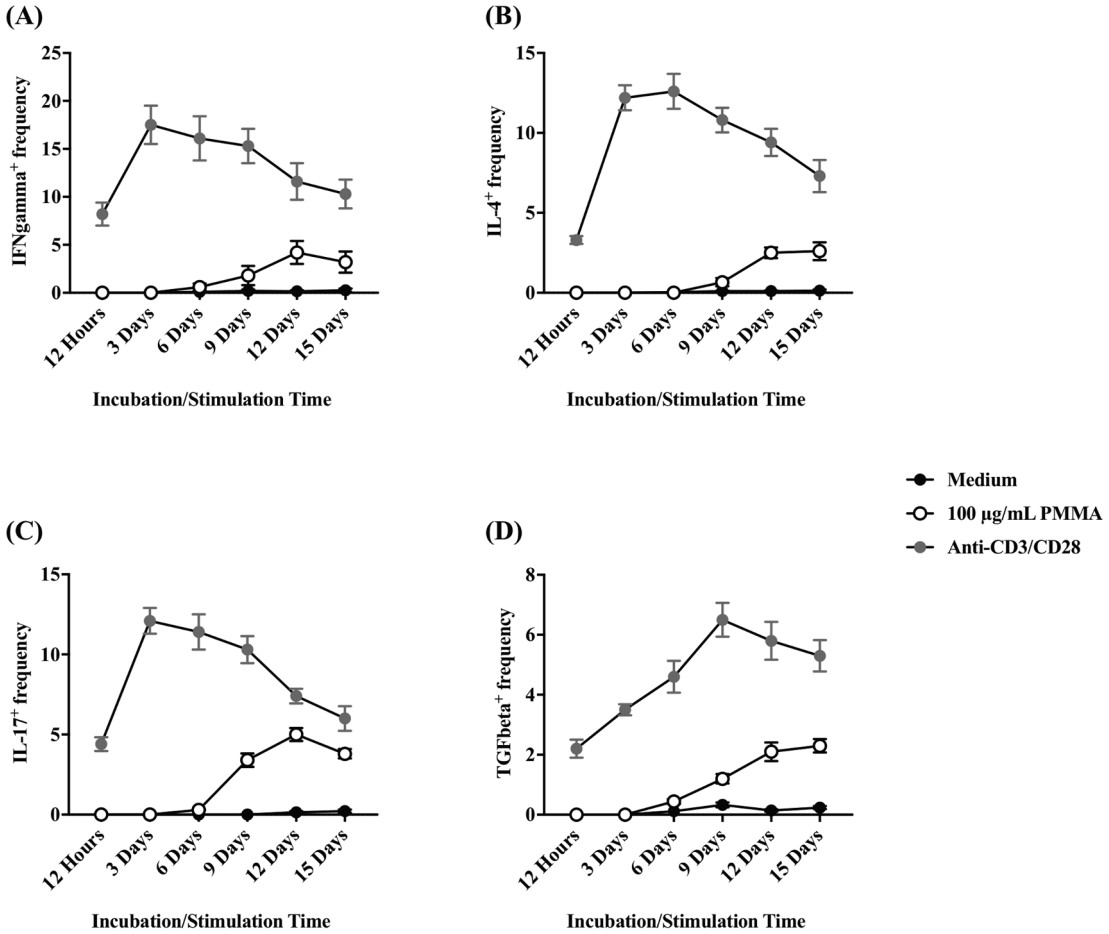


Fig. 1. Time-course examination of CD4 T cell-mediated cytokine expression following PMMA stimulation. 100 μg/mL particulate PMMA was added to PBMCs from one healthy individual. Anti-CD3 (2 μg/mL) and anti-CD28 (4 μg/mL) antibodies were used for positive control, and 0 PMMA (pure medium) was used for negative control. After 12 h to 15 days, CD4 T cells were collected and isolated, and the frequency of (A) IFNgamma, (B) IL-4, (C) IL-17, and (D) TGFbeta-expressing cells was examined using ELISpot. Each condition was triplicated. Mean ± SD was shown.

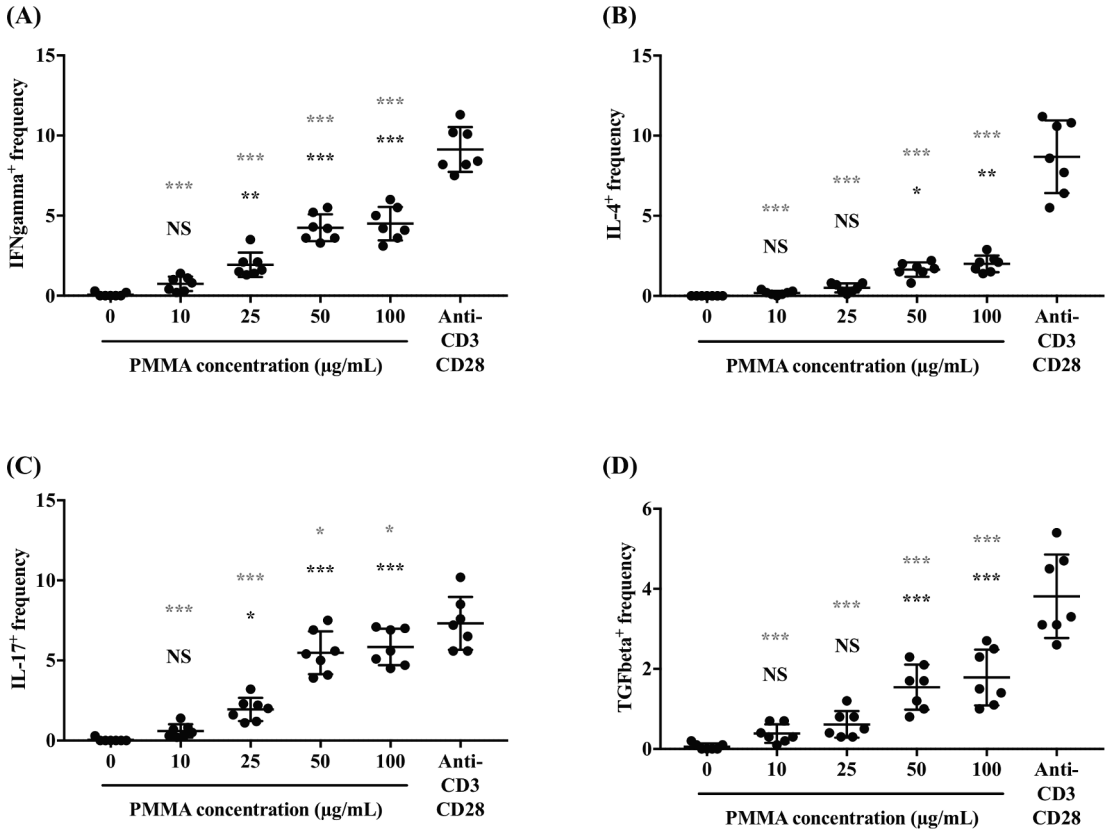


Fig. 2. Cytokine-expressing CD4 T cells in the presence of various levels of PMMA. Particulate PMMA was added to PBMCs at concentrations specified in the experiment. Anti-CD3 (2 μg/mL) and anti-CD28 (4 μg/mL) antibodies were used for positive control, and 0 PMMA (pure medium) was used for negative control. After 12 days, CD4 T cells were collected and isolated, and the frequency of (A) IFNgamma, (B) IL-4, (C) IL-17, and (D) TGFbeta-expressing cells was examined using ELISpot. One-way ANOVA followed by Tukey's multiple comparisons. NS, not significant. *P < 0.05. **P < 0.01. ***P < 0.001. Differences compared to 0 PMMA culture was labeled in black, while differences compared to anti-CD3/CD28 culture was labeled in grey.

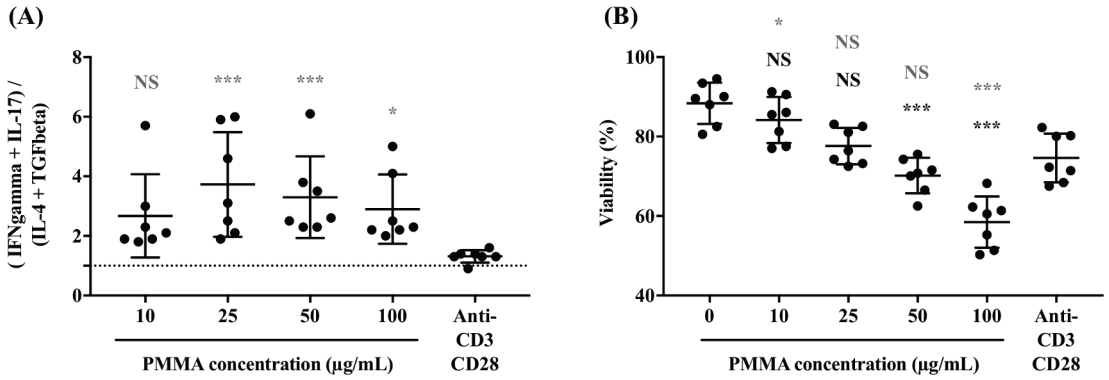


Fig. 3. Proinflammatory/antiinflammatory ratio and viability in PMMA-stimulated CD4 T cells. (A) The ratio of proinflammatory (IFNgamma + IL-17) to antiinflammatory (IL-4 + TGFbeta) CD4 T cells after PMMA stimulation, compared to the ratio of proinflammatory to antiinflammatory CD4 T cells after anti-CD3/CD28 stimulation. Kruskal-Wallis ANOVA followed by Dunn's test. (B) The percentage of live cells in PMMA-stimulated and anti-CD3/CD28-stimulated CD4 T cells. One-way ANOVA followed by Tukey's multiple comparisons. NS, not significant. *P < 0.05. **P < 0.01. ***P < 0.001. Differences compared to 0 PMMA culture was labeled in black, while differences compared to anti-CD3/CD28 culture was labeled in grey.

of stimulation. Compared to pure medium control, PMMA stimulation significantly reduced the viability of the cells in a concentration-dependent manner (Fig. 3B). While the cytokine expression was not significantly different between 50 μg/mL PMMA and 100 μg/mL PMMA, the viability was significantly lower with 100 μg/mL PMMA than with 50 μg/mL PMMA ($P < 0.01$). Moreover, compared to the viability with anti-CD3/CD28 stimulation, the viability with 10 μg/mL PMMA was significantly higher, while the viability with 100 μg/mL PMMA was significantly lower.

Overall, these data demonstrated that PMMA could stimulate

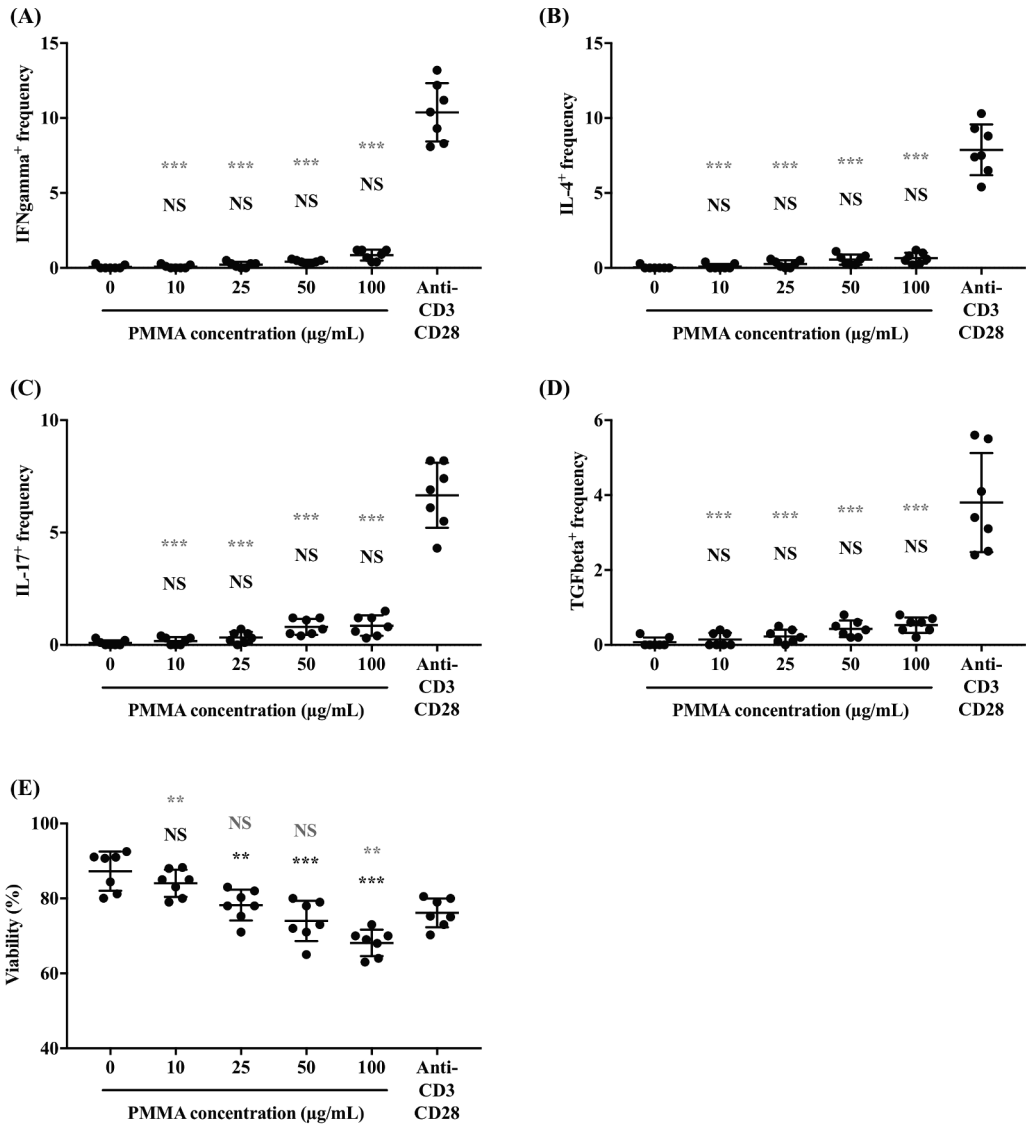


Fig. 4. Effect of PMMA treatment on isolated CD4 T cells. Particulate PMMA was added to purified CD4 T cells at concentrations specified in the experiment. Anti-CD3 (2 μg/mL) and anti-CD28 (4 μg/mL) antibodies were used for positive control, and 0 PMMA (pure medium) was used for negative control. After 12 days, the CD4 T cells were collected and isolated, and the frequency of (A) IFNgamma, (B) IL-4, (C) IL-17, and (D) TGFbeta-expressing cells was examined using ELISpot. (E) The percentage of live cells in PMMA-stimulated and anti-CD3/CD28-stimulated CD4 T cells. One-way ANOVA followed by Tukey's multiple comparisons. NS, not significant. $^{**}P < 0.01$. $^{***}P < 0.001$. Differences compared to 0 PMMA culture was labeled in black, while differences compared to anti-CD3/CD28 culture was labeled in grey.

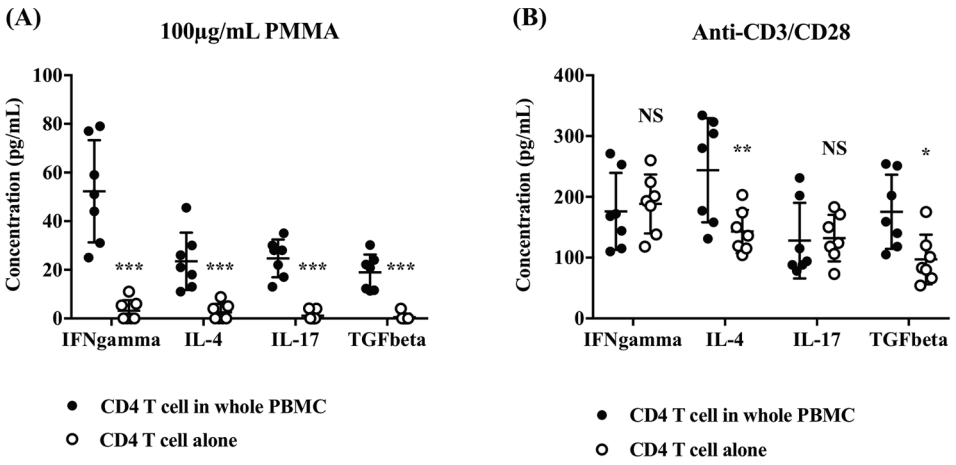
cytokine production from CD4 T cells less effectively than anti-CD3/CD28 and in a concentration-dependent manner, and was able to reduce the viability of CD4 T cells.

3.2. Effect of PMMA on isolated CD4 T cells

Previous studies demonstrated that particulate PMMA could stimulate macrophages [8,9], and in our in vitro cell culture, the CD4 T cells were stimulated in the presence of macrophages. To investigate whether PMMA could directly stimulate CD4 T cells, we performed the above experiments using CD4 T cells that were isolated using magnetic negative selection, instead of whole PBMCs. While anti-CD3/CD28 stimulation effectively promoted the expression of IFNgamma, IL-4, IL-17, and TGFbeta in purified CD4 T cells, PMMA stimulation was unable to promote the expression of these cytokines (Fig. 4A–D). However, PMMA did have a direct toxic effect on isolated CD4 T cells, by significantly reducing the viability of CD4 T cells compared to that in no PMMA control (Fig. 4E).

3.3. Effect of PMMA on the level of cytokine secretion

Subsequently, we examined the level of cytokine secretion by CD4 T cells following PMMA stimulation or anti-CD3/CD28 stimulation. Whole PBMCs or isolated CD4 T cells were incubated with 100 μg/mL PMMA for 12 days. CD4 T cells were then collected and incubated with a panel of magnetic beads that could capture IFNgamma, IL-4, IL-17, and TGFbeta. The secretion level of each cytokine was then evaluated using a Luminex assay. In PMMA stimulation experiments, while CD4 T cells in whole PBMCs presented easily detectable IFNgamma, IL-4, IL-17, and TGFbeta secretion, CD4 T cells alone had little to none cytokine secretion (Fig. 5A). In anti-CD3/CD28 experiments, both CD4 T cells in whole PBMCs and CD4 T cells alone presented IFNgamma, IL-4, IL-17, and TGFbeta secretion (Fig. 5B). The levels of IL-4 and TGFbeta were significantly higher in CD4 T cells in whole PBMCs than in CD4 T cells alone. Interestingly, the ratio of proinflammatory (IFNgamma + IL-17) cytokine levels to antiinflammatory (IL-4 + TGFbeta) cytokine levels was significantly higher in PMMA stimulation experiments than in anti-CD3/CD28 stimulation experiments (Fig. 5C).



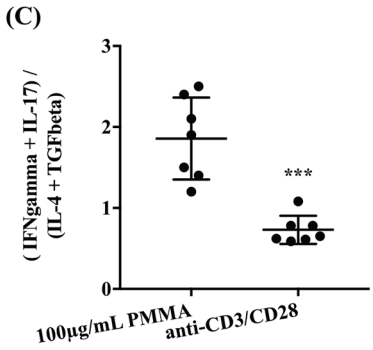


Fig. 5. Effect of PMMA treatment on CD4 T cell-mediated cytokine secretion. (A) 100 μg/mL particulate PMMA or (B) anti-CD3 (2 μg/mL) and anti-CD28 (4 μg/mL) antibodies were added to whole PBMCs or purified CD4 T cells for 12 days. In the whole PBMC experiment, CD4 T cells were isolated at the end of the stimulation. The CD4 T cells were incubated with anti-IFNgamma, IL-4, IL-17, and TGFbeta beads for 18 h, and the expression of the cytokines was examined using Luminex multiplex assay. (C) The ratio of proinflammatory (IFNgamma + IL-17) to anti-inflammatory (IL-4 + TGFbeta) cytokines after PMMA stimulation vs. after anti-CD3/CD28 stimulation. Unpaired *t* test with unequal variances. NS, not significant. *P < 0.05. **P < 0.01. ***P < 0.001.

3.4. Monocytes and CD80/CD86 costimulation are required for PMMA-mediated effects on CD4 T cells

Combining results above, it appeared that the presence of other cell types was required for PMMA-mediated effects on CD4 T cells. We hypothesized that monocytes/macrophages from the PBMCs were required, based on the findings that macrophages could phagocytize PMMA and subsequently release cytokines [8,9]. To investigate this hypothesis, we isolated monocytes from PBMCs. The monocytes and CD4 T cells were then coincubated in direct contact, or in separation with a porous membrane that separated monocytes and CD4 T cells but allowed the passage of cytokines. PMMA was then added to each cell culture at 50 μg/mL, a concentration that allowed similar cytokine production with 100 μg/mL PMMA but had significantly lower toxicity. The inclusion of monocytes resulted in IFNgamma, IL-4, IL-17, and TGFbeta expression in CD4 T cells, indicating that monocytes were required for PMMA-mediated cytokine production in CD4 T cells (Fig. 6A–D). Compared to the frequencies of cytokine-expressing CD4 T cells that were in direct contact with monocytes, the frequencies of cytokine-expressing CD4 T cells that were separated from monocytes displayed marked downregulation, indicating that direct CD4 T cell-to-monocyte contact was required.

CD80 and CD86 are critical costimulatory molecules that are expressed on the surface of antigen-presenting cells and have essential roles in promoting the proinflammatory responses from CD4 T cells. Here, we inhibited CD80 and CD86 in the CD4 T cell-monocyte coculture using blocking antibodies. Compared to isotype controls, the CD80/CD86 blocking antibodies significantly reduced the expression of IFNgamma, IL-4, IL-17, and TGFbeta by CD4 T cells (Fig. 7A–D). Interestingly, we also found that PMMA could markedly upregulate the expression of CD80 and CD86 in monocytes (Fig. 7E and F).

4. Discussion

In this study, the effect of PMMA on CD4 T cells was examined. We found that PMMA treatment of PBMCs resulted in CD4 T cell-mediated production of IFNgamma, IL-4, IL-17, and TGFbeta, which were signature cytokines for Th1, Th2, Th17, and Treg cells, respectively. However, PMMA on isolated CD4 T cells produced no significant stimulatory effect. By comparing the CD4 T cell-mediated cytokine production in PMMA-stimulated PBMCs and PMMA-stimulated isolated CD4 T cells, we found that these PMMA-mediated effects required the presence of other cell types. Further investigation revealed that the presence of monocytes was required for CD4 T cell-mediated cytokine production following PMMA stimulation. Additionally, blockade of CD80 and CD86 or physical separation of CD4 T cells from monocytes had significantly reduced the level of CD4 T cell-mediated cytokine production. Also, PMMA appeared to act as an activating adjuvant due to the upregulation of CD80 and CD86 by PMMA-treated monocytes. Overall, these data demonstrated that although PMMA resulted in the activation CD4 T cell-mediated cytokine production, PMMA could not directly act on CD4 T cells. Rather, contact-dependent mechanisms mediated by monocytes were required. In future studies, the direct effect of PMMA on monocyte/macrophage activation, including the expression of peptide-presenting major histocompatibility complexes and the M1 vs. M2 polarization markers, should be investigated. Whether other cell types, such as B cells, NK cells, and CD8 T cells were affected by PMMA should also be investigated.

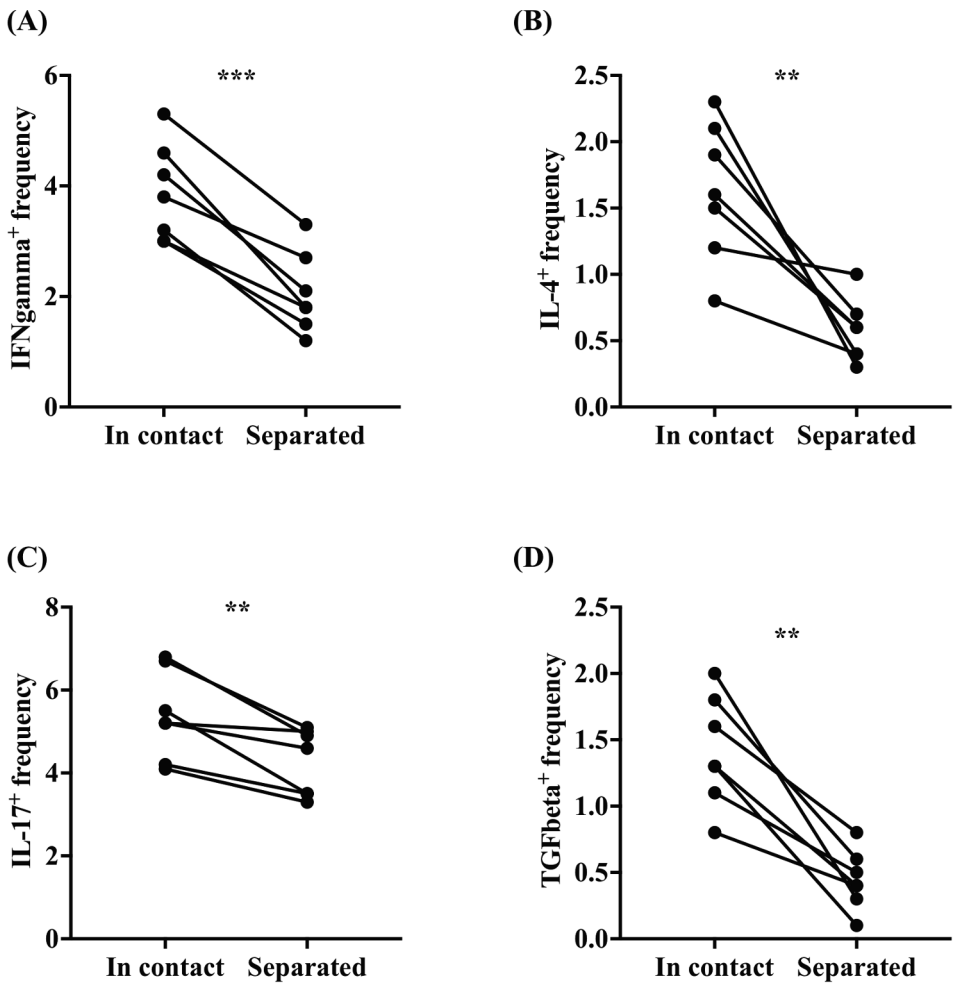


Fig. 6. Effect of PMMA in monocyte-CD4 T cell coculture. Purified monocytes and CD4 T cells from the same subjects were incubated at 1/1 ratio. The particulate PMMA was added at 50 μg/mL. In "in contact" experiments, the monocytes and CD4 T cells were incubated in the same compartment of the well, while in "separated" experiments, the monocytes and CD4 T cells were added to different compartments of the well, separated by a membrane with 0.1 μm-sized pores. After 12 days, the CD4 T cells were collected and isolated, and the frequency of (A) IFNgamma, (B) IL-4, (C) IL-17, and (D) TGFbeta-expressing cells was examined using ELISpot. Paired *t* test. **P < 0.01. ***P < 0.001.

PMMA also mediated direct death of CD4 T cells in a dose-dependent manner, and this effect was independent of autologous monocytes. Dead cells could be ingested by phagocytes, such as macrophages, and serve as antigens to mediate further inflammation. The mechanism of PMMA-mediated cell death is unclear. Whether PMMA could activate the expression of cytotoxic molecules in T cells, such as granzymes and perforin, should be investigated in further studies.

Interestingly, compared to direct anti-CD3/CD28 stimulation of CD4 T cells, PMMA preferentially promoted Th1 and Th17 responses and not Th2 or Treg responses, since the ratio of (IFNgamma + IL-17)/(IL-4 + TGFbeta) after PMMA stimulation was significantly higher than that after anti-CD3/CD28 stimulation. In general, with some exceptions, the Th1 and Th17 responses are considered proinflammatory, while the Th2 and Treg responses are considered regulatory. Aseptic loosening of orthopedic implants is characterized by a proinflammatory response in the surrounding tissues that is exacerbated by the presence of PMMA debris [15,16]. Here, stimulation with particulate PMMA directly resulted in an overabundance of proinflammatory CD4 T cell responses. The underlying mechanism for this apparent preference remains unclear and should be investigated further. Moreover, whether some implant materials favored one type of response while other materials favored other types of responses should be investigated. Theoretically, materials that preferentially induce regulatory response may be better candidates for implants. Hence, the effect of various implant materials on CD4 T cells should be investigated.

A major caveat of this study is that the in vitro cell culture system lacked some major characteristics of the in vivo environment. Indeed, among the infiltrating cells in tissues with aseptic loosening, T cells were present but were not considered the most common immune cell type [17,18], while an abundance of CD4 T cells could be found in our in vitro system. The role of T cells in the success or failure of orthopedic implants is still disputed, and whether PMMA could mediate CD4 T cell inflammation in vivo still requires more studies.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

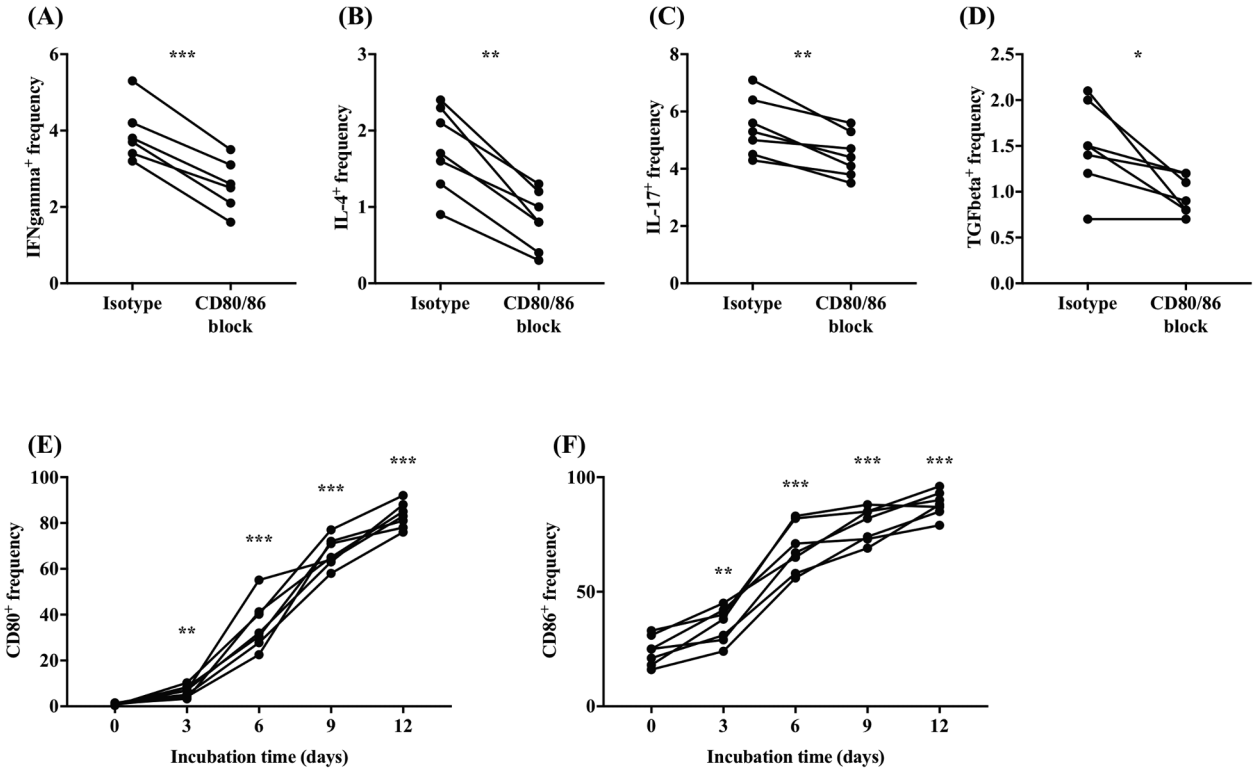


Fig. 7. The role of CD80 and CD86 in PMMA-mediated effects. (A to D) Purified monocytes and CD4 T cells from the same subjects were incubated at 1/1 ratio. The particulate PMMA was added at 50 μg/mL. Anti-CD80 and anti-CD86 blocking antibodies, or corresponding isotype control antibodies, were added at 10 μg/mL each. After 12 days, the CD4 T cells were collected and isolated, and the frequency of (A) IFNgamma, (B) IL-4, (C) IL-17, and (D) TGFbeta-expressing cells was examined using ELISpot. Paired *t* test. (E and F) Purified monocytes were incubated with 50 μg/mL PMMA for 12 days. The expression of (E) CD80 and (F) CD86 were examined by flow cytometry. Repeated-measures ANOVA followed by Tukey's multiple comparison test. Differences compared to day 0 culture were labeled. *P < 0.05. **P < 0.01. ***P < 0.001.

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