

Original Article

Oxidized low-density lipoprotein, OXPAPC, corrects defects in maturation and cytokine secretion of peripheral blood dendritic cells from sepsis patients

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Abstract: Objective: To investigate the expression differences in maturation and cytokine production of dendritic cells (DCs) from sepsis patients and the effect of oxidized phospholipid 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphorylcholine (OXPAPC) on DCs phenotypes. Methods: Peripheral blood mononuclear cells from 50 sepsis patients and 50 controls were cultured in the presence of GM-CSF, IL-4 and TNF- α to induce DCs maturation. DCs from sepsis patients were also treated with three different concentrations of OXPAPC. Cells were characterized with optical and electron microscopy, FACS analysis for CD1 α , HLA-DR and CD86 on cell surface and ELISA analysis of IL-12p70 in the supernatant. Results: DCs from sepsis patients had smaller cell bodies and nucleus and had almost no surface projection. DCs had similar CD1 α expression in sepsis patients (86.37 ± 17.24) and controls (88.58 ± 10.05). HLA-DR expression was dramatically reduced in sepsis patients (2.74 ± 5.15) compared to controls (198.35 ± 12.04). Similarly, CD86 expression was also drastically lower in sepsis patients (14.72 ± 4.83) than controls (154.56 ± 11.56). Furthermore, OXPAPC treatment of DCs from sepsis patients increased cell surface projection, HLA-DR and CD86 surface expression and IL-12p70 secretion in a dose-dependent manner. With 40 $\mu\text{g/ml}$ of OXPAPC, DCs of sepsis patients have similar phenotypes observed in healthy controls. Conclusion: DCs from sepsis patients are defective in maturation and cytokine secretion and these defects can be corrected by OXPAPC treatment.

Keywords: Sepsis, DC maturation, IL-12p70, OXPAPC

Introduction

Sepsis, a high mortality illness in intensive care units (ICU), is a systemic inflammatory response syndrome (SIRS) caused by infection. However, sepsis is not simply systemic inflammation but it also puts the body into an anti-inflammatory and immunosuppressive state. Hotchkiss [1] found that the immune function of sepsis patients was impaired and their peripheral blood showed a widespread and progressive loss of immune cells including B cells and CD4⁺ T cells. Yao [2] also found that innate immune responses in sepsis patients had defects such as macrophage malfunction and increased apoptosis in lymphoid tissues. Sepsis patients often have reduced rejection of foreign matters such as pacemakers and reduced allergic reactions to drugs [3].

Dendritic cells (DCs), the most powerful antigen presenting cells, are the originator of immune response. Only mature DCs can present antigens captured by phagocytes to T helper cells and initiate inflammatory responses. Mature DCs have dendritic shape and highly express MHC class I, MHC class II and costimulatory molecules such as CD86, CD80 and CD40 and secrete cytokines such as IL-12, IL-10, IL-6, TNF- α and IFN- γ . Sepsis inhibits DCs maturation and their secretion ability in peripheral blood of sepsis patients and animals. The recovery of DCs function contributes to the improvement of sepsis. Previous studies [4, 5] have found that in the peripheral blood of sepsis patients the expression of DCs surface molecules such as HLA-DR, a MHC class II molecule, was decreased and the secretion of proinflammatory cytokine IL-12 was reduced. Previous clinical

studies [6] also showed that the numbers of DCs in patients who died from sepsis were significantly lower than in sepsis survivors. DCs disappeared in mice which were injected with diphtheria toxin, but when bone marrow DCs were injected at the same time of diphtheria toxin injection, the survival rate of mice was greatly increased [7].

Lipopolysaccharide (LPS), an endotoxin, is a major component of gram negative bacteria. LPS is the main cause of sepsis and septic shock. The oxidized phospholipid 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphorylcholine (OX-PAPC) is an active compound of minimally oxidized low-density lipoprotein. OXPAPC can competitively inhibit LPS's effect. OXPAPC obstructs the binding of LPS to LBP and CD14. It decreases LPS-induced expression of ICAM-1, VCAM-1, E-selectin and other adhesion molecules, thereby inhibiting the adverse effect of LPS [8-10]. OXPAPC may be a potential drug for the treatment of sepsis [11]. However, it is unknown whether OXPAPC can reduce LPS-induced inhibition of DC maturation and secretion of inflammatory molecules. Therefore, we investigated the effect of OXPAPC on DC function.

Materials and methods

Study patients

Study subjects include 38 male and 12 female sepsis patients admitted to the ICU of the Second Xiangya Hospital between October 2013 and February 2014. Their ages range between 15 and 85 years with a mean of 60.57 ± 16.36 . Their APACHEII score was 6-43 with a mean of 23.19 ± 9.48 . Primary diseases were pulmonary infection in 14 cases, diffuse peritonitis in 11 cases, intracranial infection in 4 cases, blood infection in 4 cases and other disease in 9 cases. All patients had no history of autoimmune diseases, no malignancies, no use of immunosuppressive drugs.

The control group included 50 subjects (38 males and 12 females) selected from non-infectious patients in the same ICU. Their ages were 41-77 years (mean = 59.2 ± 12.6). Sepsis was defined as SIRS such as fever, tachycardia, tachypnoea or leucocytosis in response to a culture-proven or clinically suspected infection.

Chemicals

RPMI-1640 culture medium and fetal calf serum were purchased from HyClone (USA). GM-CSF and IL-4 were from Peprotech (USA). TNF- α was from Sigma (USA); FITC-labeled CD86 antibody, HLA-DR antibody and PE labeled CD1a antibody were from Biolegend (USA); PAPC (phospholipid 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphorylcholine) was from Santa Cruz Biotechnology (USA); IL-12 ELISA kit was from Dakewe (China) and lymphocyte separation medium was from Haoyang (China). OXPAPC was obtained through air-dry of PAPC for 72 hours.

Separation of mononuclear cells

Peripheral venous blood (20 ml) were obtained from each patient and diluted in lymphocyte separation medium and then centrifuged at 1800 rpm for 15 min. The tunica albuginea layers (mononuclear cells) were absorbed and added in RPMI-1640 with 10% fetal calf serum, then put in incubator for adherence.

Culture and treatment of DCs

GM-CSF (1000 U/ml) and IL-4 (500 U/ml) were added into mononuclear cells. Half culture medium was changed and half amount of cytokines was added in the third and sixth days. TNF- α (200 U/ml) was added in the sixth days. After eight days of culture DCs were collected. Different reagents were added to different study groups: 1) the control group: GM-CSF and IL-4; 2) sepsis group: GM-CSF and IL-4; 3) sepsis OXPAPC 10 group: GM-CSF and IL-4 plus OXPAPC (10 $\mu\text{g}/\text{ml}$); 4) sepsis OXPAPC 25 group: GM-CSF, IL-4 and OXPAPC (25 $\mu\text{g}/\text{ml}$); 5) sepsis OXPAPC 40 group: GM-CSF, IL-4 and OXPAPC (40 $\mu\text{g}/\text{ml}$).

Determination of surface molecules and cytokines

Cultured DCs were washed with PBS and then pelleted at 1000 rpm for 10 min. Cells were resuspended to $1 \times 10^5/\text{ml}$. FITC- or PE-labeled CD86, HLA-DR and CD1a antibodies (10 μL) were added into 100 μL cell suspensions and incubated at 4°C for 30 min. Cells were analyzed by flow cytometry for cell surface molecules. Cell culture medium was collected and used for IL-12 measurement by ELISA according to manufacturer's recommendation.

OXPAPC corrects DC defects in sepsis

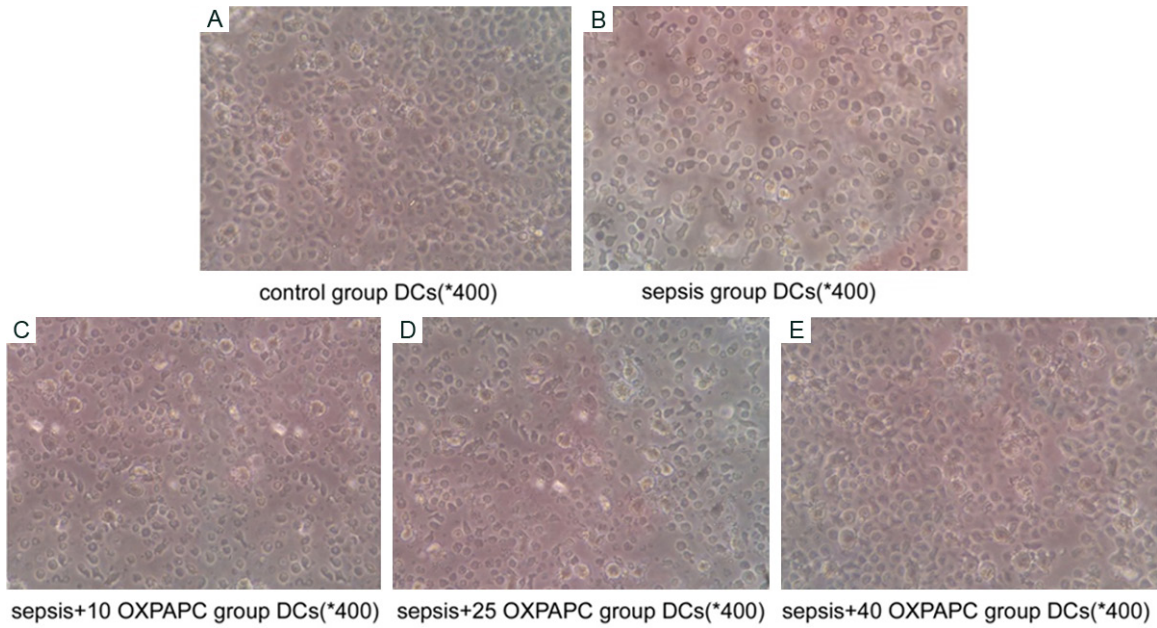


Figure 1. Morphological characteristics of DCs under an optical microscope. A: Control group; B: Sepsis group; C-E: Sepsis treated with 10, 25 and 40 µg/ml of OXPAPC.

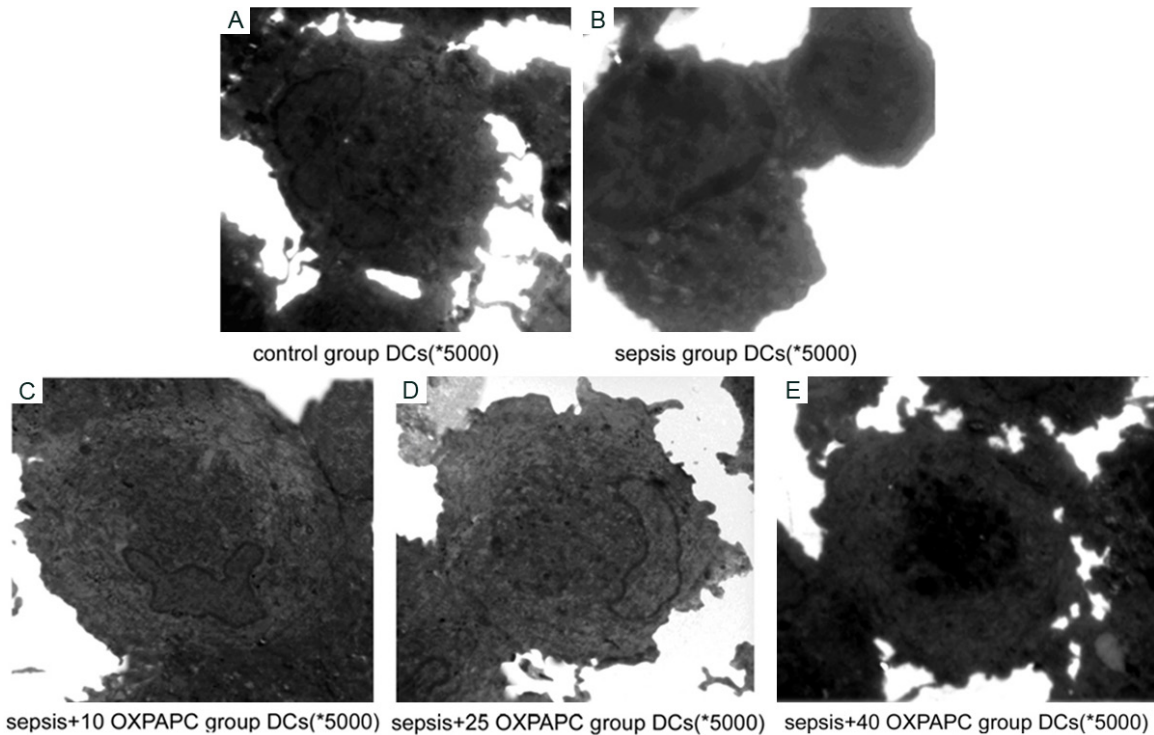


Figure 2. Morphological characteristics of DCs under an electric microscope. A: Control group; B: Sepsis group; C-E: Sepsis treated with 10, 25 and 40 µg/ml of OXPAPC.

Statistical processing

SPSS18.0 statistical software was used. Data were expressed as mean \pm SD under the condi-

tion of homogenized variance. Comparisons were done with one-way analysis of variance among groups, followed by LSD-t test. A value of $P < 0.05$ was considered statistically significant.

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Table 1. Mean and standard deviation of HLA-DR, CD86 and CD1a levels

Groups	Cases	CD1a	HLA-DR	CD86
Control	50	88.58 ± 10.05	198.35 ± 12.04	154.56 ± 11.56
Sepsis	50	86.37 ± 17.24	2.74 ± 5.15*	14.72 ± 4.83*
Sepsis OXPAPC 10	50	80.32 ± 11.73	12.57 ± 7.99 [#]	23.39 ± 4.87 [#]
Sepsis OXPAPC 25	50	90.05 ± 8.95	32.02 ± 5.86 [#]	41.81 ± 4.37 [#]
Sepsis OXPAPC 40	50	90.67 ± 16.67	169.80 ± 11.11 [#]	109.54 ± 4.92 [#]

*Comparison with the control group, $p < 0.0001$. [#]Comparison with the sepsis group, $p < 0.0001$.

Results

Morphological characteristics of DCs

DCs in the control group have extensive surface projections and large cell and nucleus sizes. In contrast, DCs in the sepsis group have smaller cell bodies and smaller nucleus and have almost no surface projection. DCs from sepsis patients with OXPAPC treatment gained more cell surface projections in an OXPAPC concentration-dependent manner (**Figures 1 & 2**).

Surface molecules

As shown in **Table 1** and **Figure 3**, the expression of CD1a was similar in the control group and sepsis patient groups with or without OXPAPC treatment. In contrast, HLA-DR expression was dramatically reduced in sepsis patients (2.74 ± 5.15) compared to controls (198.35 ± 12.04). OXPAPC treatment increased HLA-DR levels in a concentration-dependent manner (12.57 ± 7.99 , 32.02 ± 5.86 and 169.80 ± 11.11 for 10, 25 and 40 $\mu\text{g/ml}$, **Table 1**). Treatment with 40 $\mu\text{g/ml}$ of OXPAPC almost normalized HLA-DR expression. Similarly, CD86 expression was also dramatically reduced in sepsis patients (14.72 ± 4.83) compared to controls (154.56 ± 11.56) and OXPAPC treatment increased CD86 levels in a concentration-dependent manner (**Table 1**).

Cytokine secretion

IL-12p70 was measured in the culture medium of DCs from controls and sepsis patients with or without OXPAPC treatment. IL-12p70 secretion was significantly reduced in the culture medium of DCs from sepsis patients without OXPAPC treatment (3.78 ± 0.83) compared to controls (18.05 ± 1.42) (**Table 2** and **Figure 4**). OXPAPC treatment of DCs from sepsis patients increased IL-12p70 secretion in a dose-de-

pendent manner (**Table 2**). However, the secretion level did not reach the levels observed in controls even with 40 $\mu\text{g/ml}$ of OXPAPC.

Discussion

In sepsis patients, mononuclear cells with dendritic shape and characteristic expression of surface CD1a are considered as DCs. In this study, we purified and cultured mononuclear cells from peripheral blood of 50 sepsis patients and 50 matched controls. The CD1a expression levels were similar in controls and sepsis patients with or without OXPAPC treatment, suggesting that the cells are most likely DCs. We also investigated the expression of HLA-DR and CD86. HLA-DR is a MHC class II molecule and an important antigen presenting molecule [12]. CD86 is a costimulatory molecule on DC surface [13]. Peripheral blood mononuclear cell surface levels of HLA-DR and CD86 are important for the prognosis of sepsis patients. A previous study compared 23 severe sepsis patients and 26 uneventful postoperative patients and found that mononuclear cells of sepsis patients could differentiate into DCs but HLA-DR levels in patients was significantly decreased. The level of HLA-DR increased by 70% in 10 days while patients were recovering. In contrast, non-survivors were characterized by a second decrease in monocytic HLA-DR expression after day 7 or by a permanent suppression [14]. All infected patients had a loss of monocyte HLA-DR expression and the loss of HLA-DR expression on circulating monocytes was associated with a poor outcome [15]. Grimaldi [16] isolated and cultured DCs of septic shock patients in ICU and found that bone marrow DCs and plasma DCs from patients expressed lower HLA-DR compared with controls. In another study [17], peripheral blood DCs of severe sepsis patients were found to express lower level of HLA-DR, CD86, CD83 and CD11c. In animal experiments, a link also exists between sepsis and DCs. Without either increased bacteremia or plasma cytokine concentrations, intravenous injection of 10^7 wild-type DCs improved survival in sepsis mice [7]. DCs from different sepsis mouse models released less IL-12. In mice that survived sepsis, IL-12 production was sup-

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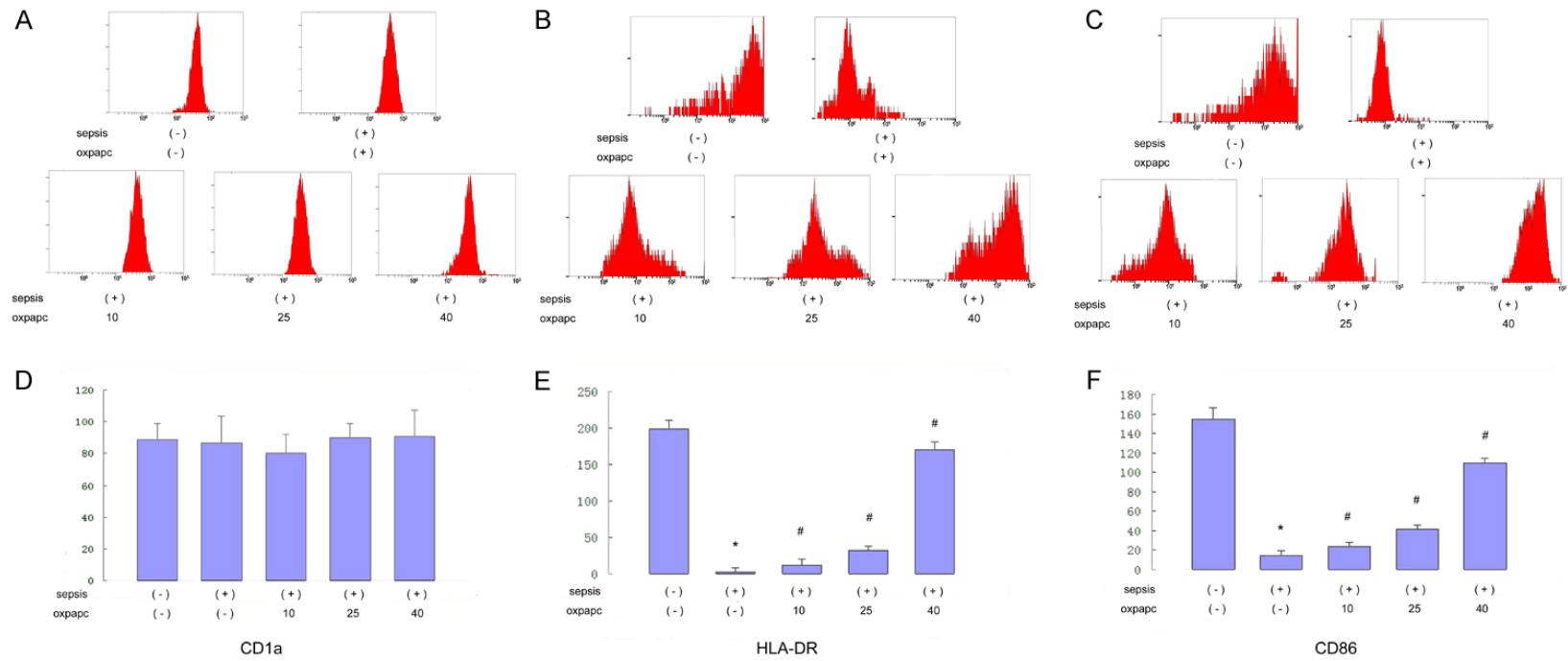


Figure 3. CD1a, HLA-DR and CD86 expression. A-C: Representative FACS profiles. D-F: Mean and standard deviation in each of the five study groups.

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Table 2. Mean and standard deviation IL-12p70 level

Groups	Cases	IL-12p70 (pg/ml)
Control	50	18.05 ± 1.42
Sepsis	50	3.78 ± 0.83*
Sepsis OXPAPC 10	50	5.18 ± 1.00 [#]
Sepsis OXPAPC 25	50	6.98 ± 0.99 [#]
Sepsis OXPAPC 40	50	9.27 ± 0.86 [#]

*Comparison with the control group, $p < 0.0001$. [#]Comparison with sepsis group, $p < 0.0001$.

pressed, providing a likely mechanism for the increased risk of secondary infections. Decreased IL-12 production *in vivo* was clearly multifactorial, involving both loss of CD11c DCs as well as alterations in the responsiveness of macrophages and remaining splenic DCs [18]. Mouse DCs of the cecal ligation and perforation (CLP) secreted less IL-12 than the control group, even though the CLP mice, which were injected with antibiotics and survived for up to 15 days, released high levels of IL-10 [19]. It is likely that cytokines like PGE2, IL-10 or TGF- β in sepsis affect the ability of DCs to secrete IL-12. The reduced production of IL-12 by DCs and increased IL-10 production would induce T helper cell transformation to a Th2 type, which weakens immune response [18].

OXPAPC is a potential drug for the treatment of sepsis. In one study [20], OXPAPC was used to treat acute lung injury infected with H5N1 avian influenza virus or the SARS-coronavirus and it was found that OXPAPC could significantly reduce the severity of lung infection. The potential mechanism is via inhibition of the LPS signaling pathway by blocking MAPKS and IKK signaling pathways and down-regulating cellular factors and inflammatory mediators. As sepsis often appeared secondary to lung infection, these results provide theoretical justification for using OXPAPC to treat sepsis. In the current study, we demonstrated that OXPAPC can reverse defects in DC maturation and cytokine secretion often observed in sepsis patients. Therefore, our study supports the concept that OXPAPC may be an excellent drug candidate for treating sepsis.

Disclosure of conflict of interest

None.

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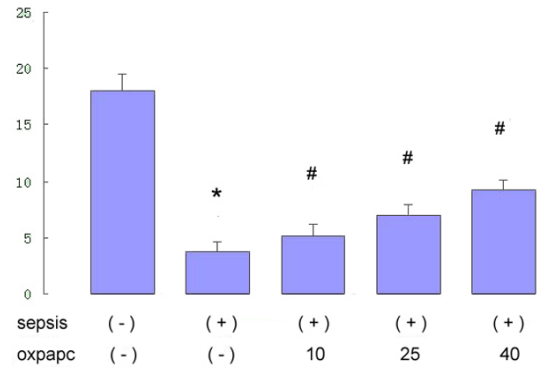


Figure 4. IL-12p70 levels in the cell culture medium. Data are expressed as mean ± standard deviation. * $p < 0.0001$, sepsis vs. control groups; [#] $p < 0.0001$, for comparisons between treated sepsis groups and untreated sepsis group.

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