ORIGINAL ARTICLES

Zymosan Induces Immune Responses Comparable with Those of Adults in Monocytes, Dendritic Cells, and Monocyte-Derived Dendritic Cells from Cord Blood

Kyoko Nohmi, MD[1], Daisuke Tokuhara, MD, PhD[1], Daisuke Tachibana, MD, PhD[2], Mika Saito, MD, PhD[1], Yuko Sakashita[1], Akemi Nakano, MD, PhD[2], Hiroyuki Terada, MD, PhD[2], Hiroko Katayama, MD, PhD[2], Masayasu Koyama, MD, PhD[2], and Haruo Shintaku, MD, PhD[1]

Objective To investigate the differences in toll-like receptor (TLR)-mediated immune responses between human neonates and adults, focusing on the cytokine profiles of monocytes, dendritic cells (DCs), and monocyte-derived DCs (MoDCs) in cord and adult blood.

Study design Purified monocytes, DCs, and MoDCs were stimulated with the following TLR ligands: lipopolysaccharide (TLR4), Pam3CSK4 (TLR1/2), flagellin (TLR5), zymosan (TLR2), polyinosinic:polycytidylic acid (TLR3), imiquimod (TLR7), and CpG (TLR9). Interleukin (IL)-8, IL-6, tumor necrosis factor, IL-1β, and IL-10 concentrations were analyzed in culture supernatants.

Results Compared with the effects in adult blood, lipopolysaccharide-, Pam3CSK4-, flagellin-, and polyinosinic: polycytidylic acid-stimulated inflammatory cytokine production in cord blood was generally weak in monocytes, comparable in DCs, and elevated in MoDCs. CpG- and imiquimod-stimulated cytokine production in DCs was comparable in cord blood and adult blood, but cytokine production was almost absent in monocytes and MoDCs in both cord blood and adult blood. In contrast, zymosan stimulation produced comparable inflammatory cytokine profiles in the monocytes, DCs, and MoDCs of cord blood and adult blood.

Conclusion The immaturity of TLR-mediated innate immunity in neonates depends on monocytes rather than on DCs. Our results indicate that zymosan-mediated TLR2 signaling may be useful for developing a neonatal vaccine adjuvant. *(J Pediatr 2015;167:155-62)*.

Neonates and infants are susceptible to infection and tend to have more severe outcomes of infection compared with older children and healthy adults.[1,2] Vaccination is the key strategy for providing sufficient protective immunity against pathogens,[3,4] but infants need more vaccinations than adults.[5,6] Although infection with important pathogens, such as respiratory syncytial virus (RSV), can be severe in the neonatal period,[7] there are no vaccines licensed for use against such diseases.

The susceptibility of neonates to infectious diseases and the difficulty in developing vaccines against them are thought to originate in the immature nature of immunity in neonates and infants, which leads to poor immune responses against pathogens or vaccine antigens (Ags).[8-10] To induce acquired immunity, T cells and B cells must be activated by Ag-presenting cells, such as dendritic cells (DCs), which are stimulated by pathogens or various Ags via the innate immune system.[11] Toll-like receptors (TLRs) are key to innate immunity by recognizing pathogen-associated molecular patterns on immune cells.[11] Therefore, previous studies of immature innate immunity have focused on comparing TLR signaling in neonates with that in adults.[12-18] Nguyen et al[12] reported that cytokine production in whole blood in response to TLR4 or TLR9 stimulation is impaired in neonatal cord blood compared with adult peripheral blood. A study of TLR-mediated cytokine profiles in whole blood cells demonstrated that compared with adult immune cells, neonatal innate immune cells are less capable of producing multiple cytokines simultaneously.[13] Levy et al[14] reported that the responses of monocytes to TLR1/2, TLR2/6, TLR4, and TLR7 stimulation were lower in cord blood than in adult blood, whereas TLR7/8 ligand stimulation elicited comparable monocyte immune responses in neonates and adults.

The evidence demonstrates that TLR-mediated innate immunity is impaired in neonates, but whether this immunologic impairment depends on the TLR type, the cell type, or the presence of plasma remains unclear. To further elucidate

Ag	Antigen	MoDC	Monocyte-derived dendritic cell
DC	Dendritic cell	pDC	Plasmacytoid dendritic cell
IL	Interleukin	poly(I:C)	Polyinosinic:polycytidylic acid
LPS	Lipopolysaccharide	RSV	Respiratory syncytial virus
mDC	Myeloid dendritic cell	TLR	Toll-like receptor
MHC	Major histocompatability complex	TNF	Tumor necrosis factor

From the Departments of [1]Pediatrics and [2]Obstetrics and Gynecology, Osaka City University Graduate School of Medicine, Osaka, Japan

Supported by the Japan Society for the Promotion of Science (Grants-in-Aid for Research Activity Start-up 24890202 [to D.T.], Scientific Research 25461558 [to D.T.], and Challenging Exploratory Research 40419700 [to M.S. and D.T.]), the SENSHIN Medical Research Foundation, the Ohyama Health Foundation, and the Osaka Research Society for Pediatric Infectious Diseases. The authors declare no conflicts of interest.

0022-3476/$ - see front matter. Copyright © 2015 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jpeds.2015.03.035

the profiles of neonatal innate immunity, we used purified cells rather than whole blood cells to investigate TLR-mediated immune responses in cord blood. We focused on how these responses depend on TLR type and cell type.

Methods

Heparinized cord blood (30-50 mL) was collected during elective cesarean deliveries without labor in healthy, full-term pregnancies (mean ± SD, 37.7 ± 0.9 weeks gestational age; n = 20) (**Table I**; available at www.jpeds.com). Heparinized peripheral blood (50-75 mL) was collected from healthy adults (mean age, 32.7 ± 5.1 years; n = 19). Mononuclear cells were separated out from cord blood or adult peripheral blood within 2 hours after blood collection. The study protocol conformed to the principles of the Declaration of Helsinki. Our institution's Ethical Committee approved the study protocol, with written informed consent.

TLR Ligands

Cells were stimulated with lipopolysaccharide (LPS; TLR4 ligand, *Escherichia coli serotype* O111 B4; Sigma-Aldrich, St Louis, Missouri), CpG (TLR9 ligand, IMG-2209Hpt; Imgenex, San Diego, California), flagellin (TLR5 ligand, IMG-2205; Imgenex), zymosan (TLR2 ligand, IMG-2212; Imgenex), imiquimod (TLR7 ligand, IMG-2207; Imgenex), polyinosinic:polycytidylic acid [poly(I:C)] (TLR3 ligand, IMG-2203-10; Imgenex), or Pam3CSK4 (TLR1 and TLR2 heterodimer ligand, IMG-2201; Imgenex), or left untreated as controls. The concentrations of the TLR ligands used for the monocyte stimulations were determined from dose–response curves with several concentrations, as in previous studies. For DC and monocyte-derived DC (MoDC) stimulation, we modified the concentrations of TLR ligands to match the cell:TLR ligand ratio.

Monocytes

Heparinized cord or adult blood was layered onto Lymphocyte Separation Medium (126-04871; Wako Pure Chemical Industries, Osaka, Japan), and the mononuclear cell layer was collected after centrifugation. Monocytes were isolated from peripheral blood mononuclear cells by positive selection using magnetic microbeads coupled to an anti-CD14 monoclonal antibody (130-050-201; Miltenyi Biotec, Auburn, California) in accordance with the manufacturer's instructions. Approximately 1×10^7 $CD14^+$ monocytes were separated from 50 mL of cord blood. Cord or adult blood monocytes were transferred to 96-well plates (10^5 cells/well) and incubated with LPS (100 ng/mL), flagellin (10 ng/mL), zymosan (1 μg/mL), CpG (1 μM), imiquimod (0.5 μg/mL), poly(I:C) (50 μg/mL), or Pam3CSK4 (100 ng/mL) for 12 hours at 37°C in humidified air containing 5% CO_2.

DCs

Peripheral blood DCs were isolated using a blood DC isolation kit (130-091-379; Miltenyi Biotec, Bergisch Gladbach, Germany) in accordance with the manufacturer's protocol. In brief, cells were isolated by a two-step magnetic-activated cell sorting separation (Miltenyi Biotec). First, B cells and monocytes were magnetically labeled and depleted with a mixture of CD19 and CD14 microbeads. Nonmagnetic "flow-through" cells were magnetically labeled and enriched using a mixture of antibodies against the DC markers blood DC Ag-4, blood DC Ag-3, and CD1c, in accordance with the manufacturer's instructions. Approximately 1×10^6 DCs were separated from 50 mL of cord blood. Cord or adult blood DCs (5×10^4 cells/well) were transferred to 96-well plates and incubated with LPS (50 ng/mL), flagellin (5 ng/mL), zymosan (0.5 μg/mL), CpG (0.5 μM), imiquimod (0.25 μg/mL), poly(I:C) (50 μg/mL), or Pam3CSK4 (50 ng/mL) for 12 hours at 37°C in humidified air containing 5% CO_2.

Generation of MoDCs

MoDCs were generated as described previously, with some modifications.[19] In brief, $CD14^+$ monocytes (5×10^5 cells/mL) isolated from cord blood or adult peripheral blood as described above were cultured for 6 days in an incubator at 37°C in humidified air containing 5% CO_2. The medium used was RPMI 1640 (Wako Pure Chemical Industries) supplemented with 10% heat-inactivated fetal bovine serum, L-glutamine, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, 50 mM 2-mercaptoethanol, 100 U/mL penicillin, 100 μg/mL streptomycin, 20 ng/mL recombinant human interleukin (IL)-4 (204-IL/CF; R&D Systems, Minneapolis, Minnesota), and 400 U/mL recombinant human granulocyte-macrophage colony-stimulating factor (215-GM/CF; R&D Systems). After washing, MoDCs (10^5 cells/well) were transferred to 96-well plates and incubated with LPS (100 ng/mL), flagellin (10 ng/mL), zymosan (1 μg/mL), CpG (1 μM), imiquimod (0.5 μg/mL), poly(I:C) (25 μg/mL), or Pam3CSK4 (100 ng/mL) for 12 hours at 37°C in humidified air containing 5% CO_2.

Cytokine Assay

After 12 hours of TLR stimulation of the monocytes, DCs, or MoDCs at 37°C in humidified air containing 5% CO_2, the concentrations of human IL-8, IL-6, tumor necrosis factor (TNF)-α, IL-1β, and IL-10 in the culture supernatants were measured with a human inflammatory cytokine cytometric bead assay kit (551811; BD Biosciences, San Jose, California) in accordance with the manufacturer's instructions. In brief, 50 μL of supernatant was mixed with 50 μL of mixed capture beads, and a standard curve was generated. After 3 hours of incubation at room temperature, the samples were washed and suspended in phosphate-buffered saline and then analyzed by flow cytometry (FACS LSR II; BD Biosciences) with CBA Analysis Software (FCAP Array version 3.0; Soft Flow, St Louis Park, Minnesota). The results are expressed in picograms per milliliter.

Flow Cytometry

The purity of isolated cord blood monocytes (>97% $CD14^+$; >99% cell viability) was confirmed by flow cytometry (FACS

Download English Version:

https://daneshyari.com/en/article/6220329

Download Persian Version:

https://daneshyari.com/article/6220329

Daneshyari.com