Effect of Interleukin-1 Receptor Antagonist on Concanavalin A Response of Peripheral Blood Mononuclear Cells from Newborn Calves

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ABSTRACT. The coexistence of interleukin (IL)-1β with IL-1 receptor antagonist (ra) in bovine colostrum and the possibility of simultaneous transfer of these cytokines to neonates via colostrum have been demonstrated. In the present study, we investigated the effect of IL-1ra on the mitogenic response of calf peripheral blood mononuclear cells (PBMC) stimulated by concanavalin A (ConA), which was mediated by IL-1. Pretreatment of PBMC with recombinant bovine (rb) IL-1ra alone significantly suppressed the proliferation of ConA-stimulated cells. However, in the presence of rbIL-1β, the suppressive activity of rbIL-1ra was counteracted. These results suggest that coexistence of IL-1ra with IL-1 in colostrum may have no effect on the activation of the neonatal immune system by IL-1β.

Key Words: IL-1β, IL-1ra, PBMC.

NOTE

Immunology

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Breast-feeding is considered to enhance T cell-mediated responses and improve B cell immunity in the immune systems of newborns [19, 20]. We previously reported the presence of a high concentration of interleukin (IL)-1β as well as IL-1 receptor antagonist (ra) in bovine colostrum [12], and Goto et al. [11] reported the possible transfer of IL-1β to neonates via the colostrum. In addition, Hagiwara et al. found that orally administered IL-1β and IL-1ra were transferred into the serum and that lymphocytes and neutrophils were activated by IL-1β in the serum of neonatal calves [13]. IL-1 contributes to protection against pathogens by activating lymphocytes and promoting production of other cytokines [7, 8]. Thus, IL-1 in the colostrum is considered to be one of the key modulators of neonatal immune systems. However, the simultaneous presence of IL-1 and IL-1ra in the colostrum and the probable simultaneous transfer of these cytokines to circulation in neonates seem to be discrepant, since IL-1ra blocks the activity of IL-1 [2, 7, 9].

It is known that concanavalin A (ConA) stimulates T cell proliferation in the presence of a costimulator such as IL-1 [1]. When peripheral blood mononuclear cells (PBMC) are stimulated with ConA, IL-1 is produced by monocytes present in the PBMC fraction. Therefore, ConA-stimulated PBMC proliferation is suppressed by IL-1ra [6]. Using this system, we investigated the effect of the simultaneous presence of IL-1β and IL-1ra on ConA response of PBMC from newborn calves before feeding colostrum.

Heparinized peripheral blood was obtained by cervical vessel puncture using a sterile vacuum syringe before feeding colostrum from 6 healthy Holstein-Friesian calves and a cow that were born and kept at Rakuno Gakuen University dairy farm. PBMC were prepared from heparinized peripheral blood by using a gradient centrifugation of 9% Ficoll (Amersham Pharmacia Biotech, Sweden)-33.4% Conray (Daiichiseiyaku Co., Tokyo, Japan) solution (specific gravity, 1.085 g/cm3) [3]. PBMC that had accumulated at the interface between the Ficoll-Conray solution and the plasma were harvested and washed three times with RPMI 1640 medium (SIGMA, U.S.A.) containing penicillin G potassium (250 U/ml; Banyu Pharmaceutical, Japan), kanamycin sulfate (25 µg/ml; Meiji Seika, Japan), streptomycin sulfate (250 µg/ml; Meiji Seika) and fungizone (1 µg/ml; Bristol-Myers Squibb, Japan).

Recombinant bovine (rb) IL-1β, rbIL-1ra, anti-rbIL-1β rabbit IgG and anti-rbIL-1β mouse IgG were prepared according to the methods described previously [11, 14].

One × 10⁵ of PBMC in 50 µl of RPMI 1640 medium containing 10% fetal calf serum was added to each well of a 96-well microculture plate (Falcon, U.S.A.) in triplicate along with 25 µl of serially diluted anti-rb IL-1β rabbit IgG or anti-rbIL-1β mouse IgG (undiluted concentration, 70 µg/ml or 60 µg/ml, respectively), rbIL-1β (22 ng), rbIL-1ra (130 ng) or both cytokines. These cytokine concentrations were determined on the basis of the results of our previous study on cytokine levels in colostrum (IL-1β and IL-1ra levels in colostrum, 884.24 and 5206.39 ng/ml, respectively) [12]. After 2 hr of incubation, 25 µl of an optimal concentration of ConA (SIGMA, U.S.A., 500 ng) was added to each well, and incubation was continued for 72 hr at 37°C in a 5% CO₂-humidified atmosphere. The blastogenic activities of the cells in cultures were evaluated by an MTT (3-[4,5-dimethylthiazol-2-y1]-2, 5-diphenyl tetrazolium bromide, Dojindo, Japan) assay [17]. Ten µl of MTT (5 mg/ml in phosphate-buffered saline) were added to each well, and the plates were incubated for 4 hr at 37°C. After incubation, 100 µl of 20% SDS-50% DMF (N,N-dimethylformamide) were added to each well, and the plates were incubated for 2 hr at 37°C. The plates were read by a microplate reader (Nihon Intermed Co., Japan) at 540 nm. The results are expressed as stimulation index (SI) according to the follow-
ing formula from triplicate assays:

\[ SI = \frac{O.D. \text{ of treated cells}}{O.D. \text{ of untreated control cells}} \]

The results are presented as means and standard deviations. Statistical significance was evaluated by ANOVA, and a P value < 0.05 was considered statistically significant.

The mitogenic response of PBMC stimulated with ConA was suppressed in a dose-dependent manner by pretreatment of the cells with rbIL-1ra as well as with anti-bIL-1α and β antibodies (Fig. 1). These results confirmed that the mitogenic response of bovine PBMC was mediated by IL-1.

Next, the effect of pretreatment with a mixture of IL-1ra and IL-1β on ConA-induced proliferation of newborn PBMC was studied. Because of variation in the SI values of ConA-induced proliferation of PBMC among individual calves, results were represented as ratio of the SI of pre-treated groups to the SI of ConA-stimulated control without pretreatment. The results are shown in Fig. 2. Pretreatment of PBMC with rbIL-1β alone resulted in the same or a higher level of proliferation compared with ConA-stimulated control cells without pretreatment. Pretreatment of PBMC with rbIL-1ra alone resulted in a significantly suppressed proliferation level. However, pretreatment with a mixture of rbIL-1ra and rbIL-1β had no inhibitory effect on ConA-stimulated proliferation of PBMC by IL-1ra.

In this study, ratio of IL-1/IL-1ra was determined on the basis of the results of our previous study on cytokine levels in colostrum. Since a 10 to 100-fold greater concentration...
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of IL-1ra was required to inhibit IL-1 activity [2, 7, 9, 14], the present results suggested that the presence of a large quantity of IL-1, even together with IL-1ra, could act as a co-stimulator of T cells stimulated by ConA. Therefore, activation of lymphocytes by IL-1 may not be influenced by the IL-1ra concentration absorbed from colostrum. Hagiwara et al. showed that oral administration of an excessive dose of IL-1 led to leukocytosis, anorexia and depression [13], and another study showed that levels of IL-1β were elevated in infants with hypoxaemia and severe perinatal distress [16]. Therefore, although the quantity of IL-1ra in colostrum might not be sufficient to inhibit the activity of IL-1β, IL-1ra might regulate the adverse effect of IL-1β in peripheral blood of newborn calves. In addition, the presence of soluble receptors to IL-6 and tumor necrosis factor (TNF)-α in human milk has been reported [5]. Human milk has been shown to have a suppressive effect on multiple human granulocyte function [4] as well as on anti-inflammatory action [10, 15, 18]. Further studies, especially studies on the influences of colostrum on neonatal immune systems, are needed.

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REFERENCES