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INTERLEUKIN-1 β IN HUMAN COLOSTRUM

C. Munoz ^(1, 2) (*), **S. Endres** ⁽¹⁾, **J. van der Meer** ⁽¹⁾, **L. Schlesinger** ⁽²⁾,
M. Arevalo ⁽²⁾ and **C. Dinarello** ⁽¹⁾

⁽¹⁾*Division of Geographic Medicine and Infectious Diseases, New England Medical Center Hospitals and Tufts University, Boston, MA 02111 (USA), and*

⁽²⁾*Immunology Unit, Instituto de Nutricion y Tecnologia de los Alimentos (INTA), Universidad de Chile, Casilla 15138, Santiago 11 (Chile)*

SUMMARY

The two forms of interleukin-1, IL-1 α and IL-1 β respectively, and tumour necrosis factor (TNF) are polypeptides sharing different biological activities which are often associated with host defence mechanisms. Because of the well-recognized benefits of breast feeding for newborns, colostrum from 9 healthy lactating women was analysed for the presence of these 3 cytokines. Specific radioimmunoassay revealed that colostrum contains a significant amount of IL-1 β (mean \pm SEM values of 1,130 \pm 259 pg/ml). The concentrations of IL-1 α and TNF were negligible.

Colostrum leukocytes are able to produce IL-1 since high activity was found after stimulation with *Staphylococcus epidermidis*. In addition, these cells produced IL-1 spontaneously *in vitro*, in contrast to resting maternal blood monocytes. As IL-1 increases resistance to infection, the presence of this cytokine represent a beneficial aspect of breast feeding.

KEY-WORDS: Colostrum, Interleukin-1, Leukocyte; Breast feeding, Resistance to infection.

INTRODUCTION

Interleukin-1 (IL-1) represents a family of polypeptides synthesized by the host mainly in response to a variety of stimuli. Two types of IL-1 (α and β)

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(*) To whom all correspondance should be addressed. Present address: Immunology Unit, Instituto de Nutricion y Tecnologia de los Alimentos (INTA), Universidad de Chile, Casilla 15138, Santiago 11 (Chile).

recognize the same receptor on lymphocytes and act as extracellular signals usually amplifying the development of antigen-specific immunity (Dinarello, 1988; Lowenthal and MacDonald, 1986; Durum *et al.*, 1985). IL-1 also possesses many non-immunological properties including effects on endocrine, nervous, vascular and mesenchymal cells (Dinarello, 1986). Both forms of IL-1 share many activities with another cytokine, cachectin or tumour necrosis factor (TNF) (Dinarello, 1986).

IL-1 activity has been demonstrated in human body fluids including plasma (Cannon and Dinarello, 1985), urine (Kimball *et al.*, 1984) and joint effusions (Fontana *et al.*, 1982; Wood *et al.*, 1983), often in association with the existence of a pathological state. Recently, the presence of TNF has also been described in serum of patients with severe infectious purpura (Girardin *et al.*, 1988) and *Plasmodium falciparum* malaria (Kern *et al.*, 1989). It remains unclear whether one or both IL-1 forms and/or TNF participate in normal physiological function in the absence of infection or injury.

Breast milk provides optimal feeding for the newborn with well-established nutritional advantages (Committee on Nutrition, 1980; ESPGAN Committee on Nutrition, 1982). In fact, human milk has many unique properties clearly beneficial for the neonate since it contains biochemical components (Lonnerdal, 1985) and immunological factors (Chandra, 1978; Pittard, 1979; Welsh and May, 1979; Victora *et al.*, 1987) which satisfy their nutritional requirements and augment their defence mechanisms against infectious diseases.

We studied the presence of both IL-1's and TNF in colostrum of healthy women during early maternal lactation using specific radioimmunoassays to measure the different cytokines. Colostral leukocytes (CL) were also studied as the possible cellular source of IL-1 and were compared, on a cellular basis, with blood mononuclear cells (BMNC) from the same mothers for IL-1 production.

MATERIALS AND METHODS

Study design.

Nine lactating Chilean mothers (age \pm SD 27 ± 5.7 years) who delivered normal full-term infants (38-40 weeks) were included in the study. All women in good health,

BMNC = blood mononuclear cell.
 CAP = colostrum aqueous phase.
 CL = colostrum leukocyte.
 IL = interleukin.

LPS = lipopolysaccharide.
 MEM = minimal essential medium.
 RIA = radioimmunoassay.
 TNF = tumour necrosis factor.

that is, without known organic disease, signs of infection or breast inflammation. The study was explained to volunteers and informed consent was obtained.

Isolation of CL, colostrum aqueous phase (CAP) and BMNC.

Milk and blood samples were obtained on the 3rd to 4th postpartum day. Colostrum (10 ml/mother) was collected by manual expression into sterile plastic tubes and immediately placed at 4°C. CL were removed by centrifugation and were over 90 % viable as determined by trypan blue exclusion. Mean cellular concentration was approximately 1.0×10^5 /ml of whole colostrum. Morphological analysis showed that the typical distribution of CL in colostrum was 60 % macrophages, 30 % polymorphonuclear leukocytes and 10 % lymphocytes. Once CL were separated, CAP was obtained by centrifugation at 10,000 g for 10 min at 4°C in order to remove particulate material and fat, and was then stored at -70°C. At the same time peripheral blood (10 ml) was obtained.

BMNC were isolated over Ficoll-Hypaque and washed three times in pyrogen-free saline. Both CL and BMNC were resuspended in minimal essential medium (MEM, Microbiological, Walkerville, MN) which had been passed through an ultrafilter in order to remove endotoxins (Dinarello *et al.*, 1987a). Cells were used at a final concentration of 2.5×10^5 /ml containing 1 % heat-inactivated human AB serum.

Immunoreactive assay.

Immunoreactive cytokines including IL-1 β (Lisi *et al.*, 1987), IL-1 α (Lonnemann *et al.*, 1988) and TNF- α (van der Meer *et al.*, 1988) were tested in colostrum itself by using a specific radioimmunoassay (RIA). There is no cross-reactivity between IL-1 β RIA and that of IL-1 α . The TNF assay recognizes TNF- α but neither form of IL-1, nor lymphotoxin (TNF- β), interferons, or colony stimulating factors (van der Meer *et al.*, 1988). Samples were spiked with known amounts of each cytokine and were recovered (95-100 %) as measured in each cytokine RIA.

Production of IL-1 by colostrum and blood cells.

Cells (CL and BMNC) were incubated in flat-bottomed microtitre plates (96 wells) overnight at 37°C in either MEM or heat-inactivated *Staphylococcus epidermidis* (bacteria/leukocyte ratio 10/1). The plates were then exposed to 3 freeze-thaw cycles and supernatants containing both extracellular and cell-associated material were frozen at -70°C until assayed. The supernatants were serially diluted and tested on the sensitive IL-1-responsive murine helper T-cell line D10.G4.1 (Kaye *et al.*, 1984), which detects less than 1 pg/ml of IL-1 (Orencole *et al.*, 1987).

Triplicate 100 μ l supernatants of CL and BMNC with/without stimulants were added to equal volumes of D10.G4.1 cell suspensions (2×10^5 /ml in complete RPMI-1640 supplemented with 5 % heat-inactivated foetal calf serum; Hyclone Laboratory, Logan, UT). The cultures were incubated for 48 h at 37°C in 5 % CO₂, pulsed with 1.0 μ Ci of tritiated thymidine (6.7 Ci/mmol; New England Nuclear, Boston, MA), and incubated for an additional 18 h then harvested. Incorporated radioactivity was determined by liquid scintillation. The amount of IL-1 was calculated using half-maximal proliferative responses to recombinant human IL-1 β as the definition of 1 unit/ml as previously described (Dinarello *et al.*, 1987b). Because the number of monocytes differed in CL compared to BMNC cell preparations, the data were expressed as IL-1 units/ 10^4 monocytes \pm SEM.

RESULTS

We measured the concentrations of immunoreactive IL-1 β , IL-1 α and TNF in colostrum. Table I shows the amounts of these cytokines in CAP from 9 healthy mothers. High levels of IL-1 β were found with a mean value of 1,130 pg/ml. Concentrations of IL-1 α and TNF were 10 times lower, that is, about 100 pg/ml for both cytokines; these values were close to the detection limit of the technique. We also evaluated IL-1 β in "early mature" milk (day 7) from a subgroup of lactating mothers; although the concentration varied, it was generally about half that in colostrum (data not shown).

After finding that CAP from normal mothers contained significant amounts of IL-1, we then studied the production of this cytokine by CL *in vitro*. There was high spontaneous IL-1 activity from unstimulated CL from mothers (fig. 1); in comparison, unstimulated maternal blood monocytes produced very low amounts of IL-1. In addition, *S. epidermidis*-stimulated CL produced considerably more IL-1 than stimulated BMNC from the same mothers.

DISCUSSION

In this report, we demonstrate the presence of IL-1 β in human colostrum of healthy mothers in the absence of infection or injury, clinical events which are often associated with the presence of this cytokine (Dinarello *et al.*, 1987b). In addition, other investigators have also described IL-1 activity in normal amniotic fluid (Brody *et al.*, 1987).

TABLE I. — Immunoreactive cytokines in human CAP.

Mother	Cytokine (pg/ml)		
	IL-1 β	IL-1 α	TNF
1	1,100	100	120
2	1,500	110	80
3	360	240	80
4	1,200	150	110
5	400	110	90
6	380	90	110
7	700	130	90
8	2,500	150	100
9	2,000	170	110
Range	360-2,500	90-240	80-120
Mean \pm SEM	1,130 \pm 239	139 \pm 14	99 \pm 5

CAP was tested in duplicate in the respective RIA for IL-1 β , IL-1 α and TNF. The detection limit was 80 pg/ml for all three cytokines at the 95 % confidence level.

Recently, Soder (1987) observed that human milk from healthy Swedish mothers, 1-3 months after the onset of lactation, contains an IL-1-like factor that is very similar to macrophage-derived IL-1 α . However, the presence of other IL-1-like factors derived from the active components of milk was not excluded.

Using specific RIA techniques, we found both forms of IL-1 in colostrum; the concentration of IL-1 α being extremely low and close to the detection limit of the assay. Furthermore, IL-1 β was also detected in "early mature milk" (day 7), although the concentration was about half that found in colostrum. Thus, it is possible that the kinetics of the production of IL-1 α and IL-1 β vary during the course of lactation in a similar manner to other immunological factors (Goldman *et al.*, 1982).

Using a T-cell bioassay, we could not accurately detect IL-1 in unfractio-nated colostrum because of the presence of inhibiting substances. The presence of inhibitors of T-cell proliferation in human milk is not surprising because inhibitory substances are found in most human body fluids such as plasma, urine, peritoneal fluid and joint effusions (Cannon and Dinarello, 1985; Kimball *et al.*, 1984; Fontana *et al.*, 1982; Wood *et al.*, 1983). In order to re-move these inhibitors, we chromatographed CAP by gel-filtration and, using a bioassay, we detected IL-1 activity in the 40-50- and 15-20-kD fractions. We were able to confirm that this immunostimulatory activity in CAP was due to IL-1 by specific neutralization with anti-IL-1 antibodies.

Since there were high levels of IL-1 β in CAP, we investigated whether the colostrual cells were the source of IL-1. We found that *S. epidermidis*-stimulated CL produced significant amounts of IL-1 *in vitro*. On the other hand, CL "spontaneously" produced this cytokine. When IL-1 production by unstimu-

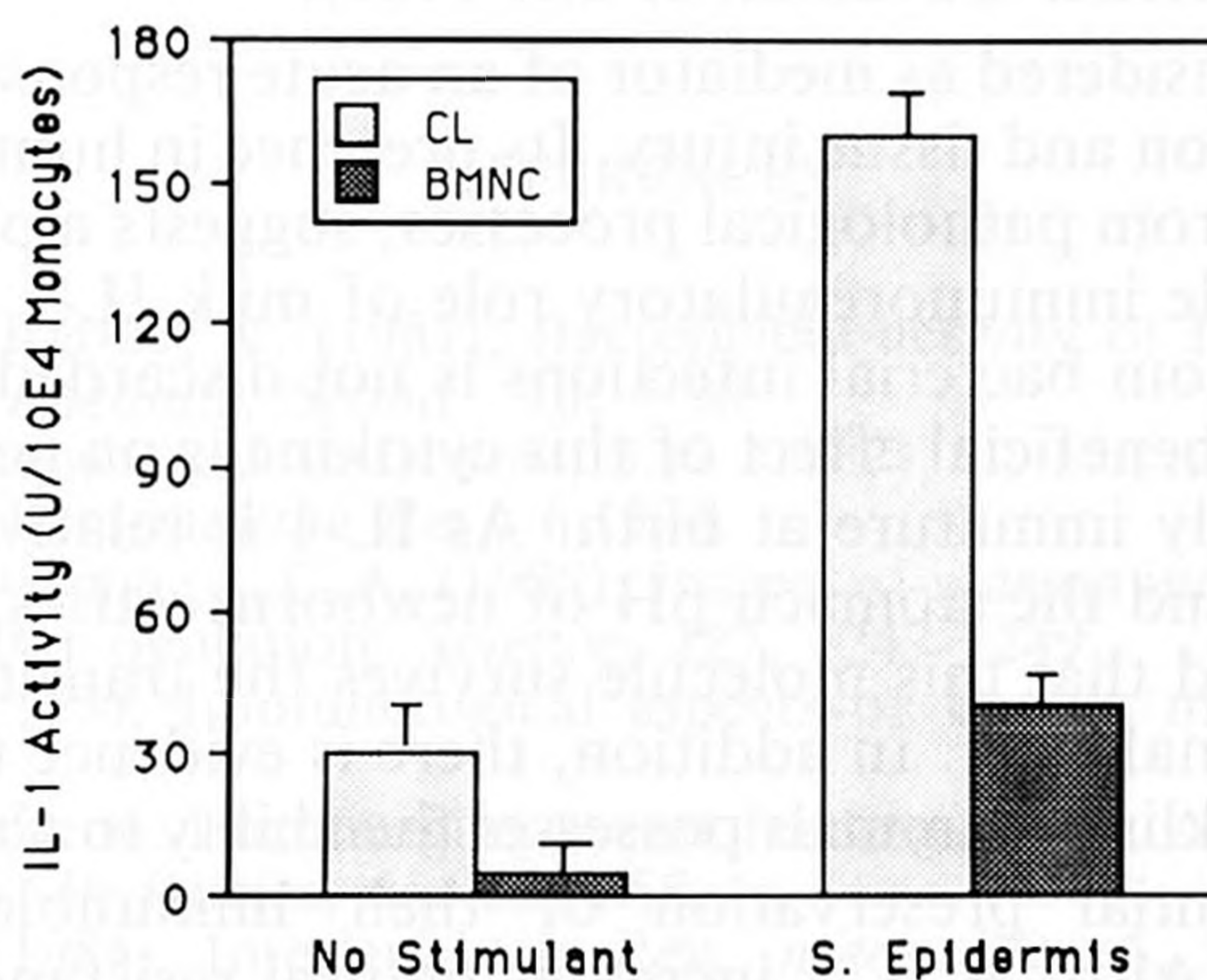


FIG. 1. — IL-1 production by CL compared with BMNC.

Bars represent the mean \pm SEM IL-1 activity of CL and BMNC from 9 healthy mothers who delivered normal full-term infants.

lated CL and blood monocytes was compared, the activity was negligible in maternal BMNC, indicating that culture conditions were not responsible for the spontaneous production of IL-1 by CL. This result was achieved by employing ultra-filtered media during the incubation period, a step which excludes endotoxin contamination (Dinarello *et al.*, 1987a).

We have shown that BMNC of over 100 individuals and the 9 mothers in the present study do not produce IL-1 spontaneously. Thus, the spontaneously produced IL-1 by CL was probably due to activation *in vivo*. These findings are consistent with the spontaneous oxidative metabolic activity (Pickering *et al.*, 1980) and the phagocytosis and killing of bacteria and fungi (Robinson *et al.*, 1878; Bhaskaram and Reddy, 1981) reported for milk macrophages.

Since staphylococcal infections are relatively common in children in developing countries, we chose to show data on IL-1 production by CL using *S. epidermidis* as a stimulus. Although some authors have found that capsular material from periodontal pathogens alone may have some IL-1-like activity (Harvey *et al.*, 1987), we used heat-inactivated *S. epidermidis* as it does not interfere with the magnitude of responses of either colostrum macrophages or blood monocytes.

Recently, Subiza *et al.* (1988) reported a limited ability of human breast milk macrophages to produce IL-1 *in vitro* in response to bacterial lipopolysaccharide (LPS) from *Escherichia coli*. It is possible that CL are more sensible to *S. epidermidis* than to LPS and/or that the capacity of LPS to induce IL-1 varies depending on the concentration and the strain source. For example, blood monocytes from normal adults produce higher amounts of both immunoreactive IL-1 β and TNF in response to *S. epidermidis* compared with LPS (Endres *et al.*, 1988). On the other hand, LPS (1 μ g/ml) from *E. coli* is 3-4 times less potent than LPS (1 μ g/ml) from *Neisseria meningitidis* for inducing IL-1 (Haeflner-Cavaillon *et al.*, 1989).

IL-1 is often considered as mediator of an acute response to microbial invasion, inflammation and tissue injury. Its presence in human colostrum, an environment free from pathological processes, suggests a physiological role. Although a possible immunoregulatory role of milk IL-1 in protecting the mammary gland from bacterial infections is not discarded, we suggest that preferentially, the beneficial effect of this cytokine is on neonates since they are immunologically immature at birth. As IL-1 is relatively acid resistant (Dinarello, 1984) and the stomach pH of newborns varies between 2.3-3.6, it can be speculated that this molecule survives the transit and is absorbed in the gastrointestinal tract. In addition, there is evidence that the gastrointestinal tract of suckling mammals possesses the ability to absorb various proteins with substantial preservation of their immunological properties (Lawrence, 1985). Also, as IL-1 increases natural resistance to infection in granulocytopenic mice (van der Meer *et al.*, 1988), and is identical to haemopoietin-1 (Mochizuki *et al.*, 1987), transfer of this colostrum-derived cytokine to the neonate may play a role in augmenting host defence mechanisms and improving resistance to infection.

RÉSUMÉ

L'INTERLEUKINE-1 β DANS LE COLOSTRUM HUMAIN

Les deux formes d'interleukine-1 (IL-1 α and IL-1 β) et le "tumour necrosis factor" (TNF) sont des cytokines possédant des activités diverses, souvent associées aux mécanismes de défense de l'hôte. En raison de l'effet bénéfique et bien reconnu de l'allaitement maternel, la présence de ces trois cytokines a été analysée dans le colostrum de 9 jeunes femmes. A l'aide de tests radioimmunologiques spécifiques, il a été montré que le colostrum contenait des quantités significatives d'IL-1 β (moyenne \pm SEM = 1130 \pm 259 pg/ml). Les concentrations d'IL-1 α et de TNF étaient au contraire extrêmement faibles.

Les leukocytes présents dans le colostrum sont capables de produire de l'IL-1 puisqu'une forte activité IL-1 a été retrouvée après stimulation de ces cellules par *Staphylococcus epidermidis*. Par ailleurs, nous avons observé que ces cellules produisent spontanément de l'IL-1 *in vitro*, contrairement aux monocytes provenant des personnes examinées. Dans la mesure où l'IL-1 augmente la résistance à l'infection, la présence de cette cytokine dans le colostrum peut être considérée comme l'un des facteurs bénéfiques de l'allaitement maternel.

MOTS-CLÉS: Colostrum, Interleukine-1, Leucocyte; Allaitement, Résistance aux infections.

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Correspondence to Dr G. M. H. Hermans, Department of Cell Biology, Division Haematology, Medical Centre, Vrije Universiteit, Van der Boerhorststraat 7, 1053 BT Amsterdam, The Netherlands.