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Cytokine-Stimulated Human Natural Killer Cytotoxicity: Response to Rotavirus-Infected Cells

STEVE KOHL,[32] MAURICE W. HARMON, AND JY PING TANG

Department of Pediatrics and Program in Infectious Disease and Clinical Microbiology, University of Texas Health Science Center [S. K., J. P. T.], and Departments of Microbiology and Immunology, Baylor College of Medicine [M. W. H.], Houston, Texas, USA

Summary

The ability of rotavirus-infected cells to stimulate leukocytes to release a cytokine which enhanced the subsequent leukocyte cytotoxicity to a second set of [^{51}Cr] labeled rotavirus-infected cells was analyzed. Human interferon increased leukocyte cytotoxicity to Simian rotavirus (SA-11)-infected target cells. Similarly, 11 of 12 supernates of SA-11-stimulated peripheral blood leukocyte cultures increased the killing of SA-11-infected cells ($P < 0.005$). This resulted in a calculated cytokine-dependent cellular cytotoxicity value of 9.6 ± 1.9%. Three of five of the supernates tested contained measurable levels of interferon (12–48 unit/ml). In contrast, SA-11-stimulated colostral leukocyte culture supernates neither increased leukocyte cytotoxicity nor contained measurable levels of interferon.

Abbreviations

CDCC, cytokine-dependent cellular-cytotoxicity
CPE, cytopathic effect
cpm, counts per minute
HBSS, Hank's balanced salt solution
HSV, herpes simplex versus
MC, mononuclear cells
NKC, natural killer cytotoxicity
SA-11, Simian rotavirus
SMEM, supplemented minimal essential medium
VSV, vesicular stomatitis virus

The ability of lymphocytes to destroy virus-infected cells in the absence of antibody-NKC is thought to be an important early immune response to viral infection (12, 18, 23, 29). We and others have documented the enhancement of human NKC by human leukocyte interferon (6, 10, 11, 24, 27). A cytokine is a cell-produced substance. We have demonstrated recently that cytokine-containing supernates derived from cultures containing HSV-infected cells and either human peripheral blood or colostral leukocytes can stimulate the NKC of lymphocytes from either adult or neonatal humans (14). Although interferon may not be the only cytokine involved (9), we have termed this interaction CDCC.

The most common cause of gastroenteritis in infants is rotavirus infection (1). Breast-fed infants are more resistant to gastroenteritis than are bottle fed infants (2, 3, 5, 7, 17). This is not entirely correlated with the presence of antiviral antibody in the breast milk (19, 21, 22, 26). Although the serologic and breast milk humoral response to rotavirus has been studied (1, 4, 20, 25, 30), there is little data on the cellular response to rotavirus. We now report the ability of rotavirus-stimulated peripheral blood, but not colostral leukocytes, to produce interferon and cytokine(s), which stimulate the killing of rotavirus-infected cells.

MATERIALS AND METHODS

Tissue culture cells. MA-104 monkey kidney cells (Microbiological Associates, Bethesda, MD) were utilized to grow SA-11. The cells were grown in MEM (Grand Island Biological Company, Grand Island, NY) supplemented with 10% heat-inactivated fetal calf serum (Flow Laboratories, Rockville, MD), penicillin (50 unit/ml), streptomycin (50 μg/ml) and L-glutamine (final concentration 1.5%).

Virus. The rotavirus used was the cultivatable SA-11 (originally obtained from Dr. Richard H. Conklin, University of Texas Medical School, Houston, TX). The virus was grown in MA-104 cells in the presence of Porcine grade IV Pancreatin (1 μg/ml, Sigma Chemical Co., St. Louis, MO). There was 40–50% CPE after 18 h of infection.

Interferon. Human leukocyte interferon (10,000 unit/ml) was obtained by Sendai virus stimulation of human buffy coat leukocytes in the laboratory of Dr. Kari Cantell, and was kindly provided by the Antiviral Substances Program of the National Institute of Allergy and Infectious Disease.

Interferon assay. As previously described (8), a yield reduction assay was used to measure interferon activity. Before assay, the pH of each sample was reduced to 2.0 by addition of 0.1 N HCl and held at 4°C for 24 h. After restoring the pH to 7.4 by addition of 0.1 N NaOH, samples were diluted in MEM containing 5% fetal bovine serum, and 1.0-ml aliquots of each dilution were added in duplicate to monolayers of human foreskin fibroblasts (cell strain HR-218 from HEM Research, Inc). After incubation at 37°C for 18–24 h, the medium was removed and the cells were washed once with phosphate buffered saline. Cell monolayers were challenged with VSV at a multiplicity of at least 1. After absorption for 1 h at 37°C, the inoculum was removed by washing the monolayers four times with phosphate buffered saline. Monolayers were refed with 2.0 ml of MEM containing 2.0% fetal bovine serum and incubated at 37°C. After 24 h, medium was collected and centrifuged at 1400 × *g* for 10 min, and the supernatant fluid was frozen at −70°C until assayed for virus. VSV yields were determined by plaque formation in L-cells. A laboratory human interferon (α type) standard was included in each assay. One unit of interferon was defined as the dilution of the original sample that reduced the VSV yield by 50% when compared to controls. A unit of interferon was equivalent to 0.4 unit of human interferon research standard B(69/19) from the Medical Research Council, Mill Hill, London, England. All interferon values are expressed in human interferon standard B(69/19) units.

Colostral cells. As previously described, colostrum was obtained after informed consent from mothers on days 2–4 postpartum using an Egnell type SMB breast pump (13). All specimens were obtained between 8 and 10 AM before nursing. None of the women had fever, evidence of mastitis, or had received drugs to suppress milk production. Colostral cells were washed

four times in HBSS (Gibco, Grand Island, NY) and diluted to desired concentrations in SMEM.

Blood leukocytes. Heparinized (30 unit/ml) blood was obtained after informed consent from healthy laboratory workers and postpartum lactating women. Blood was sedimented in 10% (v/v) dextran (3%, Sigma Chemical Co.). The leukocyte rich plasma was then subjected to ficoll-hypaque (Pharmacia, Piscataway, NJ) bouyant-density centrifugation. The separated MC, obtained at the interface, were washed four times in HBSS and resuspended in SMEM at the desired concentrations described previously. The MC layer contained a combination of lymphocytes and monocytes-macrophages (11, 13, 14).

Cytokine culture preparations. Leukocytes from blood or colostrum were cultured in SMEM in 12 x 75 mm sterile polystyrene capped culture tubes (Scientific Products, McGraw Pk, IL). Where indicated, leukocytes were cultured in the presence of MA-104 cells (2.5×10^4/ml) or MA-104 cells infected the day previously with SA-11 rotavirus. The final concentrations of leukocytes were utilized to result in ratios of stimulating (MA-104 cells) to responding leukocyte cells of 1:100 (2.5×10^6 leukocyte/ml) to 1:60 (1.5×10^6 leukocyte/ml). Cells were cultured at 37°C in a humidified atmosphere of 95% air and 5% CO_2 for 18 h, after which time the cultures were centrifuged (400 $\times$ *g* for 15 min) and the cell-free supernates collected and stored at −80°C before use. Supernates from cultures containing SA-11-infected MA-104 cells and leukocytes are referred to as virus-stimulated cytokine preparations. Those supernates from cultures containing uninfected MA-104 cells and leukocytes are referred to as unstimulated cytokine preparations.

Microcytotoxicity assay. The assay for cellular cytotoxicity was performed as described previously (11–14). Target cells were [^{51}Cr]MA-104 cells, which had been infected with SA-11 rotavirus 18 h previously. Each microcytotoxicity assay was carried out in triplicate in rigid polystyrene "U" bottom well microtiter plates (Cooke, Alexandria, VA). To each well were added 100 μl of MC effector cells (1.5×10^5 cell/well), 50 μl of target cells (5×10^3 cell/well), and 50 μl of cytokine preparation. The covered plates were then incubated at 37°C in a humidified atmosphere of 95% air and 5% CO_2.

After 4 h incubation, 100 μl were aspirated from the top of each well without disturbing the cell bottom. To each well, 100 μl of 1 M NaOH was added and the total volume aspirated into a separate container. All samples were counted in a Beckman Biogamma 4000 Gamma Counter for 1 min. Chromium release was calculated according to the following formula:

$$[^{51}\text{Cr}]\text{released} = \frac{2A}{A + B} \times 100$$

Where A equals cpm in the top 100 μl, and B equals cpm in the bottom 100 μl to which NaOH was added.

Natural killer cytotoxicity (%NKC) was defined as:

$$\frac{\left[\%[^{51}\text{Cr}]\text{release of target cells + MC effector cells} - [^{51}\text{Cr}]\text{release of target cells}\right]}{100 - (\%[^{51}\text{Cr}]\text{release of target cells})} \times 100$$

% CDCC was defined as:

$$\frac{\left[\%[^{51}\text{Cr}]\text{release of target cells + MC effector cells + virus stimulated cytokine preparation}\right] - \left[\%[^{51}\text{Cr}]\text{release of target cells + MC effector cells + unstimulated cytokine preparation}\right]}{100 - \left[\%[^{51}\text{Cr}]\text{release of target cells + MC effector cells + unstimulated cytokine preparation}\right]} \times 100$$

Spontaneous [^{51}Cr] release of uninfected MA-104 cells was 14.2 ± 3.3% and spontaneous [^{51}Cr] release of SA-11 rotavirus infected cells was 24.3 ± 2.1% after 4 h incubation. The [^{51}Cr] release of infected cells approached 100% after 18 h incubation and they were therefore not utilized in long term assays. These values were not affected by purified interferon, or virus-stimulated or unstimulated cytokine preparations alone. All assays were performed in triplicate with standard deviations of less than 5%.

Statistical method. Data are expressed as the mean ± S.E.M. of experiments. The significance of differences of mean values was determined by Student's paired two-tail *t* test.

RESULTS

NKC of peripheral blood MC to rotavirus-infected cells. Initial experiments defined NKC to SA-11-infected and uninfected MA 104 cells. Using an MC effector to target cells ratio of 30:1 in a 4 h [^{51}Cr] release assay, NKC of similar magnitude was detected against uninfected MA-104 cells (NKC, 12.5 ± 5.5%) and SA-11-infected cells (NKC, 14.3 ± 2.2%) (Table 1). Using both types of target cells, the addition of purified human leukocyte interferon at the onset of the assay, without preincubation, augmented NKC. Although there was an 8.1% increase in NKC to uninfected cells, and a 15% increase in NKC to infected cells in the presence of interferon, this difference is difficult to compare due to the higher spontaneous [^{51}Cr] release of infected cells (24.3%) than uninfected cells (14.2%) (Table 1).

Exhaustive attempts to induce increased cytotoxicity by the addition of antirotavirus antibody produced experimentally in chickens, guinea pigs, and mice, as well as that that found naturally in convalescent serum of children recovering from documented rotavirus enteritis were unsuccessful (data not shown). Similarly, omission of fetal calf sera, a possible source of antirotavirus antibody, failed to diminish cellular cytotoxicity. Although NKC was detected both to rotavirus-infected and uninfected MA-104 cells, antibody-dependent cellular cytotoxicity was never demonstrated in this system.

CDCC to rotavirus-infected cells stimulated by blood leukocyte cultures incubated with rotavirus-infected cells. Because interferon stimulated NKC to rotavirus-infected target cells, we next

Table 1. *Natural killer cytotoxicity (NKC) of peripheral blood mononuclear cells (MC) to rotavirus-infected and uninfected cells*

	Uninfected target cells[1]	SA-11-infected target cells[2]
% [^{51}Cr] release		
Target cells alone[3]	14.2 ± 3.3	24.3 ± 2.1
Target cells + MC[4]	23.6 ± 5.7	35.2 ± 2.7
Target cells + MC + interferon[5]	30.4 ± 4.4	46.8 ± 3.9
%NKC[6]	12.5 ± 5.5	14.3 ± 2.2
%NKC + interferon[7]	20.6 ± 4.7	29.3 ± 4.1

[1] MA-104 cells utilized as target cells in a 4-h assay, mean ± S.E.M. of two experiments.

[2] SA-11-infected MA-104 cells utilized as target cells in a 4-h assay, mean ± S.E.M. of nine experiments.

[3] [^{51}Cr] released by target cells alone in the 4-h cytotoxicity assay (spontaneous [^{51}Cr] release).

[4] [^{51}Cr] released by target cells in the presence of human MC at an effector to target cell ratio of 30:1 in the 4-h cytotoxicity assay.

[5] [^{51}cr] released by target cells in the presence of human MC and 50 units (250 unit/ml) of interferon added at the onset of the cytotoxicity assay.

[6] $$\%\ \text{NKC} = \frac{[^{51}\text{Cr}]\ \text{release of target cells + MC}] - [\%\ [^{51}\text{Cr}]\ \text{release of target cells}]}{100 - [\%\ [^{51}\text{Cr}]\ \text{release of target cells}]} \times 100.$$

[7] % NKC in the presence of 50 units (250 units/ml) of interferon added at the onset of the cytotoxicity assay.

determined whether human peripheral blood MC, upon incubation with rotavirus, would produce a cytokine which stimulated NKC. Preliminary experiments demonstrated that cultures of leukocytes and virus-infected cells, containing 60 leukocytes per infected cell in an 18-h culture, were optimum for cytokine production. Unlike the HSV system (11, 14), preincubation of the effector leukocytes with the virus-stimulated cytokines supernates was not necessary to demonstrate increases in NKC. Table 2 lists the results of 12 experiments comparing the [^{51}Cr] release induced by MC from normal human adults in the presence of supernates prepared from cultures of MC from lactating women incubated with SA-11-infected or uninfected cells. In all but one of these experiments (number 2), there was enhanced [^{51}Cr] release when MC cells were incubated with supernates of cultures of leukocytes and rotavirus infected cells. This increased cytotoxicity was highly significant ($P < 0.005$) and resulted in a CDCC of from 5.6–24.7% (mean 9.6 ± 1.9%), (Table 2).

Seven experiments using MC from lactating women and SA-11-viral infected cell cultures utilizing 100 MC per infected cell similarly revealed a CDCC of 7.6 ± 2.9%. Experiments using virus-stimulated MC supernates from five nonlactating adults (60 MC per virus-infected cell) revealed a CDCC value of 11.1 ± 3.2% (range, 4.6–11.4%). There was, therefore, no significant difference in CDCC mediated by MC supernates from either lactating or nonlactating adults, using varying ratios of virus-infected cells to leukocytes. Human peripheral blood MC, upon incubation with rotavirus infected cells, released a cytokine which enhanced MC cytotoxicity to rotavirus-infected cells, mediating CDCC.

Lack of CDCC to rotavirus-infected cells stimulated by colostral leukocytes cultures incubated with rotavirus-infected cells. Because peripheral blood MC from lactating women, upon rotavirus stimulation released a cytokine stimulating CDCC, colostral cells were similarly examined. Table 3 reveals the results of 12 experiments utilizing colostral cells simultaneously obtained from the same subjects as in Table 2. The result of [^{51}Cr] release induced by MC in the presence of supernates of colostral cell cultures stimulated by SA-11-infected or uninfected cells is listed. In contrast to the experiments using MC cultures, only one colostral culture (number 1) induced CDCC of over 5% (12.5%). These was no statistically significant difference in [^{51}Cr] release induced by colostral cell supernates in the presence of SA-11-infected *versus* uninfected MA 104 cells. The CDCC value of 1.7 ± 1.5% was not significant.

To determine if the process of cell preparation itself affected the ability of leukocytes to produce the NKC-stimulating cytokine, a limited number of experiments were performed using alternate cell preparations. In these experiments, colostral cells were subjected to ficoll-hypaque density separation as was previously used to purify blood leukocytes. These cells were primarily MC. In contrast, blood leukocytes were obtained by gravity sedimentation, without further purification. These cells were a mixture of mononuclear and polymorphonuclear leukocytes. Due to lower cell yields, three experiments from different donors were performed using cultures of 30 (not 60) leukocytes per stimulating cell. The supernatants were then utilized in the cytotoxicity assay as described in Tables 2 and 3. The %CDCC mediated by the supernatants of the gravity sedimented leukocytes was 4.9 ± 1.2% ($P < 0.05$). As seen with unpurified colostral cells, supernatants for ficoll-hypaque purified colostral cell cultures again failed to mediate CDCC (1.0 ± 0.5%, P = NS). Although rotavirus-infected cells stimulate blood MC to release

Table 2. *Cytokine-dependent cellular cytotoxicity (CDCC) stimulated by blood leukocyte cultures (MC) incubated with rotavirus-infected cells*[1]

	% [^{51}Cr] release stimulated by leukocyte culture supernate		
Exp. no.	MC + SA-11-infected cell[2]	MC + uninfected cells[3]	% CDCC[4]
1	61.5	48.9	24.7
2	76.6	82.3	0
3	57.5	54.1	7.4
4	40.0	34.6	8.3
5	44.8	39.1	9.5
6	43.0	36.9	9.7
7	40.3	36.3	6.3
8	47.4	34.0	20.3
9	40.8	33.9	10.5
10	32.5	27.5	6.9
11	35.3	31.5	5.6
12	44.3	40.4	6.5
	P[5] < 0.005		9.6 ± 1.9

[1] MC incubated with SA-11-infected [^{51}Cr] labeled target cells at an effector to target cell ratio of 30:1 in the presence of blood leukocyte culture supernates in a 4 h cytotoxicity assay.

[2] % [^{51}Cr] release in the cytotoxicity assay using a final dilution of ¼ of a supernate of MC and SA-11-infected MA-104 cells from an 18-h culture using 60 MC per SA-11-infected MA-104 cell.

[3] % [^{51}Cr] release in the cytotoxicity assay using a final dilution of ¼ of supernate of MC and uninfected MA-104 cells from an 18-h culture using 60 MC per uninfected MA-104 cell.

[4] CDCC = calculated cytokine-dependent cellular cytotoxicity

$$\frac{(\text{column 2} - \text{column 3})}{100 - \text{column 3}} \times 100$$

[5] P = significance of difference between [^{51}Cr] release in the presence of blood leukocyte cytokine stimulated by SA-11-infected *versus* uninfected MA-104 cells.

Table 3. *Cytokine-dependent celular cytotoxicity (CDCC) stimulated by colostral leukocyte cultures incubated with rotavirus-infected cells*[1]

	% [^{51}Cr] release stimulated by leukocyte culture supernate		
Exp. no.	Colostral cells + SA-11-infected cells[2]	Colostral cells + uninfected cells[3]	% CDCC[4]
1	61.8	56.3	12.5
2	56.4	56.8	0
3	30.8	38.3	0
4	36.2	41.8	0
5	34.2	36.6	0
6	38.3	37.5	1.3
7	37.2	40.4	0
8	34.8	38.1	0
9	37.5	34.7	4.3
10	23.4	26.3	0
11	36.5	35.1	2.2
12	38.0	42.1	0
	P = NS[5]		1.7 ± 1.1

[1] MC incubated with SA-11-infected, [^{51}Cr] labeled target cells at an effector to target cell ratio of 30:1 in the presence of colostral leukocyte culture supernates in a 4-h cytotoxicity assay.

[2] % [^{51}Cr] release in the cytotoxicity assay using a final dilution of ¼ of supernate of colostral cells and SA-11-infected MA-104 cells from an 18-h culture using 60 colostral cells per infected MA-104 cell.

[3] % [^{51}Cr] release in the cytotoxicity assay using a final dilution of ¼ of supernate of colostral cells and uninfected MA-104 cells from an 18-h culture using 60 colostral cells per uninfected MA-104 cells.

[4] CDCC = calculated cytokine-dependent cellular cytotoxicity

$$\frac{(\text{column 2} - \text{column 3})}{100 - \text{column 3}} \times 100$$

[5] P = significance of differences between [^{51}Cr] release in the presence of colostral cell cytokine stimulated by SA-11-infected *versus* uninfected MA-104 cells.

Table 4. *Interferon production of peripheral blood leukocytes and colostral cells during incubation with rotavirus-infected cells*[1]

Experiment no.	MC + SA-11-infected cells[2]	Colostral cells + SA-11-infected cells[3]
1	<2	<16
2	48	<2
3	42	<16
4	<6	<2
5	12	<2

[1] The lowest dilution tested was determined by the available volume of each sample.

[2] Interferon levels (unit/ml) in 18-h culture supernates of peripheral blood mononuclear cells plus SA-11-infected cells.

[3] Interferon levels (unit/ml) in 18-h culture supernates of colostral cells plus SA-11-infected cells.

cytokine mediating CDCC, they did not stimulate colostral cells to do so.

Interferon production by peripheral blood leukocytes and colostral cells after incubation with rotavirus-infected cells. Because supernates of rotavirus-stimulated peripheral blood leukocyte cultures mediated cytotoxicity whereas similar colostral cell supernates failed to mediate cytotoxicity, the interferon content of culture supernates was assayed (Table 4). Whereas measurable levels of interferon were detected in three of five peripheral blood MC cultures, interferon was not detected in any of five cultures of colostral cells and SA-11-infected cells (Table 4).

The antiviral activity was partially characterized as interferon by stability to pH 2.0 for 24 h, lack of activity on heterologous cells (mouse L-cells), and susceptability to digestion by trypsin. The activity was not neutralized by rabbit antiserum to human interferon-β(kindly provided by Dr. J. Vilcek) but was partially neutralized by rabbit antiserum to human interferon-α(obtained from NIH Research Reagents).

DISCUSSION

We have demonstrated that human peripheral blood leukocytes mediated NKC to rotavirus-infected cells. This cytotoxicity was enhanced by human leukocyte interferon as previously described using other virus-infected cells (11, 24, 27). Rotavirus-infected cells, but not uninfected cells, stimulated peripheral blood cells to produce a cytokine that enhanced NKC to rotavirus-infected cells (Table 2). Similar experiments using colostral cells failed to demonstrate CDCC. Because interferon is one of the lymphokines able to stimulate NKC, cultures of leukocytes and virus-infected cells were analyzed for the production of interferon. Although most blood leukocyte cultures had measurable levels of interferon, none was detected in the colostral cell cultures. The interferon was characterized by stability at acid pH and neutralization with anti-leukocyte interferon antiserum.

The results using blood leukocytes are not unlike our previous experiments, which demonstrated HSV-infected cell stimulation of peripheral blood cells to produce a cytokine mediating CDCC (14). They differ in the lower magnitude of CDCC and lower levels of interferon produced by rotavirus-infected cell stimulation (14). Unlike the HSV system, in which colostral cells produced cytokine(s) mediating CDCC (14), none was detected using rotavirus-infected cells. It is possible that the simian rotavirus is merely a poor stimulator of cytokines, as supported by the low levels of interferon produced (16). When available, it is hoped that this work may be extended to the human rotavirus, which is closely related, but by no means identical to SA-11.

In the HSV system, colostral cells tended to produce lower levels of cytokine than did blood leukocytes (14). It is possible that differences in cytokine production of colostral cells compared with blood cells in both the HSV and rotavirus system are related to the presence of different types and amounts of leukocytes in the compartments. When the cytokine is further identified, and the cell responsible for the production characterized, this can be ascertained.

Other lymphokines such as interleukin-2, which may enhance NKC (9), have not been assayed for in our system. It has recently been shown that prostaglandin E_2, a known inhibitor of NKC, may be released by stimulated macrophages (15). It is possible that colostral macrophages (a prodominant colostral cell) override any stimulatory cytokines with inhibitory prostaglandin production. Indeed, one could view such production as a mechanism to protect the neonate's intestinal cells from allogeneic cell directed maternal NKC. Other possible NKC inhibitors, as colostral lipids (13) or even IgA, may be slowly released by colostral macrophages, especially after phagocytosis (28), and may similarly be responsible for NKC inhibition.

Although our previous (13) and present results fail to support the role of colostral cells as highly active intestinal effector cells, or even producers of substances that stimulate neonatal effector cells during rotavirus infection, they raise important questions regarding the role of colostral cells as immunoregulators *in vivo.*

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32. Requests for reprints should be addressed to: Dr. Steve Kohl, Program in Infectious Diseases and Clinical Microbiology, 6431 Fannin Street, Houston, Texas 77030.
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Behavioral and Cardiac Rhythmicity during Mother-Father-Stranger Infant Social Interaction

MICHAEL W. YOGMAN,[25] BARRY M. LESTER, AND JOEL HOFFMAN

Department of Pediatrics, Harvard Medical School, and Child Development Unit, Children's Hospital Medical Center, Boston, Massachusetts, USA

Summary

In an effort to understand the temporal organization of infant physiologic and behavioral systems during social interaction with adults, spectral and cross-spectral analyses of infant heart rate and mother-father-stranger-infant behavioral data are described herein for a 3-month-old infant during face-to-face social interaction with her parents and a stranger. This infant's heart rate rhythms were stronger during social interaction with both mother and father than with a stranger. Infant behavioral rhythms were associated with (high coherence) the parents' behavioral rhythms, but not with those of the stranger. Furthermore, infant heart rate and infant behavioral rhythms showed high coherence with all three adults. Although these findings are preliminary, based on a case study and need replication, they generate intriguing hypotheses. This technique of studying the rhythmicity of infant social interaction with spectral analysis suggests that the 3-month-old infant's behavior and heart rate are synchronized during interaction with all three adults; however, the infant and adult behavioral rhythms are synchronous only with mother and father and not with an unfamiliar stranger.

Research on biologic rhythms suggests that a wide range of rhythmic phenomena in the human organism have a biologic basis deriving from the periodic transmission of impulses from a specialized area of the central nervous system, a biologic clock (13). The development of an adaptive fit between the rhythms of the newborn and the extrinsic rhythmicity of the environment is an evolving area of research. For example, Condon and Sander (5) have suggested that the human neonate synchronizes its body motions to the phonemes of adult speakers. Recent work has emphasized the contributions made by caregivers to facilitate the temporal organization of the infant's various functions such as sleeping and feeding, which become coordinated within the baby and linked with the regular periodicities of the physical and social environment (15). Although the presence of rhythmicity in both behavioral and physiologic systems in the infant may have a biologic basis, the relationship between behavioral and physiologic rhythms may be influenced by the sensitivity of the infant's caregiving environment. One might also expect that the rhythmic structure of the infant's behavior would vary if the feedback provided by a strange adult differs from that of the parent during social play.

The concept of rhythmicity in infant-adult social interactions has long been of interest to scientists studying the structure, development, and biologic basis of social communication (2, 16, 19). Studies of mother-infant interaction (18, 1, 3, 11) assume that rhythmicity is part of the underlying structure of the interaction and are attempting to uncover the rules that govern the relationship between the behavior of the two participants. When the two partners cycle together, the interaction is described as synchronous and the degree of synchrony has been suggested as a measure of the quality of the interaction (3, 4).

Descriptive studies of infant behavior with other adults suggest that infants interact differently with mothers than with fathers (7, 22, 23) or with strangers (6), although the rhythmic structure of these interactions has not been studied systematically or quantified. With mothers and infants, one of the patterns described has been the build-up and withdrawal of attention. Infants and mothers appeared to increase and decrease their behavioral attention and arousal in a periodic, cyclical fashion (2) that may reflect a relationship between infant behavioral and physiologic rhythms. The authors suggested that both the infant's behavioral state or arousal and his physiologic systems are related