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Lack of Suppression by Concanavalin A-Activated Neonatal Mononuclear Cells

MARGARET SODERBERG-WARNER, BONNIE ANK, AND E. RICHARD STIEHM

Division of Immunology, Department of Pediatrics, UCLA Center for the Health Sciences, Los Angeles, California 90024

ABSTRACT. We studied the mixed leukocyte culture suppression generated by cord and newborn mononuclear cells stimulated by concanavalin A (Con A) compared to normal adult cells and cells from patients with systemic lupus erythematosus. Also we studied the effect of supernatants from cord or adult cells stimulated with Con A linked to sepharose (Con A sepharose). Peripheral blood mononuclear cells from 15 adults, 10 normal newborns, 23 cord blood samples, and 11 patients with systemic lupus erythematosus were preincubated with Con A for 48 hr, irradiated, and added to a one-way mixed leukocyte culture. Adult Con A-activated cells suppressed the mixed leukocyte culture by 30 ± 6.5%. By contrast cord cells and newborn cells had no suppressive activity; these cells resulted in stimulation of 18 ± 12.1 and 18 ± 7%, respectively. This lack of suppression also was present in systemic lupus erythematosus cells (11.2 ± 13.3). The supernatants of both Con A sepharose-stimulated cord and adult cells showed significant suppressive activity and there was some suppressive activity of sepharose-stimulated cells alone. These results suggest that the mixed leukocyte cultures suppressive activity observed previously by newborn cells is radiosensitive and dependent on ongoing cell division for its expression. It also is independent of prior mitogenic stimulation. (*Pediatr Res* 19: 927–929, 1985)

Abbreviations

MNC, mononuclear cells
MLC, mixed leukocyte culture
Con A, concanavalin A
SLE, systemic lupus erythematosus

The human newborn immune system may have excessive suppressive activity. In 1974, Olding and coworkers (1, 2) showed that 90% of dividing cells in a MLC consisting of half infant male lymphocytes and half adult female lymphocytes were male cells by chromosome analysis, suggesting suppression of division of the adult female cells. Subsequent studies (3, 4) identified that this was in part due to the secretion of a soluble, suppressive substance formed by the neonatal T lymphocytes. Using a murine model, Bassett *et al.* (5) demonstrated that newborn murine splenic lymphocytes were weaker stimulators of a MLC than were adult murine lymphocytes from the same strain and that this effect was due to inhibition of proliferation mediated by a soluble substance in the supernatant. Others have shown that neonatal T cells also inhibit the proliferation of and immunoglobulin synthesis by neonatal and adult B cells (6).

Con A has been used to selectively activate normal adult suppressor T cells (7, 8). Using this procedure, deficient suppressor cell activity has been noted in patients with certain human autoimmune diseases, particularly SLE (7, 9). Williams and Korsmeyer (10) have shown that supernatants from Con A-stimulated human cord blood lymphocytes inhibit the MLC response. Abedin and Kirkpatrick (11) reported that both cord blood cells and cell supernatants suppressed antigen and mitogen-induced proliferation of adult T cells even without prior mitogenic activation, implying a high degree of spontaneous suppressor activity.

The present studies sought to identify functional differences between adult and newborn suppressor activity. We postulated that Con A-treated irradiated newborn lymphocytes and their supernatants would demonstrate more suppression than adult cells. To our surprise, we found that Con A-activated newborn cells had less suppressive activity than adult cells, and that while the supernatants did exhibit some suppressive activity, this was independent of the presence of Con A. We believe these results indicate that neonatal T suppression requires active cell division for its expression.

METHODS

Subjects. We studied cells from 15 normal adult controls ages 25 to 50 yr, 23 freshly obtained cord blood specimens, 10 normal newborns 24 to 72 h of age, and 11 patients with SLE. The latter included nine females and two males aged 21 to 59 years, four of whom had active disease, nine of whom were on prednisone alone, and one of whom was on prednisone plus Imuran at the time of the study. We also studied the supernatants from 10 cord blood specimens and 11 adults.

Suppressor assay. Peripheral blood mononuclear cells separated by Ficoll-Hypaque centrifugation (12) were suspended at a concentration of 3×10^6 cells/ml in RPMI with 15% AB serum. Con A (Sigma Chemical), 120 μg, was then added to 2 ml of this suspension. This and a second suspension of 2 ml lacking Con A were incubated for 48 h at 37° C in 5% CO_2. These "preincubated" cells were washed twice with Hanks' balanced salt solution, irradiated with 4000 R, washed once more with Hanks' balanced salt solution, and resuspended in RPMI at a concentration of 1×10^6 cells/ml. The incubated cells were then added to a one-way MLC utilizing two nonrelated normal adults as responders or stimulators (the stimulator population was prepared by similar means). Only experiments in which the stimulation index of the control MLC was greater than 5 were included. Thus each assay (in triplicate in microtiter plates) contained 1×10^5 (0.05 ml) responder cells, 1×10^5 (0.05 ml) irradiated stimulator cells, 1×10^5 (0.1 ml) irradiated Con A-treated suppressor cells (or control irradiated suppressor cells), and 0.05

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Reprints Dr. E. Richard Stiehm, UCLA Department of Pediatrics, Los Angeles, CA 90024.
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ml of pooled O positive human serum. After 5 days of incubation 2 μCi of tritiated thymidine were added per well and cultures were harvested 16 to 24 h later with a MASH apparatus. Radioactivity was determined in a beta scintillation counter. Suppression was calculated by the formula:

$$\% \text{ suppression} = \frac{\text{Control cpm} - \text{Expt. cpm}}{\text{Control cpm}}$$

Supernatant assay. Peripheral blood mononuclear cells were cultured for 72 h with sepharose-conjugated (insoluble) Con A (100 μg/ml) as described by Williams and Korsmeyer (10). We used lymphocytes isolated from heparinized cord blood specimens from normal newborn infants 1 to 3 days old and from 11 normal laboratory volunteers. After separation with Ficoll-Hypaque centrifugation, 1×10^6 lymphocytes were cultured for 72 h with Sepharose-conjugated Con A (100 μg/ml). Preliminary experiments had shown that maximum suppression occurred after a 72-h incubation. Controls included cells incubated with sepharose particles alone and cells incubated with culture media alone. After 72 h, the mix was centrifuged and the supernatants stored at −70° C until assayed.

The thawed cell culture supernates were added to the MLC system described above. Aliquots (0.20 ml) of supernate was added to an MLC containing 0.10 ml (1×10^5) responding and 0.10 ml (1×10^5) irradiated stimulating cells in triplicate. Supernatants also were added to responding cells alone to determine any possible effect on unstimulated cell cultures. After 7 days tritiated thymidine was added to the cultures and the cells were harvested and counted 16 to 24 h later.

Statistical analyses were performed by Student's *t* test.

RESULTS

The results of the Con A-induced suppression are presented in Figure 1. Seventy percent of the Con A-activated mononuclear cells from normal adults caused a significant suppression of as MLC with a mean degree of suppression of −30%. Con A-activated mononuclear cells of patients with SLE showed significantly less suppression, with a mean stimulation of 11%. Indeed, 11 of 22 (50%) of the MLC assays incubated with SLE MNCs showed stimulation rather than suppression. Cord and newborn Con A activated MNCs showed less suppression than adult MNCs; both exhibited a mean stimulation of 18%. Only five of 19 (26%) newborn cell MNC assays and five of 40 (12.5%) cord blood MNC assays exhibited significant suppression. The degree of suppression of Con A-activated cord or newborn samples did not differ significantly from each other or from the SLE samples. All differed significantly from the adult normal controls ($p < 0.05$).

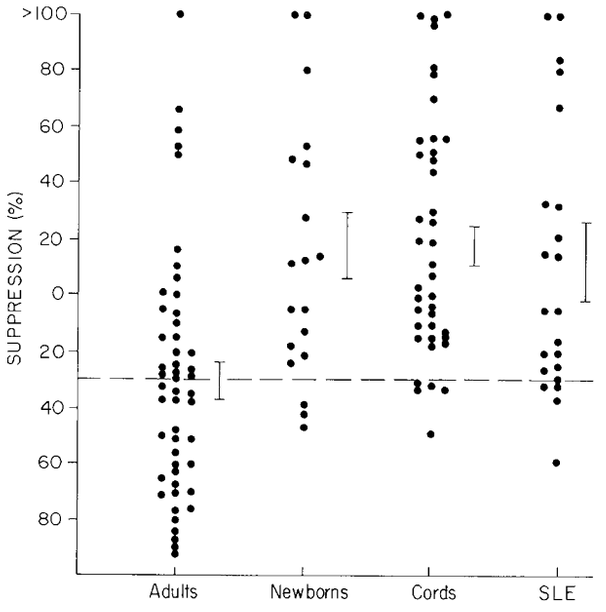

Fig. 1. Suppression of a MLC by mononuclear cells of adults, newborns, cord blood, or SLE patients following activation with Con A. The vertical axis represents the degree of suppression; the horizontal line at −30% represents the mean adult suppression. The *vertical bars* represent ±1 SE of the mean. The mean suppression for adults (15 subjects, 49 assays) was −30 ± 6.5%; for newborns (10 subjects, 19 assays) it was 18 ± 12.1% for cord blood samples (23 subjects, 40 assays) it was 18 ± 7% and for SLE patients (11 subjects, 22 assays) it was 11.2 ± 13.3%.

The suppressive effect of supernatants from adult and cord MNCs are shown in Figure 2 and Table 1. Supernatants of unstimulated cord or adult cells had minimal effects on a MLC. By contrast, the supernatants from sepharose-treated adults and cord cells had significant suppressive effects (21 ± 2 and 28 ± 5%, respectively) on the MLC reaction. Supernatants from Con A-sepharose activated adult and cord cells showed even more suppressive effects. The suppression of 47 ± 5% for adult supernatants was significantly greater ($p < 0.02$) than that of the cord blood cell supernatants (31 ± 5%).

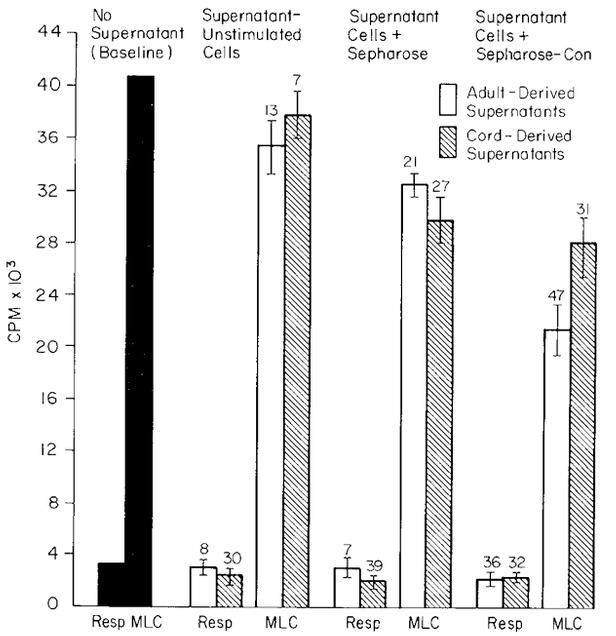

Fig. 2. Inhibition of a MLC or unstimulated responder cells (*Resp*) by supernatants of adult or cord derived mononuclear cells. The first *lane* is the baseline stimulation. The other *lanes* represent the effect of supernatants from unstimulated cells (*lanes 2 and 3*), from cells stimulated with sepharose (*lanes 4 and 5*), and from cells stimulated with sepharose-Con A (*lanes 6 and 7*). The *numbers* above each *bar* indicate the human suppression ±1 SEM. N = 11 for the adult group and 10 for the cord group. Difference in MLC suppression was significant only for *lanes 6 and 7* ($p < 0.02$).

Table 1. *Suppression of a MLC by supernatants from cord or adult mononuclear cells*

Supernatant source	% Suppression of a MLC		
	Adult cells ($n = 11$)	Cord cells ($n = 10$)	p
Cells alone	13 ± 5	7 ± 5	NS
Cells + sepharose	21 ± 2	27 ± 5	NS
Cells + sepharose + Con A	47 ± 5	31 ± 5	<0.02

There was minimal suppressive activity of the various supernatants on responder cells alone, as indicated in Figure 2. However, the low baseline responses of the responder cell population minimize the significance of this low level suppression.

DISCUSSION

Using a culture mix of maternal and male infant phytohemagglutinin-stimulated cells, Olding and Oldstone (2) first suggested a suppressive effect of newborn cells on dividing adult cells. They found that mitogen stimulated nonirradiated lymphocytes from human newborns inhibited the phytohemagglutinin responses of adult lympocytes when the cell populations were separated by a cell impermeable membrane. They attributed this effect to the secretion of a "suppressor substance" by increased numbers of highly active newborn suppressor cells. In the same system phytohemagglutinin-stimulated lymphocytes from nonrelated adults did not exhibit inhibition nor did mitogen-stimulated lymphocytes from newborns inhibit cells from another newborn.

Altman *et al.* (13) showed that Con A-activated newborn cells have suppressive activity, but it was significantly less than that of Con A-activated cells from older children. Recently Hayward and Malmberg (14) reported that the higher frequency of dividing newborn cells in a 2-way newborn-adult MLC was due to increased proliferation by newborn non-T cells rather than newborn suppression of the adult T cells (10). This increased proliferation could be eliminated by irradiating the non-T cells.

Others have demonstrated that newborn T cells incubated with either adult or neonatal B cells significantly inhibit B cell differentiation and immunoglobulin synthesis (14–16). These suppressive effects are also abolished by irradiation, suggesting the need for ongoing cell division for suppression to occur. Cell division may also be necessary for the secretion of soluble suppressors such as observed by Olding and Oldstone (2) and Williams and Korsmeyer (10).

In the Con A suppressor system utilized in our experiments, suppressor cells are generated with Con A during a preincubation period and then irradiated to prevent further cell division. Under these conditions, normal adult cells show suppressive properties on MLC-, antigen-, or mitogen-induced proliferation (7, 8, 16). The present studies indicate that newborn and cord cells under these conditions are not suppressive, but instead are stimulatory, and resemble cells from patients with SLE, a condition associated with diminished T suppressor activity. These results suggest that newborn suppressor cells are highly radiosensitive and require ongoing cell division to express suppression.

Knaub and Jeannet (17) reexamined the Con A-induced suppressor cell assay and found no evidence of cytotoxicity as a cause for the suppression. They also concluded that in their assay (as in ours) it was unlikely that suppression following Con A activation results from competition for nutrients as the Con A-stimulated cells had been irradiated and therefore exhibit little ongoing cell division.

In our experiments, there was little difference in the suppressive effects of supernatants derived from cord blood cells incubated with Con A-Sepharose or Sepharose alone. This suggests that the suppressive effect of newborn cells is independent of the presence of Con A. This is in agreement with the findings of both Hayward and Kurnick (18) and Abedin and Kirkpatrick (11), who demonstrated neonatal suppressive activity in the absence of mitogenic stimulation. Hayward and Kurnick (18) showed that newborn T cells were capable of suppression without lectin activation, that the suppressor activity was an intrinsic characteristic of the cell, and that suppression could not be further increased above this basal level.

We conclude that neonatal and cord blood Con A-activated T cells are not always suppressive, that newborn suppressor cells are radiosensitive, and that supernatants from cord blood cells are suppressive but do not require lectin activation for the expression of suppression. Thus, significant functional differences exist between the neonatal and the adult suppressor cell systems particularly in terms of activation stimuli.

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