Announcement

The Editorial Office of *PEDIATRIC RESEARCH* will be moving April 1, 1984.
After April 1, 1984, all manuscripts should be mailed to:

Delbert A. Fisher, M.D.
Editor-in-Chief
Harbor-UCLA Medical Center
Research & Educational Institute
1124 W. Carson St.
Torrance, CA 90502

Suppressor T-Cell Activity in Newborns and Mothers

YEHUDITH ALTMAN, ZEEV T. HANDZEL,¹³⁸³ AND STANLEY LEVIN

Pediatric Research Institute of the Kaplan Hospital, Rehovot, Affiliated to the Hebrew University—Hadassah Medical School, Jerusalem, Israel

Summary

We studied autologous and allogeneic concanavalin A (Con A)-induced suppression of proliferative responses to phytohemagglutinin (PHA) and Con A in peripheral blood mononuclear cells in 24 normal newborns and compared the results with those obtained from 20 normal older children. Three concentrations of PHA and one of Con A were used to stimulate responder cells. Suppressor activity, elicited in the stimulated cell population after 48 h pre-incubation with Con A, was expressed as percentage inhibition of the proliferative response to PHA. There was an inverse relationship between PHA concentration and suppressor activity, and autologous suppression was greater than allogeneic suppression within each group of patients and at each mitogen concentration. In both the autologous and allogeneic systems, older children showed more suppressor activity than newborns. Feto-maternal pairing showed that newborns efficiently suppress their mother's mitogenic responses, but the mothers do not suppress their own or other newborn's lymphocytes, despite having normal autologous suppressor capability. We suggest that suppressor activity by the fetus and its inhibition in the mother may play a part in the mechanism for controlling maternal-fetal immune rejection.

Abbreviations

Con A, concanavalin A
LIF, leukocyte migration inhibition factor
PBS, phosphate buffered saline
PHA, phytohemagglutinin
PWM, pokeweed mitogen
MIF, migration inhibition factor

The immune competency of lymphocytes from newborn infants has been investigated in several studies because of the known increased susceptibility of newborns to infections (15, 23, 34). Some parameters of T-cell function in cord blood have been shown to be "normal" or similar to that found in healthy older children, and these include the quantity and proportion of E-rosette-forming cells (5, 34), lymphocyte response to mitogens (8, 12), and production of certain lymphokines such as viral and immune interferon and LIF (15). Other newborn cellular immune functions have been reported to be diminished, and these include reduction of PHA-induced cytotoxicity (8), diminished lymphotoxin (12), and MIF production (34) as well as lymphocyte cAMP levels (14). There are, as yet, few studies of the quantitation or functional abilities of subpopulations of T-lymphocytes in newborn infants because techniques for their evaluation have only become available recently. In one report, Olding and Oldstone (27) found that cord blood T-lymphocytes suppress PHA-induced mitosis in maternal lymphocytes, and that DNA synthesis in maternal and other female lymphocytes is inhibited by proliferating newborn lymphocytes (26). Con A-activated T-

cells have been shown to suppress proliferative responses to soluble antigens, mitogens, and allogeneic cells (32) as well as antibody (19) and LIF production (21).

The present study was designed to investigate this suppressor cell subpopulation of lymphocytes in newborn infants by evaluating the Con A-induced suppressor activity of mitogenesis in autologous and allogeneic cells, and comparing the results with those found in older, healthy individuals. The same technique was used to evaluate the feto-maternal immunoregulatory relationship at birth.

MATERIALS AND METHODS

Heparinized cord blood was obtained from 24 normal newborn infants immediately after delivery. The control group consisted of 20 children aged 1–10 yr who had been hospitalized for elective surgery or for observation after minor traumata and who were undergoing venipuncture for routine laboratory studies.

In addition, seven pairs of maternal blood samples, with their newborn's cord bloods, were assayed reciprocally for suppressor activity. At the same time these maternal cells were assayed with cells of other newborns in an allogeneic system. As controls for the maternal regulatory capacity, maternal autologous suppressor activity was evaluated, as well as allogeneic suppressor activity of two mothers upon lymphocytes of young healthy adults. After explaining the purpose of the study to the mothers and obtaining their consent, blood was drawn and lymphocytes were separated by Ficoll-Hypaque (Pharmacia, Uppsala, Sweden) density gradient centrifugation (7) and the cells washed 3 times in PBS. They were then resuspended in RPMI 1640 medium (Bio-Lab, Jerusalem) containing 15% heat-inactivated pooled human AB serum (Magen David Blood Bank, Jaffa, Israel), antibiotics and 2 mM glutamine/ml of medium.

Con A preactivation (32). A suspension of lymphocytes at a concentration of $2-4 \times 10^6$ cells/ml of the medium mixture was cultured in two plastic tubes (Falcon No. 3033, Oxnard, CA). In the first, cells were incubated in the presence of $25 \mu\text{g/ml}$ of Con A (Miles-Yeda Laboratories, Rehovot, Israel), for 48 h at 37°C in humidified air with 5% CO_2 for the activation of suppressor cells. In the second (control) tube cells were incubated without Con A. Cells from both tubes were then washed 3 times with PBS and resuspended in RPMI medium containing 10% pooled human AB serum. They were counted and treated with $50 \mu\text{g/ml}$ mitomycin C (Sigma Chemicals, OH) for 30 min at 37°C , followed by four washes in PBS before resuspension in culture medium.

Suppressor induction in autologous co-cultures. Experiments were performed in microculture wells (Costar No. 4506, Cambridge, MA) in triplicate. One-tenth ml medium containing 0.5×10^5 2-day-old responder cells in fresh medium were co-cultured with 0.1 ml medium containing 0.5×10^5 autologous, Con A preactivated and mitomycin-C-treated "suppressor cells." The control consisted of 0.1 ml with 0.5×10^5 2-d-old cells co-cultured with 0.1 ml containing 0.5×10^5 autologous cells,

mitomycin-treated but not Con A-preactivated. These mixtures of cells were then stimulated by PHA or Con A. In the above experiments 48-h-old cells that were kept in culture medium at 37°C were used in order to avoid drawing blood from the newborns and a second one from the controls. We have found that 48-h-old responder cells yield similar results to that of fresh cells, and this has also been reported by Dwyer (11) and Feighery *et al.* (13).

Mitogens. We used PHA (Burroughs-Welcome Beckenham, England) and Con A (Miles-Yeda, Rehovot, Israel). We evaluated a concentration range of 0.125–2.5 µg/ml PHA. For Con A, the mitogenic dose of 5 µg/ml gave optimal suppression, and therefore was used throughout this study.

Suppression induction in allogeneic co-cultures. The assay is the same as described above, only the cells cultured with Con A and subsequently treated with mitomycin C were allogeneic to the responder cells. The responder cells in these experiments were fresh cells separated from blood of healthy donors age 1–10 yr.

Termination of cultures. Cultures were incubated for 72 h at 37°C in 5% CO₂ in air in a humid incubator, and were pulsed for the last 4 h with 2 Ci/well of [³H]thymidine (Nuclear Research Center-Negev, Israel). Cells were then harvested on filters with a cell harvester (Yeda, Rehovot, Israel) and incorporation of radioactive thymidine was measured in a liquid scintillation counter (Packard).

Calculation of suppression in cultures. Suppressor cell activity was calculated by the following formula (32):

$$\% \text{ suppression} = \left(1 - \frac{A}{B}\right) \times 100$$

where A is the proliferative response in counts per minute (cpm) of the culture to which Con A-activated lymphocytes were added, and B is the proliferative response of the culture to which control lymphocytes were added.

RESULTS

Suppression by Con A-activated autologous cells. Lymphocytes of both newborn and normal children exhibited the ability to suppress the autologous response to mitogens; however at all concentrations of both mitogens, lymphocytes from older children showed greater suppressor activity than those from newborns. A statistically significant difference was demonstrated only with the lowest concentration of PHA and with Con A (Table 1, A and B).

Suppression by Con A-activated allogeneic cells. Here too lymphocytes from normal children showed greater suppressor ability than those from newborns, with significance being found only with two lower concentrations of PHA (Table 1, C and D).

Comparison of autologous and allogeneic suppressor activity indicated greater suppression by autologous cells in both newborns and older children. The differences were significant in both groups (Table 1, A and C) for the lowest PHA concentration, as well as the intermediate PHA dosage in the newborn group (Table 1, B and D).

In the materno-fetal pairing experiments (Table 2) a definite pattern emerged, showing that newborns efficiently suppress their mother's lymphocyte reactivity, but the mothers' lymphocytes have almost no suppressor activity on mitogenic responses of their offsprings or of other newborn lymphocytes. The lymphocytes of these mothers suppress their own mitogenic responses. A single experiment is described in Table 3 (Newborn—Mother pair C) that gives data from which suppressor activity was calculated.

DISCUSSION

Evaluation of the activity of suppressor cell precursors by Con A preactivation of lymphocytes has been found to be a useful tool in clinical immunology (11, 16, 18, 25, 31). It has been shown that Con A preactivation leads to the induction of suppressor cell activity of *in vitro* proliferative mitogenic responses in healthy adult donors (18, 32), and that it is specifically T-cell in nature (30), requiring cell to cell contact (22). Our study took advantage of the fact that Con A activation enables the exploration of mutual suppressor activity between allogeneic cell populations. We demonstrated that lymphocytes from newborn infants and healthy children, when precultured with Con A are able to suppress both autologous and allogeneic proliferative cell responses to T mitogens; however, some interesting differences emerged between these two groups. First, autologous suppression was greater than the allogeneic one, and second, the suppressor activity of lymphocytes in normal children was generally somewhat greater than in lymphocytes of newborn. We also found that with increasing concentrations of PHA, suppressor activity decreased. It appears, therefore, that high concentrations of PHA may provide enough stimulation to overcome much of the suppressor activity.

Gupta *et al.* (14) showed that Con A pretreatment of lymphocytes enhances generation of T-cells as well as suppression of immunoglobulin production *in vitro*. Miyawaki *et al.* (24) demonstrated enhanced suppressor activity, residing in Fc-IgG-bearing

Table 1. Con A-induced suppressor activity in newborns and older children*

Cell combination	"Suppressor" cells from	Responding cells	Suppression† of mitogenic response (%) to:			
			PHA 0.125 µg/ml	PHA 0.25 µg/ml	PHA 2.5 µg/ml	Con A 5 µg/ml
A	Normal children	Autologous	75 ± 14.5 (n = 20)	58 ± 25 (n = 20)	27 ± 14 (n = 7)	53 ± 23 (n = 20)
B	Newborns	Autologous	65 ± 17.5 (n = 22)	53 ± 23 (n = 18)	16 ± 17 (n = 18)	34 ± 24 (n = 22)
C	Normal children	Allogeneic	61 ± 20 (n = 16)	46 ± 29 (n = 15)	17 ± 15.5 (n = 6)	36 ± 27 (n = 15)
D	Newborns	Allogeneic	44 ± 24 (n = 24)	24 ± 24 (n = 20)	11 ± 13 (n = 19)	23 ± 22 (n = 23)
<i>P</i> values (Student's <i>t</i> test)		A:B	=0.05	=0.5	=0.10	0.02
		C:D	=0.02	=0.01	=0.30	=0.10
		A:C	<0.05	<0.20	<0.3	=0.05
		B:D	<0.005	<0.005	...	=0.10

* *n*, number of cases; PHA, phytohemagglutinin; and Con A, concanavalin A.

† Data represent means ± 1 SD of percentage suppression of tritiated thymidine uptake of responding cells in the presence of Con A-activated mitomycin C-treated cells compared with the uptake of responding cells in the presence of only mitomycin C-treated (control) cells (for details see "Materials and Methods").

Table 2. Con A-induced suppressor activity* of newborn and mother lymphocytes in co-cultures

Con A suppressors	Responding cells	% Suppression of DNA synthesis in seven individual experiments							mean $\pm$ 1 SD
		A	B	C	D	E	F	G	
Newborn	His mother	53	45	23	45	49	53	50	45 $\pm$ 10
Newborn	Another newborn	9	11	0	0	3	25	13	9 $\pm$ 8
Newborn	His own	33	18	58	39	66	51	54	45 $\pm$ 16
Newborn	Child X	...	49	18	71	...	...	...	46 $\pm$ 26
Mother	Her newborn	0	0	0	22	12	7	8	7 $\pm$ 7
Mother	Another newborn	...	...	0	0	5	0	0	1 $\pm$ 2
Mother	Her own	63	...	...	0	57	37	50	41 $\pm$ 25
Mother	Young adult	...	...	23	...	...	...	41	32

* % suppression was calculated as in "Materials and Methods."

Table 3. Con A-induced-suppressor activity in a representative experiment (C)

Con A suppressors*	Responding cells from	Mitogenic response (cpm)	With PHA (%)
		0.25 μ g/ml	suppressions
Newborn C	—	His mother	41720
Newborn C	+	His mother	32366
Newborn C	—	Another newborn	96093
Newborn C	+	Another newborn	104740
Newborn C	—	His own	24667
Newborn C	+	His own	10423
Newborn C	—	Child X	95857
Newborn C	+	Child X	78683
Mother C	—	Her newborn	34700
Mother C	+	Her newborn	34946
Mother C	—	Another newborn	87743
Mother C	+	Another newborn	105920
Mother C	—	Young adult	62290
Mother C	+	Young adult	48160

* Responding cells were mixed either with non-activated control cells (—) or with Con A-preactivated cells (+).

ing cord blood T-cells, of PWM-driven immunoglobulin synthesis by adult cocultured lymphocytes. This suppressor capacity appeared to regress with age, in contrast to the system which we used, which presumably assays a T-T cell suppressor function, and not a T-B cell interaction. Oldstone *et al.* (28) concluded that the newborn IgG receptor-bearing suppressor T-cells inhibit mitosis and immunoglobulin production of lymphocytes from their mothers. In addition, they found that newborns effectively suppress the proliferative responses of lymphocytes from their mothers or even other nonpregnant females, suggesting that inhibition of maternal lymphocytic proliferation occurs via a soluble suppressor factor (26). Others have claimed that these infants also have spontaneously activated T subpopulations that inhibit B-cell maturation *in vitro* (10). Lawler *et al.* (20) demonstrated an inhibitory effect of newborn lymphocytes on division of maternal cells in one-way and two-way mixed lymphocyte culture reactions, but have excluded the possibility of serum factors being involved. A similar suppressive effect of cord blood T-cells on adult lymphocytes was also described (1). In an attempt to identify T-cell subsets in cord blood, using specific monoclonal anti-T antibodies (OK-T), Hayward and Kurnick (17) recently reported no quantitative increase in the suppressor cell subpopulation (OK-T₈) to match a decreased functional suppression of PWM-induced B-cell differentiation. On the other hand, Yachie *et al.* (37) demonstrated a decrease in OK-T₈ bearing cells as well as a "reversal" of the helper-suppressor functions in cord blood between T₄ and T₈ positive cells.

Unanue and Olding (36) demonstrated suppressor cell activity in hemopoietic cells of fetal liver cells as early as the 8th wk of gestational age, and at the 15th wk in peripheral blood cells.

Read *et al.* (29) claimed that the suppressor activity of the fetus decreases with increasing gestational age, thus being lowest at birth in full-term newborns and more pronounced in premature neonates. Another possible fetal suppressor mechanism may be mediated by alpha-feto protein, which has been implicated as an inhibitor of mitogenic response of maternal lymphocytes. It is believed that its selective effect on proliferative responses is mediated by suppressor cells (2). In other studies, humoral cord blood suppressor factors have been described (9, 26).

The results of the second part of our study, in which the fetomaternal immune relationship was investigated can be interpreted to corroborate the evidence for fetal suppressor activity. We demonstrated paralysis of maternal ability to develop suppressor activity on newborn lymphocytes, regardless of whether or not the offspring was her own or someone else's; and the paralysis was present in mothers with normal autologous maternal suppressor activity. Indeed, Stahn *et al.* (33) found a marked decrease in T-cell colony formation in pregnancy. These findings together with reported changes in T-cell numbers (35) and functional impairment in cell-mediated immunity (3), found during pregnancy, and the decrease in maternal alloreactivity to paternal and fetal cells at delivery (6) indicate a generalized depression of maternal thymus-dependent immunity. It seems that a delicate immune balance is established between the mother and her fetus—a balance dependent in part on their reciprocal histoincompatibility (4), which is essential for fetal survival as an allograft. An important factor in maintaining this balance probably resides in fetal capacity for inhibiting maternal T-effector and suppressor functions as demonstrated in the present study. Upsetting this balance could contribute to the emergence of a preeclamptic state (5) or result in early abortion. The advantage of the Con A activation method is to enable the study of mutual functional suppressor activity between different individuals.

REFERENCES AND NOTES

1. Abedin, M. and Kirkpatrick, C. H.: Immunosuppressive activity of cord blood leukocytes. *Pediatrics*, 66: 405 (1980).
2. Albert, E., Dienstag, J. L., Sepersky, S., Littman, B., and Rocklin, R.: Immunosuppressive characteristics of human AFP effect on tests of cell mediated immunity and induction of human suppressor cells. *Immunol. Commun.*, 7: 163 (1978).
3. Baines, M. G., Press, H. F., and Millar, K. G.: Lymphocyte populations in peripheral blood during normal human pregnancy. *Clin. Exp. Immunol.*, 28: 453 (1977).
4. Beer, A. E. and Billingham, R. E.: Immunoregulatory aspects of pregnancy. *Fed. Proc.*, 37: 2374 (1978).
5. Birkeland, S. A. and Kristofferson, K.: Pre-eclampsia: a state of mother-fetus immune imbalance. *Lancet*, 2: 720 (1979).
6. Birkeland, S. A. and Kristofferson, K.: The fetus as an allograft: a longitudinal study of normal human pregnancies studied with mixed lymphocyte cultures between mother-father and mother-child. *Scand. J. Immunol.*, 11: 311 (1980).
7. Boyum, A.: Ficoll-Hypaque method for separating mononuclear cells and granulocytes from human blood. *Scand. J. Clin. Lab. Invest.*, 21: (suppl.): 97 (1969).
8. Campbell, A. C., Waller, C., Wood, J., Aynsley-Green, A., and Yu, V.: Lymphocyte subpopulations in the blood of newborn infants. *Clin. Exp. Immunol.*, 18: 469 (1974).

9. Chardonnens, X. and Jeannet, M.: Immunobiology of pregnancy: evidence for a fetal immune response against the mother. *Tissue Antigens*, **15**: 401 (1980).
10. Durancy, A., Fischer, A., and Griscelli, C.: Active suppression of B lymphocytes maturation by two different newborn T lymphocyte subsets. 4th International Congress of Immunology (Paris) (abstract), 4.2.06 (1980).
11. Dwyer, J. M.: Failure of Concanavalin A-stimulated lymphocytes to suppress responsiveness to PHA and allogeneic cells as a marker of disordered immunoregulation. In: D. O. Lucas (Ed.) *Regulatory Mechanisms in Lymphocytes Activation*, pp. 773-775 (Academic Press, Tucson, 1977).
12. Eife, R. F., Eife, G., August, C. S., Kuhre, W. L., and Staehr-Johansen, K.: Lymphotoxin production and blast cell transformation by cord blood lymphocytes: dissociated lymphocytes function in newborn infants. *Cell. Immunol.*, **14**: 435 (1974).
13. Feighery, C., Wheelan, C. C., Weir, D. G., and Greally, J. F.: In vitro studies of suppressor cell function in human peripheral blood mononuclear cells. *Clin. Exp. Immunol.*, **32**: 459 (1978).
14. Gupta, S., Schwartz, S. A., and Good, R. A.: Subpopulation of human T lymphocytes. VII. Cellular basis of concanavalin A-induced T-cell mediated suppression of immunoglobulin production by B lymphocytes from normal humans. *Cell. Immunol.*, **44**: 242 (1978).
15. Handzel, Z. T., Levin, S., Dolphin, Z., Schlesinger, M., Hahn, T., Altman, Y., Schechter, B., Shneyour, A., and Trainin, N.: Immune competence of newborn lymphocytes. *Pediatrics*, **65**: 491 (1980).
16. Hallgren, H. M. and Yunis, E. J.: Suppressor lymphocytes in aged humans. *J. Immunol.*, **118**: 2004 (1977).
17. Hayward, A. R. and Kurnick, J.: Newborn T-cell suppression: early appearance, maintenance in culture and lack of growth factor suppression. *J. Immunol.*, **126**: 50 (1981).
18. Hubert, C., Delespesse, G., and Govaerts, A.: Concanavalin A-activated suppressor cell in normal human peripheral blood lymphocytes. *Clin. Exp. Immunol.*, **26**: 95 (1976).
19. Hynes, B. F. and Fauci, A. S.: Activation of human B lymphocytes. V. Kinetics and mechanisms of suppression of plaque-forming cell responses by concanavalin A-generated suppressor cells. *J. Immunol.*, **120**: 700 (1978).
20. Lawler, S. D., Ukaejiofo, E. O., and Reeves, B. R.: Interaction of maternal and neonatal cells in mixed lymphocyte cultures. *Lancet*, **2**: 1185 (1975).
21. Lomnitzer, R. and Rabson, A. R.: Suppression of leucocyte inhibitory factor (LIF) production and (³H) Thymidine incorporation by con A-activated mononuclear cells. *Cell. Immunol.*, **43**: 41 (1979).
22. Lomnitzer, R. and Rabson, A. R.: The mechanism of suppression of mitogen induced blastogenesis by con-A treated human blood mononuclear cells. 4th International Congress of Immunology (Paris) (abstract), 4.7.41 (1980).
23. Miller, M. E.: Host defences in the human neonate. *Pediatr. Clin. N. Am.*, **24**: 413 (1977).
24. Miyawaki, T., Seki, H., Kubo, M., and Taniguchi, N.: Suppressor activity of T-lymphocytes from infants, assessed by co-culture with unfractionated adult lymphocytes in the pokeweed mitogen system. *J. Immunol.*, **123**: 1092 (1979).
25. Newman, B., Blank, S., Lomnitzer, R., Disler, P., and Rabson, A. R.: Lack of suppressor cell activity in systemic lupus erythematosus. *Clin. Immunol. Immunopathol.*, **13**: 187 (1979).
26. Olding, L. B., Murgita, R. A., and Wigzell, H.: Mitogen stimulated lymphoid cells from human newborns suppress the proliferation of maternal lymphocytes across a cell impermeable membrane. *J. Immunol.*, **1109**: (1977).
27. Olding, L. B. and Oldstone, N. B. A.: Lymphocytes from human newborns abrogate mitosis of their mother's lymphocytes. *Nature*, **249**: 161 (1974).
28. Oldstone, B. A., Tishon, A., and Moretta, L.: Active thymus derived suppressor lymphocytes in human cord blood. *Nature*, **269**: 333 (1977).
29. Read, S. E., Dyson, A. E., and Kudo, J.: Suppressor lymphocyte activity in premature neonates. 4th International Congress of Immunology (Paris) (abstract), 4.7.51 (1980).
30. Sakane, T. and Gree, I.: Suppressor T-cells induced by concanavalin A: suppressor T-cells belong to distinctive T-cell subclasses. *J. Immunol.*, **119**: 1169 (1978).
31. Sakane, T., Steinberg, A. D., and Green, I.: Studies of immune functions of patients with systemic lupus erythematosus. I. dysfunction of suppressor T-cell activity related to impaired generation of rather than response to suppressor cells. *Arthritis Rheum.*, **21**(6): 657 (1978).
32. Shou, L., Schwartz, S. A., and Good, R. A.: Suppressor cell activity after concanavalin-A treatment of lymphocytes from normal donors. *J. Exp. Med.*, **143**: 1100 (1976).
33. Stahn, R., Fabricius, H. A., and Hartleitner, N.: Suppression of human T-cell colony formation during pregnancy. *Nature*, **276**: 831 (1978).
34. Stiehm, E. R., Winter, H. S., and Bryson, Y. J.: Cellular (T-cell) immunity in the human newborn. *Pediatrics*, **64**: 814 (1979).
35. Strelkauskas, J. A., Davis, I. J., and Dray, S.: Longitudinal studies showing alterations in the levels and functional response of T and B lymphocytes in human pregnancy. *Clin. Exp. Immunol.*, **32**: 53 (1978).
36. Unanue, A. M. and Olding, L. B.: Ontogeny and postnatal persistence of a strong suppressor activity in man. *J. Immunol.*, **27**: 1182 (1981).
37. Yachie, A., Miyawaki, T., Nagaoki, T., Yokoi, T., Mukai, M., Uwadana, N., and Taniguchi, N.: Regulation of B cell differentiation by T-cell subsets defined with monoclonal OKT₄ and OKT₈ antibodies in human cord blood. *J. Immunol.*, **131**(4): (1981).
38. Requests for reprints should be addressed to: Dr. Z. T. Handzel, Pediatric Research Institute of the Kaplan Hospital, Rehovot, Israel.
39. This study was supported in part by a grant from the Hebrew University—Hadassah Research Fund, Jerusalem.
40. Received for publication July 28, 1982.
41. Accepted for publication April 13, 1983.