Lymphocyte Reactivity in Pregnant Women and Newborn Infants

V. Y. H. YU, C. A. WALLER, I. C. M. MACLENNAN, J. D. BAUM

Summary
The mitotic response to phytohaemagglutinin (PHA) was determined in lymphocytes of mothers and their newborn infants obtained at delivery and seven days later by measuring the rate of $^{125}$I-idoxuridine uptake into DNA in lymphocytes cultured in their own plasma and after washing and resuspension in fetal bovine serum. There was no difference in the unstimulated counts of maternal lymphocytes taken at delivery, whether un-washed or washed, compared with those from non-pregnant controls. With PHA stimulation the mitotic response of the maternal lymphocytes cultured in their own plasma was reduced compared with that of the control lymphocytes but washed maternal cells showed a similar response to the controls. These findings suggest that the reduced lymphocyte mitotic response to PHA in pregnancy is due to a plasma inhibitory factor. This inhibition was not evident in maternal blood taken seven days after delivery.

DNA synthesis in unstimulated cultures from newborn infants at birth and seven days after birth was greater than that in adult control cultures. With PHA stimulation the mitotic response of cord-blood lymphocytes cultured in their own plasma paralleled that of control lymphocytes but washed newborn cells showed a greater response. Thus plasma suppression similar to that observed in the mother seems also to affect infants at birth. This inhibition was not demonstrable in blood taken from infants of 7 days.

Introduction
In most pregnant women the mitotic response of their lymphocytes to phytohaemagglutinin (PHA) is reduced (Purtilo et al., 1972), but the process by which this response, which seems to be predominantly the property of T cells, is reduced is uncertain (Finn et al., 1972). Divergent results have been obtained regarding the suppressive effect of serum from pregnant women on the PHA response of lymphocytes from normal healthy donors (Purtilo et al., 1972; Walker et al., 1972). A recent study indicated the presence of a serum inhibitor in pregnancy (St Hill et al., 1973) but its nature and origin is unknown. There are several conflicting reports on the response of cord-blood lymphocytes to PHA (Lindahl-Kiessling and Böök, 1964; Leikin et al., 1968; Meuwissen et al., 1968; Jones, 1969; Campbell et al., 1975).

We have studied the mitotic responses to PHA of the lymphocytes of mothers and their newborn infants, obtained at delivery and seven days later. To investigate the presence of an inhibitory plasma factor we also compared the PHA responses of lymphocytes cultured in the presence of autologous plasma with those after washing the cells and resuspending them in fetal bovine serum.

Patients and Methods
Collection of Blood Samples.—Blood samples were obtained by venepuncture from 24 mothers at delivery, and 24 samples of cord blood were collected from their newborn infants at the same time. In each case 5 ml of blood was anticoagulated with preservative-free heparin and an EDTA sample was taken for a white cell and differential count. All samples were cultured within 24 hours of collection and in most cases within 12 hours. With each batch of samples tested control samples of venous blood, taken from age-matched healthy non-pregnant women, were treated in the same manner. Blood samples were also obtained from 13 of the mothers and their infants seven days after delivery and were similarly treated.

Preparation of Cultures.—A whole-blood microtechnique similar to that described by Maini et al. (1973) was used in setting up the cultures. Tubes were set up in triplicate each containing 1 ml of minimal essential medium (M.E.M.) supplemented with glutamine, non-essential amino-acids, and antibiotics to which 0.1 ml of the blood sample was added. PHA (Wellcome, dried reagent, lot K6888) was added to a concentration of 1/100. Triplicate tubes were also set up without added PHA. Preparations of washed cells were made by first diluting 2 ml of the blood sample with 20 ml of M.E.M. under which about 4 ml of heat-inactivated fetal bovine serum (Biocult, batch 000239) was layered. The cells were then centrifuged through the layer of fetal bovine serum and the autologous plasma removed by sucking off the supernatant until 2 ml remained. After resuspension of the cells 0.1 ml was added to each culture tube and similarly treated as the unwashed cell cultures.

Assessment of Lymphocyte Response to PHA.—The cultures were incubated for 72 hours at 37°C. DNA synthesis was estimated by incorporation of $^{125}$I-idoxuridine (Craig et al., 1969). A stock solution of this reagent (Radiochemical Centre, Amersham) was made up with added cold deoxyuridine to a concentration 10 mg/ml and an activity of 20 mCi/l. From this solution 0.05 ml (1 μCi) was added to each tube three hours before the end of culture after taking off 0.5 ml of culture medium. At the end of the culture the cells were spun down and the supernatant containing the excess isotope decanted off. Red cells were lysed by washing.
Results
The average counts per minute of the triplicate cultures were plotted and expressed logarithmically. The mitotic response to PHA was derived by subtracting the count per minute in the unstimulated culture from that of the PHA-stimulated culture of the same sample. Statistical analyses were by Student’s t test for data between subject groups and the paired t test for un-washed and washed cell cultures in the same group. The results of all lymphocyte cultures are summarized in tables I and II.

There was no significant difference in unstimulated counts of non-pregnant adult controls, mothers at delivery, and mothers seven days after delivery, and similar unstimulated counts were obtained for both un-washed and washed cell cultures. The mitotic response to PHA of maternal lymphocytes, however, was, in contrast, higher than that of the lymphocytes of non-pregnant controls when the cells were cultured in the presence of their own plasma (P < 0.01). Washed cells, in contrast, had a similar mitotic response to that of non-pregnant control cells and the responses of the un-washed and washed cell cultures were significantly different (P < 0.001; fig. 1). Lymphocytes from mothers seven days after delivery had a mitotic response similar to the control response and there was no difference between un-washed and washed cell cultures.

DNA synthesis in unstimulated cultures of cells from newborn infants at birth and at 7 days of age was significantly greater than that in the cells of adult controls (P < 0.001). Similar unstimulated counts were obtained for both un-washed and washed cell cultures. Mitotic response to PHA of cord-blood lymphocytes paralleled that of adult control lymphocytes when the cells were cultured in the presence of their own plasma. Washed cells, however, had a greater mitotic response than adult control cells and the responses of the un-washed and washed cell cultures were significantly different (P < 0.001; fig. 2). Lymphocytes from 7-day-old infants had a greater mitotic response than adult control cells (P < 0.005) and there was no difference between un-washed and washed cell cultures.

Discussion
Lymphocyte response to PHA is an expression of a facet of T-cell immunity (Bloom, 1971) though some B cells may respond in some circumstances (Phillips and Weisrose, 1974). The maternal lymphocyte response to PHA may be depressed during pregnancy (Purtito et al., 1972; Finn et al., 1972), and our results indicate that this persists up to the time of delivery. Other evidence which indicates lowered maternal immunity includes impaired in-vitro lymphocyte response to purified protein derivative (Smith et al., 1972) and prolonged homo-

**TABLE I—Lymphocyte Response of Phytohaemagglutinin in Mothers and Children.** Results are expressed as Logarithmic Means ± S.D. Arithmetic Means are shown in Parenthesis

<table>
<thead>
<tr>
<th>Culture</th>
<th>Non-pregnant Controls</th>
<th>Mothers at Delivery</th>
<th>Mothers 7 Days after Delivery</th>
<th>Infants at Birth</th>
<th>Infants 7 Days after Birth</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 16)</td>
<td>(n = 24)</td>
<td>(n = 13)</td>
<td>(n = 24)</td>
<td>(n = 13)</td>
</tr>
<tr>
<td>Unstimulated (c.p.m.)</td>
<td>2.094 ± 0.24 (121.00)</td>
<td>2.080 ± 0.21 (120.00)</td>
<td>N.S.</td>
<td>2.011 ± 0.32 (102.00)</td>
<td>2.092 ± 0.30 (121.00)</td>
</tr>
<tr>
<td>PHA stimulated (c.p.m.)</td>
<td>2.95 ± 0.23 (26.930)</td>
<td>4.92 ± 0.26 (26.690)</td>
<td>N.S.</td>
<td>4.355 ± 0.41 (26.230)</td>
<td>4.82 ± 0.36 (26.460)</td>
</tr>
<tr>
<td>Mitotic response*</td>
<td>4.43 (27.100)</td>
<td>4.48 (27.700)</td>
<td>N.S.</td>
<td>4.43 ± 0.43 (21.440)</td>
<td>4.56 ± 0.36 (26.690)</td>
</tr>
</tbody>
</table>

* Mitotic response = stimulated – unstimulated c.p.m. per culture.

N.S. = Not significant—that is, P > 0.05.

**TABLE II—Lymphocyte Response of Phytohaemagglutinin: Statistical Comparison of Results Between Different Groups**

<table>
<thead>
<tr>
<th></th>
<th>Unstimulated Counts</th>
<th>Mitotic Response</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unwashed Cells</td>
<td>Washed Cells</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-pregnant Controls (1) v. Mothers at Delivery (2)</td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
<tr>
<td>Non-pregnant Controls (1) v. Mothers 7 Days After Delivery (3)</td>
<td>4.19, P &lt; 0.001</td>
<td>4.19, P &lt; 0.001</td>
</tr>
<tr>
<td>Non-pregnant Controls (1) v. Infants at Birth (4)</td>
<td>5.1, P &lt; 0.001</td>
<td>5.1, P &lt; 0.001</td>
</tr>
<tr>
<td>Non-pregnant Controls (1) v. Infants 7 Days After Birth (5)</td>
<td>5.1, P &lt; 0.005</td>
<td>5.1, P &lt; 0.005</td>
</tr>
</tbody>
</table>
Comparison of Aerosol Ipratropium Bromide and Salbutamol in Chronic Bronchitis and Asthma

G. R. PETRIE, K. N. V. PALMER

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Summary

The effects of inhaling 200 µg of salbutamol were compared with those of inhaling 40 µg of ipratropium bromide singly and in combination with salbutamol in eight patients with bronchitis and eight asthmatic patients in a double-blind controlled trial. Changes in airways resistance were assessed by measuring the forced expiratory volume in 1 second and specific airways conductance. Both drugs were significantly better in relieving airways obstruction than placebo. Salbutamol was significantly more effective than ipratropium bromide in patients with asthma, but in the patients with bronchitis there was no significant difference between salbutamol and ipratropium bromide. The combination of the two drugs produced a slightly greater and longer response than either drug alone but this was not significant.

References