Lymphocytosis and Histamine Sensitization of Mice by Fractions from Bordetella pertussis

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Administration of Bordetella pertussis cell extracts induced in mice hypersensitivity to histamine, as well as pronounced leukocytosis and hypoglycemia. The leukocytosis was mainly caused by an increase in the small lymphocytes in the circulating blood, and it was most pronounced 3 to 4 days after injection of B. pertussis extracts. Rabbit antimouse lymphocyte serum produced a decrease in the lymphocyte count in normal mice, as well as in mice treated with B. pertussis extracts. This depression in lymphocytes was observed whether the antilymphocyte serum was given 1 day or 2 days after the administration of B. pertussis extracts. The increased histamine sensitivity and hypoglycemia of mice treated with B. pertussis extract were not affected by treatment with antilymphocyte serum, although a marked lymphopenia was present. These observations indicate that the three phenomena observed in pertussis-treated mice are independent of each other.

Administration of Bordetella pertussis cells or cell extracts induces a variety of physiological changes in mice. The animals become more sensitive to the deleterious effects of histamine, serotonin, anaphylaxis, endotoxin, and other agents (3, 5). In addition, mice develop hypoglycemia, hypoalbuminemia, and marked lymphocytosis (5). Sensitivity to histamine, serotonin, and endotoxin increases to a peak by the 3rd or 4th day after treatment with B. pertussis. Similarly, lymphocytosis (4) and hypoglycemia (7) reach a peak at that time. All these phenomena are produced by a heat-labile substance of the B. pertussis cell.

In this paper, the interrelationship among lymphocytosis, hypoglycemia, and histamine sensitivity is examined.

MATERIALS AND METHODS

Soluble saline extract (SE) of B. pertussis cells was prepared as previously described (6).

Female CFW mice weighing 20 to 22 g each, purchased from Carworth Farms, were employed. Total white blood cell counts and differential counts were performed by standard procedures, with blood obtained by clipping the tail a few millimeters from the distal end. Blood for glucose determination was obtained from the infraorbital sinus by means of a capillary pipette.

Antilymphocyte serum (ALS) was prepared in rabbits immunized with two weekly subcutaneous injections of a suspension of 10⁶ mouse lymph node cells emulsified in complete Freund’s adjuvant. The rabbits were bled 2 weeks following the last inoculation. Intrapertoneal injection of 0.2 ml of ALS into normal CFW mice produced a pronounced lymphopenia within 4 hr after administration. The lymphocyte count returned to normal in 72 hr (Table 1).

Glucose determinations were performed by the glucose oxidase method utilizing Glucostat (Worthington Biochemical Corp., Freehold, N.J.).

Sensitization of mice to histamine was accomplished by intravenous injection of 50 µg of SE dissolved in 0.15 M saline.

Histamine challenge was performed by giving 0.2 mg of histamine (expressed as free base) as the diphasphate salt intraperitoneally to each mouse. Since most deaths occurred within 30 min after challenge, the final results were recorded 4 hr after challenge.

RESULTS

A group of mice was given SE, and the number of white blood cells and lymphocytes was determined daily for 5 days. The average values for each day are given in Table 2. On day 0, before injection of SE, the white blood cell count was 17,095 and the lymphocyte count was 13,064 per mm³ of blood. These numbers increased sharply 1 day after SE treatment and reached a peak on the 3rd day when the total white blood cell count was 110,110 and the lymphocyte count 75,648 per mm³ of blood. By the 5th day,
TABLE 1. Effect of ALS on the blood count of CFW mice

<table>
<thead>
<tr>
<th>Time after serum treatment</th>
<th>No. of mice</th>
<th>Normal rabbit serum</th>
<th>ALS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Avg no. of WBC/mm³ (range)</td>
<td>Avg no. of lymphocytes/mm³ (range)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>11,734 (4,554-18,661)</td>
<td>5,613 (2,860-7,920)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>18,794 (13,213-24,501)</td>
<td>6,584 (2,750-10,340)</td>
</tr>
<tr>
<td>4 hr</td>
<td>24</td>
<td>19,794 (11,176-22,028)</td>
<td>9,567 (7,480-11,650)</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>21,405 (18,684-25,410)</td>
<td>17,818 (10,010-23,650)</td>
</tr>
</tbody>
</table>

a White blood cell (WBC) and lymphocyte counts were performed on blood from individual mice at various time intervals after intraperitoneal administration of 0.2 ml of either normal rabbit serum or of rabbit ALS.

TABLE 2. Effect of saline extract of B. pertussis on the blood count of CFW mice

<table>
<thead>
<tr>
<th>Day</th>
<th>No. of mice</th>
<th>Avg no. of WBC/mm³ (range)</th>
<th>Avg no. of lymphocytes/mm³ (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>12</td>
<td>17,095 (8,250-30,140)</td>
<td>13,064 (5,690-23,200)</td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>66,990 (55,770-80,850)</td>
<td>47,435 (37,924-57,404)</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>91,410 (81,180-105,600)</td>
<td>65,778 (60,340-71,300)</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>110,110 (92,730-119,790)</td>
<td>75,648 (62,129-95,832)</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>57,970 (52,360-63,580)</td>
<td>40,332 (38,784-41,880)</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>54,490 (50,490-58,190)</td>
<td>34,718 (29,284-40,151)</td>
</tr>
</tbody>
</table>

a WBC and lymphocyte counts performed daily on blood from different mice after intravenous administration of 50 μg of saline extract.

the cell count dropped to about one-half of its peak value. These results confirmed the observations of Morse (4), who employed whole-cell pertussis vaccine as the lymphocyte-stimulating factor. All mice sensitized with a similar dose of SE and challenged 3 days later with 0.2 mg of histamine died, whereas all normal controls survived this challenge.

Since ALS has a cytotoxic effect on mouse lymphocytes in vivo and in vitro (2), the effect of ALS on the lymphocytosis and histamine sensitivity of mice treated with SE was studied. ALS and SE administered a few hours apart produced a toxic reaction resulting in death of 30% of the mice. Therefore, CFW mice were inoculated intraperitoneally with 0.2 ml of ALS or normal rabbit serum 1 or 2 days after sensitization with SE. Total white blood cell counts and absolute lymphocyte counts were determined for 3 days and were compared to counts obtained from mice treated only with SE. ALS sharply reduced the white blood cell count in mice treated with SE (Fig. 1), as it did that of normal mice (Table 1). Figure 1 shows that the white blood cell count 1 day after SE treatment was approximately 70,000 cells per mm³. Injection of ALS at this time depressed the total count to 7,000 cells per mm³ in 24 hr (subgroup I). The total count approached 100,000 cells per mm³ 2 days after SE treatment. Administration of ALS at this time also lowered the blood count to the same level (7,000 per mm³) in 24 hr (subgroup II). The total number of white blood cells in SE-treated mice receiving normal rabbit serum was not significantly different from that of SE-treated control mice. The marked alteration of total count was primarily caused by changes in lymphocyte population.

To determine the effect of ALS on histamine sensitivity, mice were inoculated intravenously with 0.2 ml of saline or 0.2 ml of saline containing 50 μg of SE. Two days later one group of control mice was given normal rabbit serum and another group ALS. Similarly, one group of SE-treated mice was injected with normal rabbit serum and another group with ALS. On the 3rd day of the experiment, all mice received 0.2 mg of histamine base intraperitoneally. None of the mice in the two saline control groups but all the SE-treated mice died (Table 3). These results indicate that an increased sensitivity to histamine exists despite the absence of leukocytosis.

Another similarly conducted experiment in which the blood glucose of mice was determined showed (Table 4) that ALS or normal rabbit serum had no effect on the hypoglycemia produced by SE.

**DISCUSSION**

The results indicate that the histamine sensitization and hypoglycemia observed in SE-treated
mice do not depend on the presence of leukocytosis. Other investigators (C. Fishel, personal communications), as well as one of us (R. K. Bergman, unpublished data), have noticed that the hypoglycemia per se is not responsible for histamine sensitization. Thus, it appears that histamine sensitivity, lymphocytosis, and hypoglycemia are produced independently, although possibly by a similar active principle in the B. pertussis cell. The ability to produce lymphocytosis and increased histamine sensitivity is found in the purest preparations of the histamine-sensitizing factor (HSF) that we have thus far obtained. From these observations, it seems possible that HSF produces many physiological changes in the mouse. Fishel et al. (1) have postulated that HSF acts by interfering with the function of the hormone epinephrine. This hormone is known to have profound effects on many physiological functions of the animal (8). Whether HSF as found in SE produces only one fundamental alteration responsible for the many physiological changes is not known now, but we believe that this may be the case.

**LITERATURE CITED**


![Graph showing effect of ALS on white cell and lymphocyte count of saline extract-treated mice](image-url)