THE EFFECT OF BACTERIAL ADJUVANTS ON TRYPANOSOMA LEWISI INFECTIONS IN RATS

I. R. TIZARD and C. P. RINGLEBERG

Department of Veterinary Microbiology and Immunology and University of Guelph, Guelph, Ontario

Abstract. The effect of Escherichia coli endotoxin, B.C.G. vaccine and Bordetella pertussis vaccine on the parasitemia of Trypanosoma lewisi was studied in rats. It was found that in general, B. pertussis administration resulted in higher and more prolonged parasitemias than normal. In contrast E. coli endotoxin tended to reduce both the intensity and duration of parasitemia. Possible reasons for this phenomenon are discussed.

Experiments were undertaken to determine whether bacterial products commonly employed as immunological adjuvants could influence the parasitemia in rats infected with Trypanosoma lewisi.

MATERIALS AND METHODS

T. lewisi (donated by Dr. T. J. Styles, Schenectady, N.Y.) maintained by rapid sequential passage in rats, was obtained at peak parasitemia by cardiac puncture. The adjuvants employed were B.C.G. vaccine* (0.1 mg organisms i.m.); Pertussis vaccine* (1.5 x 10^6 dead Bordetella pertussis s.c.), and E. coli endotoxin (50 μg s.c.**). The design of the experiment was a 3 x 4 factorial; three groups each of twenty rats were infected with T. lewisi. One group received adjuvant simultaneously with infection. One group received adjuvant one week prior to infection and the remaining group received both adjuvant and 1 x 10^6 dead trypanosomes one week prior to infection. Five additional rats were included in this latter group to serve as a control on the influence of dead trypanosomes administered alone. Each of these groups was subdivided into four subgroups each of five animals. Three subgroups each received a different adjuvant. The remaining five animals served as a control. Animals were bled daily from the tail and trypanosomes counted in a Neubauer Hemocytometer after dilution in Hayen's solution. Results were subjected to one-way analyses of variance at days 6, 8, 10, 12 and 15.

RESULTS AND DISCUSSION

The results of these experiments, (summarised in Table 1) indicated that, in general, E. coli endotoxin could reduce the parasitemia of T. lewisi while B. pertussis treatment resulted in significantly higher parasite levels than in control animals.

*) Connaught Laboratories, Toronto, Ontario.

**) Obtained from E. coli 045 by saline extraction and T.C.A. precipitation and purified by gelfiltration. Kindly donated by Dr.R.B. Truscott
Table 1. The effect of bacterial adjuvants on the *T. lewisi* parasitemia of groups of 5 rats

<table>
<thead>
<tr>
<th>Mean parasite numbers / mm³ blood</th>
<th>Adjuvant</th>
<th>Day</th>
<th>6</th>
<th>8</th>
<th>10</th>
<th>12</th>
<th>15</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>62.8</td>
<td>69.0</td>
<td>28.0</td>
<td>22.0</td>
<td>27.0</td>
</tr>
<tr>
<td>Adjuvant given simultaneously</td>
<td>A</td>
<td></td>
<td>47.0*)</td>
<td>73.0</td>
<td>109.0</td>
<td>148.0*)</td>
<td>86.0</td>
</tr>
<tr>
<td>with infection</td>
<td>B</td>
<td></td>
<td>29.8*)</td>
<td>53.0</td>
<td>36.4</td>
<td>45.2</td>
<td>84.0</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td></td>
<td>44.4*)</td>
<td>48.4*)</td>
<td>32.6</td>
<td>10.2*)</td>
<td>3.2*)</td>
</tr>
<tr>
<td>Adjuvant given 1 week prior to</td>
<td>A</td>
<td></td>
<td>114.0</td>
<td>124.6</td>
<td>87.4</td>
<td>64.4</td>
<td>50.8</td>
</tr>
<tr>
<td>infection</td>
<td>B</td>
<td></td>
<td>56.4*)</td>
<td>88.2</td>
<td>79.0</td>
<td>58.6</td>
<td>31.8</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td></td>
<td>104.2</td>
<td>102.0</td>
<td>80.5</td>
<td>45.5</td>
<td>5.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>253.2*)</td>
<td>188.4*)</td>
<td>237.0*)</td>
<td>221.6*)</td>
<td>220.6*)</td>
</tr>
<tr>
<td>Adjuvant</td>
<td>10⁶</td>
<td>A</td>
<td>95.6</td>
<td>41.2</td>
<td>41.0</td>
<td>10.0</td>
<td>0.0</td>
</tr>
<tr>
<td>dead organisms</td>
<td>B</td>
<td></td>
<td>161.0*)</td>
<td>90.4</td>
<td>65.0</td>
<td>49.6</td>
<td>40.0</td>
</tr>
<tr>
<td>given 1 wk prior to infection</td>
<td>C</td>
<td></td>
<td>(P &lt; 0.01).</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>to infection</td>
<td>D</td>
<td></td>
<td>(P &lt; 0.01).</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control level</td>
<td>E</td>
<td></td>
<td>89.0</td>
<td>98.8</td>
<td>83.5</td>
<td>57.2</td>
<td>32.5</td>
</tr>
</tbody>
</table>


*) Results marked differ significantly from the control level E (P < 0.01).

Specifically, of the adjuvants administered simultaneously with infection, *B. pertussis* vaccine resulted in higher mean parasite counts than in the control group. B.C.G. infected and *E. coli* endotoxin treated animals while having lower parasite counts did not differ significantly from normal. When adjuvants were administered one week prior to infection, a similar effect was noticed. The parasitemia in endotoxin treated animals being statistically significantly different from controls at 6, 8, 12 and 15 days.

Administration of 1 × 10⁶ frozen and thawed organisms together with adjuvant one week prior to infection resulted in highly significant changes in the blood parasite levels. This was most marked in that group of rats which received *B. pertussis* together with dead organisms, and resulted in a massive prolonged biphasic parasitemia and a delayed decline in parasite numbers which did not commence until 15 days after onset of infection. This was statistically different (P < 0.01) from both the control group which had received no treatment and the group which had received dead trypanosomes only. Rats which had received dead parasites alone also exhibited a significantly higher parasitemia than the control group, but only around 6 days after onset of infection.

Neither B.C.G. nor *E. coli* endotoxin caused a significant change in peak parasite levels when administered with dead trypanosomes although the parasitemia in B.C.G. infected rats was relatively transient, being terminated 10 days post-infection.

Three significant observations arise from these experiments with respect to *T. lewisi* infection. These are that *E. coli* endotoxin may serve to reduce the parasitemia, *B. pertussis* vaccine may enhance the parasitemia and that prior inoculation with dead trypanosomes may also result in higher parasite levels.

It has been shown previously by Styles (1965) that *E. coli* endotoxin could depress the parasitemia of *T. lewisi* in rats. This was especially the case when administered in small serial doses. However it was reported that a single dose given prior to or at the time of infection could cause higher parasite levels. Since endotoxins have several
biological activities it is difficult to ascribe the results reported here to any one of them. The important activities which may contribute to the effect on parasite levels include reticuloendothelial stimulation as well as stimulation both of antibody production and of preformed antibody release (Hill and Rowley 1967). Since these rats had never been exposed to T. lewisi previously and the low parasitemias were observed before antibody production could be considered to reach significant proportions, it is probable that reticuloendothelial stimulation accounted for the observed effect, at least in part. It is also possible that endotoxin exerts a direct toxic effect on T. lewisi.

Ever since Lewis and Loomis (1924) showed that tuberculous guinea pigs produced higher antibody levels than normal animals, acid fast bacteria have been employed as adjuvants. In general these organisms are capable of stimulating the antibody response, especially reaginic antibody (Lipton et al. 1956) and of enhancing the production of delayed type hypersensitivity. However cell mediated immunity is not a significant factor in protection against T. lewisi (Tizard, Styles and Holmes, unpublished observations). Mycobacteria are capable of producing a state of acquired immunity in macrophages (Coppel and Youmans 1969) and it is possible that reticuloendothelial stimulation may account for the slightly depressed parasitemia B.C.G. induces in T. lewisi infection.

That deleterious results can arise from the use of material which stimulates the immune response is very obvious from the observation that B. pertussis vaccine can enhance the parasitemia of T. lewisi. B. pertussis does however possess a number of biological properties which may influence trypanosome infections. It releases a histamine sensitising factor (Munoz 1964), has an adjuvant effect through its endotoxin (Farthing and Holt 1962), and specifically stimulates reaginic antibody formation in rats (Mota 1963).

It is difficult to hypothesise how any of these activities may result in increased parasite levels in normal rats. However the administration of $1 \times 10^{22}$ dead B. pertussis may result in at least a temporary reticuloendothelial blockade. Two other hypotheses may also be suggested to account for higher than normal parasitemias. One is that B. pertussis specifically stimulates reaginic antibody in rats and it is possible that this antibody is particularly ineffective in clearing T. lewisi. An alternative hypothesis is that a “blocking” antibody may be produced which reduces the ability of infected animals to clear the organism. These blocking factors have been demonstrated to exist in T. brucei infection in rabbits (Woo, P.T.K., personal communication) and may also account for the lack of protection afforded by dead trypanosomes as well as the synergistic effect of dead trypanosomes.

ВОЗДЕЙСТВИЕ БАКТЕРИАЛЬНЫХ СТИМУЛЯТОРОВ
НА ЗАБОЛЕВАНИЯ, ЗВЫВАЕМЫЕ TRYPANOSOMA LEWISI У КРЫС

И. Р. Тизард и С. И. Ринглберг

Резюме. Авторы изучали воздействие эндотоксина Escherichia coli, вакцины В. С. Г. и Bordetella pertussis на паразитемию Trypanosoma lewisi у крыс. В общем установлено, что инъекции B. pertussis в результате вызывали более высокую и длительную паразитемию чем нормально. А наоборот, эндотоксин E. coli способствовал понижению как интенсивности так длительности паразитемии. В работе обсуждаются возможные причины этого явления.
REFERENCES


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I.R.T., Dept. of Veterinary Microbiology and Immunology, University of Guelph, Guelph, Ontario, Canada