EFFECTS OF HYPOBARIC HYPEROXIA ON THE DEVELOPMENT AND ACTIVITY OF IMMUNE SPLEEN CELLS

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The purpose of this study was to evaluate the effect of intermittent exposure to hypobaric hyperoxic conditions (380 mm Hg and 100% O₂) on cellular aspects of resistance to an infectious disease. Mice were immunized against Francisella tularensis while being exposed to the test environment. Spleen cells from these immunized-exposed donors were transferred to nonexposed recipients that were maintained in a normal ground-level environment. These recipient mice were then challenged with F. tularensis to compare their resistance with that of control animals receiving spleen cells from immunized donors which had not been exposed to the test environment. The reverse was also accomplished by immunizing groups of donor mice - maintained at ground level and challenging the recipients (of their spleen cells) that were exposed to the test environment. The results indicate that exposure of the donor animals to hypobaric hyperoxia did not affect the development of cellular immunity; but, in exposed recipients, the activity of immune cells was impaired.
Effects of Hypobaric Hyperoxia on the Development and Activity of Immune Spleen Cells

TONY D. DAVID and JEROME P. SCHMIDT


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PUBLISHED REPORTS concerning the effects of exposure to modified environments on host resistance to infectious disease are difficult to interpret and evaluate because of the variations in test conditions and the numerous infectious agents employed by different investigators. Extrapolation of results has been further hindered by the complexity of host-parasite relationships and the lack of knowledge concerning specific cellular defense mechanisms. Although the exact mechanisms involved are not well-defined, it is quite evident that modified barometric pressures and gaseous atmospheres do alter host resistance to various pathogenic microorganisms.² ¹² ¹⁴ The present study was designed to evaluate environmental effects on cellular immunity. Tularemia in the mouse was selected as the model because resistance to that disease in this host is primarily dependent upon phagocytic cells.¹

MATERIALS AND METHODS

Environmental Test Conditions: The hypobaric hyperoxic test environment (380 mm Hg and 100% O₂) was maintained in an altitude chamber having an interconnected airlock and work space. The temperature was constantly controlled at 20°C, and the relative humidity ranged from 40% to 60%. The mice were otherwise maintained in an adjacent well-ventilated animal room with a constant temperature of 20°C.

Experimental Groups: In each of our two studies, animals were separated into four groups, designated respectively as A-A, A-G, G-A, and G-G. The “A” signified exposure to the test environment (hereafter referred to as “altitude”) 4 hrs per day for 14 consecutive days; and “G” represented ambient ground conditions. In one study (Table I), donor mice were separated into the four groups for immunization against tularemia. Group A-A animals were subjected to altitude for 14 days and then given their first immunization. After 14 more days of altitude conditioning, they were given their second immunizing dose. Group A-G animals were acclimatized similarly, but were retained at ground level after the 14-day period. Group G-A was a reversal of group A-G, and group G-G was maintained at ground level throughout the immunization period. To allow time for maximal response to the second injection, exposure to altitude (groups A-A and G-A) or ground level (groups A-G and G-G) was continued for 10 more days before the animals were sacrificed and their spleens harvested. Spleen cells from these four groups of immunized donors were transferred to recipient animals which were maintained at normal ground conditions. The four groups of recipient animals were identified according to the environmental conditioning of their donor animals, and were challenged 24 hrs after the cell transfer. Each group was composed of 20 mice and these were separated into two cages of 10 mice each.

The same procedure was used in the other study (Table II) except that the recipients, instead of the
TABLE I. THE EFFECTS OF HYPOBARIC HYPEROXIA ON DEVELOPMENT OF IMMUNE CELLS.

<table>
<thead>
<tr>
<th>Exposure schedule of donors**</th>
<th>Challenge of ground-level recipients**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groups</td>
<td>Before first immunization (14 days)</td>
</tr>
<tr>
<td>A-A altitude</td>
<td>altitude</td>
</tr>
<tr>
<td>A-G altitude</td>
<td>ground level</td>
</tr>
<tr>
<td>G-A ground level</td>
<td>altitude</td>
</tr>
<tr>
<td>G-G ground level</td>
<td>ground level</td>
</tr>
<tr>
<td>Pooled standard deviation</td>
<td>31.1</td>
</tr>
</tbody>
</table>

*Differs from group A-A p < 0.1
**Differs from group A-G p < 0.05
***Differs from group A-G p < 0.01
††Differs from group G-G p < 0.005
†††Differs from group G-G p < 0.0005

TABLE II. THE EFFECTS OF HYPOBARIC HYPEROXIA ON THE EXPRESSION OF CELLULAR IMMUNITY.

<table>
<thead>
<tr>
<th>Exposure schedule of recipients*</th>
<th>Mortality Dead/total</th>
<th>Mean survival time (Days)</th>
<th>Survival Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groups</td>
<td>Before challenge (14 days)</td>
<td>After challenge (14 days)</td>
<td>Percent</td>
</tr>
<tr>
<td>A-A altitude</td>
<td>altitude</td>
<td>altitude</td>
<td>30/30</td>
</tr>
<tr>
<td>A-G altitude</td>
<td>ground level</td>
<td>ground level</td>
<td>30/30</td>
</tr>
<tr>
<td>G-A ground level</td>
<td>altitude</td>
<td>altitude</td>
<td>30/30</td>
</tr>
<tr>
<td>G-G ground level</td>
<td>ground level</td>
<td>ground level</td>
<td>19/20</td>
</tr>
<tr>
<td>Pooled standard deviation</td>
<td>2.7</td>
<td>0.48</td>
<td>1.5</td>
</tr>
</tbody>
</table>

*Spleen cells from donors maintained at ground level were transferred to recipients 24 hrs prior to challenge.
††Differs from group A-A p < 0.1
†††Differs from group A-G p < 0.05
**Differs from group A-G p < 0.01
†††Differs from group G-G p < 0.005

Donor mice, 8 wks of age or older, were immunized by infection with the LVS strain. Immunization consisted of an intraperitoneal injection of 175 CFU and, 14 days later, a booster dose of 10,000 CFU. In preliminary work less than 10 CFU of the LVS strain proved to be as lethal as 1,000 CFU. Although shorter survival time was associated with the higher dosage, the end mortality rate was not dose-dependent. To prevent mortality among donor animals, they were treated with 4·40 µg of streptomycin subcutaneously for 3 consecutive days, beginning 2 days after each immunizing dose.

Donor animals were anesthetized with methoxyflurane and killed by exsanguination. Siliconized glassware was used throughout the preparation of the cells for transfer. The spleens were aseptically removed and placed in petri dishes containing cold Hanks' Balanced Salt Solution (BSS), pH 7.2-7.4, with 100 µg of dihydrostreptomycin and 100 units of penicillin/ml. Spleens from eight to 10 mice were pooled for processing to yield approximately 10⁸ cells/ml of the final dilution to transfer to recipient animals. Suspensions were prepared by cutting each spleen in five or six pieces and then expressing the splenic pulp in a loose-fitting tissue homogenizer with 5 ml of BSS. This preparation was filtered through a 250-mesh stainless-steel sieve into a beaker containing an additional 5 ml of BSS. The cells were then sediment-
Splenocytes had been most affected. The resistance of mice receiving immune spleen cells from exposed donors was not different from that of recipients of cells from nonexposed donors. Therefore, the development of cellular resistance was probably not affected. On the other hand, exposure to the chamber environment did reduce the survival index of animals receiving immune spleen cells derived from nonexposed donors. Hence it can be inferred that the in vivo protective activity of immune cells was impaired in the exposed mice, or that the hypobaric hyperoxia caused the recipients to be less capable of deriving passive protection from transferred spleen cells. The exact mechanisms involved were not defined, but cellular resistance appears to have been affected by the environment of the host.

ACKNOWLEDGEMENTS

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REFERENCES