Infection and Immunoglobulin Concentrations in Chediak-Higashi Mice

RONALD J. ELIN, JOSEPH B. EDELIN, AND SHELDON M. WOLFF
National Institute of Allergy and Infectious Diseases, Laboratory of Clinical Investigation, Bethesda, Maryland 20014

Received for publication 4 March 1974

The Chediak-Higashi syndrome (CHS) has been reported in man, cattle, mink, and mice. CHS humans and cattle have an increased incidence of pyogenic infections, whereas CHS mink are more susceptible to Aleutian disease. Age- and sex-matched groups of CHS mice (mutant strain) and C57 Bl/6N (parent strain) were challenged with Candida albicans, Escherichia coli, Klebsiella pneumoniae, and Staphylococcus aureus intravenously and Streptococcus pneumoniae intraperitoneally. A significant increase ($P < 0.001$) in mortality rate for the CHS mice was demonstrated with all five challenge organisms. Heterozygous mice challenged with C. albicans had a mortality rate essentially the same as C57 Bl/6N mice. The serum immunoglobulin concentrations of CHS mice were found to be the same or greater than the control or heterozygous mice. CHS mice have an increased susceptibility to pyogenic infections, which is not due to immunoglobulin deficiency. These mice may provide a useful laboratory model for the study of increased susceptibility to infection.

The Chediak-Higashi syndrome (CHS) is a rare autosomal recessive disease first described in man in 1943 (1). A similar syndrome has been described in mink (6), cattle (8), and mice (7). The primary characteristics of this syndrome are pigmented dilution (partial oculocutaneous albinism), frequent and severe pyogenic infections (man, mink, and cattle), and giant lysosomal granules that can be demonstrated in all granule-containing cells (2). The fundamental defect which leads to an increased susceptibility to pathogenic microorganisms has not been delineated. However, studies in humans have demonstrated defects in leukocyte regulation, structure, and function that may contribute to increased susceptibility to bacterial infections (12). Thus far, the responses of CHS mice to challenge with microorganisms have not been reported. Therefore, the following experiments were done to evaluate the susceptibility of CHS mice to infectious agents and to detect a possible impairment of humoral immunity by measuring serum immunoglobulin (Ig) concentrations.

MATERIALS AND METHODS

Animals. All mice were bred at the National Institutes of Health, Bethesda, Md. The control mice were C57 Bl/6N (parent strain), and CHS "beige" mice were the mutant strain (bg). These two strains of mice are readily separated by their different coat colors. The F1 hybrid of these two strains produced the heterozygous mice (C57 Bl/6N-bg). The mice weighed from 20 to 24 g and were approximately 16 weeks old when studied. All mice were fed Purina Lab Chow and water ad libitum.

Infection. The inoculum of Candida albicans strain B311 was prepared from organisms grown in Sabouraud broth for 18 h at 37 °C with constant agitation. The yeast cells were harvested and washed three times with sterile, 0.85% phosphate-buffered saline at pH 7.4. After direct counting with a hemocytometer, the inoculum was adjusted to a concentration of $10^8$ organisms per 0.2 ml with phosphate-buffered saline. Each animal was injected intravenously with $10^8$ organisms in the lateral tail vein. In addition, serial 10-fold dilutions of the inoculum were plated on Sabouraud agar to determine viable organisms. A close agreement between the hemocytometer count and colony count was found.

Streptococcus pneumoniae type 25 (ATCC 6325) were stored in rabbit blood under Vaseline petroleum jelly at −20 °C until subcultured in Todd-Hewitt broth at 37 °C for 20 h. The bacteria were washed twice with modified Hank solution, their concentration was estimated by turbidity using a Coleman Junior spectrophotometer, and they were diluted with modified Hank solution to a concentration of $10^8$ organisms per ml. Each animal was injected intraperitoneally with $10^8$ organisms. The number of viable organisms in the inoculum was determined by serial 10-fold dilutions and growth on Columbia agar with 10% sheep red blood cells.

Coagulase-positive S. aureus (ATCC 14154), Escherichia coli (ATCC 12014), and Klebsiella...
pneumoniae (ATCC 8045) were stored at room temperature on semisolid agar slants and subcultured in Trypticase soy broth at 37 C for 18 h. After the cultures were washed twice with modified Hank solution, the concentration of organisms was estimated by turbidity using a spectrophotometer and diluted to \( 5 \times 10^{3} \) organisms per 0.2 ml for S. aureus, 2 \( \times 10^{3} \) organisms per 0.2 ml for E. coli, and 2.5 \( \times 10^{3} \) organisms per 0.2 ml for K. pneumoniae. Each mouse was injected intravenously with 0.2 ml of the inoculum. The viable number of organisms in the inocula was determined by serial 10-fold dilution and growth on Trypticase soy agar.

**Experimental design.** For each study, all mice were inoculated with the same concentration of organisms at the same time. Mice were observed at 8- or 12-h intervals and the number of surviving mice was tabulated. Deaths within the first 4 h were considered to be due to technical causes, and these mice were excluded from the study. The study with each micro-organism was repeated one or two times with consistent results. The mortality rates were compared statistically for significant differences using the Wilcoxon test (11).

**Ig concentrations.** Healthy, uninfected mice were exsanguinated by transection of the axillary artery and vein. The blood was allowed to clot and the serum was separated by centrifugation. All sera were stored at \(-20 \) C prior to Ig determination. The IgG1, IgG2, IgA, and IgM concentrations were determined on individual mouse serum samples by radial immunodiffusion (4). The concentrations were expressed as the mean ± the standard error of the mean, and groups were compared using the Student's t test.

**RESULTS**

The CHS mice challenged with C. albicans, S. pneumoniae, K. pneumoniae, S. aureus, and E. coli showed a significant increase \((P < 0.001)\) in mortality rate compared with age- and sex-matched control mice (Fig. 1-6). The C57 Bl/6N and heterozygous mice challenged with C. albicans had essentially the same mortality rates (Fig. 1). With C. albicans, S. pneumoniae, and K. pneumoniae as the challenge organisms, essentially all the mice died; however, the mortality curves for CHS mice were significantly shifted to the left of the control mice. When S. aureus and E. coli were used as the challenge organisms, 78 and 96% of CHS mice

![Fig. 1](http://iai.asm.org/)

**Fig. 1.** Mortality rate of CHS, C57 Bl/6N, and heterozygous mice challenged with \( 10^6 \) C. albicans intravenously. Numbers in parentheses indicate the number of mice in each group.

![Fig. 2](http://iai.asm.org/)

**Fig. 2.** Mortality rate of CHS and C57 Bl/6N mice challenged with \( 10^4 \) S. pneumoniae intraperitoneally. Numbers in parentheses indicate the number of mice in each group.

![Fig. 3](http://iai.asm.org/)

**Fig. 3.** Mortality rate of CHS and C57 Bl/6N mice challenged with \( 10^6 \) K. pneumoniae intravenously. Numbers in parentheses indicate the number of mice in each group.
Numbers in each group. challenged with group. succumbed, two these showedpared with the other significantly serum IgA
bers in parentheses indicate the number of mice in each group.

**FIG. 4.** Mortality rate of CHS and C57 Bl/6N mice challenged with $5 \times 10^8$ S. aureus intravenously. Numbers in parentheses indicate the number of mice in each group.

succumbed, while only 7 and 50% of control mice succumbed, respectively. Therefore, with these two challenge organisms, there was an increase in the total mortality for CHS mice in addition to earlier deaths in these animals.

The results of serum Ig concentrations showed a significant increase ($P < 0.001$) in serum IgM concentrations of CHS mice compared with the other two groups (Table 1). The serum IgA concentrations of CHS mice were significantly greater ($P < 0.001$) than those of the heterozygous mice. No significant differences between the Ig concentrations of C57 Bl/6N and heterozygous mice were found.

**DISCUSSION**

An increased susceptibility to infection has previously been documented in CHS humans, minks, and cattle (9). This study demonstrates an increased susceptibility of CHS mice to challenge with gram-positive bacteria, gram-negative bacteria, and a fungus. Therefore, all animal species that have been shown to have the CHS as characterized by large lysosomal granules have been shown to have an increased susceptibility to infection.

The mechanism of the increased susceptibility to infection is probably related to impaired cellular function and is not humoral in origin. The serum Ig concentrations of CHS mice were found to be the same or greater than the control or heterozygous mice in this study. These results are consistent with the reported Ig concentrations in CHS in man (2). Although not measured in mice, antibody production has been reported to be normal in human beings with CHS (2). Therefore, the increased susceptibility to infection in man and mice is not due to serum Ig deficiency. On the other hand, in vitro studies with peripheral blood granulocytes of CHS humans (10), cattle (W. C. Davis, Fed. Proc. 29:1379, 1970), and recently mice (5) have documented delayed intracellular killing of bacteria. Also, abnormal granulocyte chemotaxis occurs in man (3), mink (G. A. Padgett, personal communication), and mice (5). Therefore, these in vitro observations in mice and other species establish a cellular defect in the CHS which relates to the in vivo findings in this study of nonspecific increased susceptibility to infection.

It is of interest that, despite the results reported in this study, CHS mice appear to

**FIG. 5.** Mortality rate of CHS and C57 Bl/6N mice challenged with $2 \times 10^8$ E. coli intravenously. Numbers in parentheses indicate the number of mice in each group.

**FIG. 6.** Comparison of the mortality curves for CHS and C57 Bl/6N mice. On the ordinate is shown the Wilcoxon test expressed as a standard normal deviate; that is, as (normal variate minus expected mean)/standard deviation. The challenge organisms are on the abscissa. The levels of significance are shown by the horizontal lines with the $P$ values on the right.
TABLE 1. Serum immunoglobulin concentrations of CHS, C57Bl/6, and heterozygous mice

<table>
<thead>
<tr>
<th>Mice</th>
<th>IgG&lt;sub&gt;1&lt;/sub&gt;</th>
<th>IgG&lt;sub&gt;2&lt;/sub&gt;</th>
<th>IgA</th>
<th>IgM</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHS (21)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.20 ± 0.13</td>
<td>3.56 ± 0.18</td>
<td>2.54 ± 0.16</td>
<td>0.53 ± 0.06</td>
</tr>
<tr>
<td>C57Bl/6 (22)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.20 ± 0.14</td>
<td>3.07 ± 0.09</td>
<td>2.06 ± 0.15</td>
<td>0.28 ± 0.01</td>
</tr>
<tr>
<td>Heterozygous (18)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.91 ± 0.21</td>
<td>2.95 ± 0.11</td>
<td>1.52 ± 0.17</td>
<td>0.23 ± 0.01</td>
</tr>
</tbody>
</table>

<sup>a</sup> Mean ± standard error of the mean.
<sup>b</sup> The numbers of animals used for the determination.

have a normal life expectancy and do not appear to have an increased frequency of infections when left alone in the laboratory. Whether this model proves to be only of laboratory interest or not remains to be seen. Nonetheless, these animals, in comparison to the other species with the CHS, should prove to be a useful experimental model for studying increased susceptibility to infection.

ACKNOWLEDGMENTS

We thank Richard Asofsky for providing the materials and methods for the mouse immunoglobulin assay.

LITERATURE CITED