Relative Immunologic Capacity of Leukemic and Low-leukemic Strains of Mice To Resist Infection*

WILLIAM H. MURPHY, JR., AND JEROME T. SYVERTON†

(Department of Bacteriology, University of Minnesota, Minneapolis, Minn.; and Department of Bacteriology, University of Michigan, Ann Arbor, Mich.)

SUMMARY

A comparative study was made to determine whether young mice predisposed to a high incidence of spontaneous leukemia displayed a distinctive response to infection which could be related to either a hyperactivity of their reticuloendothelial system (RES) or, conversely, some immunologic deficiency. The relative susceptibility to experimental infection of young mice (7–10 weeks old) of "leukemic" strains (AKR and C58) was compared with "low-leukemic" strains (C57BL/6, Swiss, BALB). Test agents included: *Candida albicans*, *Blastomyces dermatitidis*, *Histoplasma capsulatum*, *Sporotrichum Schenckii*, *Streptococcus pyogenes*, and *Salmonella typhosa Ty2*. When the dose levels of mycotic agents were carefully adjusted, it was possible to demonstrate marked differences in the response of male and female mice of the same strain to infection. Relative differences in the susceptibility of diverse inbred strains to mycotic infection were pronounced. Generally, predisposition to leukemia failed to correlate with either resistance or susceptibility to mycotic infection. Results with the bacterial agents presented the same general pattern. Experimental findings were discussed in regard to relative differences in the immunologic competence of "leukemic" and "low-leukemic" mice to resist infection and its possible relationship to early detectable changes in the RES of hosts predestined to manifest spontaneous malignant neoplasms.

The recent stimulating publication of Old and his associates (15) serves to direct attention toward changes in the phagocytic activity of the reticuloendothelial system (RES) in relation to the pathogenesis of cancer. Apart from the presentation of experimental data designed to reveal the manner in which the RES may be influenced during progressive growth of malignant cells, evidence was presented to show that certain transplantable tumors enhanced the phagocytic activity of the RES of recipient hosts. These intriguing studies and their possible applications to design of experiments devised to test the effect of RES function on progressive tumor growth seem, however, to have only limited applicability to the early natural changes occurring in hosts predestined to succumb to fatal spontaneous malignancies. For example, the clinical diagnosis of leukemia and its dismal prognosis yield essentially no information concerning the early changes in tissues characteristic of subjects which will clinically manifest leukemia at some later date. One can extend this line of reasoning to other classes of spontaneous malignancies, each class possessing its distinctive features. With regard to experimental spontaneous leukemia in mice, it is possible to propose two parameters which may help to distinguish the host predisposed to cancer. Thus, one may assume that the RES of normal animals predisposed to leukemia may be char-
acterized by either a hyperactivity of RES cells or, antithetically, that there may be some basic defect in the RES of such animals detectable at an early age when leukemia is still occult. Although these hypotheses are unquestionably oversimplified, they are subject to experimental tests in a variety of ways (15). Accordingly, this report provides experimental data from studies designed to assess the relative immunologic capacity of “leukemic” strains of mice to resist infection by a variety of mycotic and bacterial agents. For obvious reasons it was necessary in these studies to compare the innate resistance of mice exhibiting a high incidence of spontaneous leukemia (C58 and AKI) with those known to be essentially free of leukemia (BALB, Swiss, and C57BL/6).

MATERIALS AND METHODS

Mice.—Pen-bred Swiss mice were obtained exclusively from one source (Tumblebrook mice, Taconic Farms, Germantown, New York). Mice of the AKR and C57BL/6 strains were purchased from Jackson Memorial Laboratory and have been described in detail in publications from that source. BALB and C58 mice, derived from breeding stocks originally provided by Dr. E. C. MacDowell (20), were obtained from colonies carefully inbred and selected for either absence of leukemia (less than 1 per cent in BALB) or a high incidence of leukemia (60-80 per cent in the C58 strain). A careful record of the incidence of neoplasms in substrains of inbred mice was kept, since Law has pointed out (13) that some BALB strains of mice may have a high natural incidence of spontaneous leukemia. Mice were from 7 to 10 weeks old when used, except as noted under certain experiments recorded below.

Fungi.—Stock Minnesota strains (3) were employed throughout all experiments. Procedure for growth and preparation of Candida albicans has been reported (18). Histoplasma capsulatum, Blastomyces dermatitidis, and Sporotrichum Schenckii were grown at 37°C on Francis-glucose-cystine agar slants in 25 X 150-mm. screw-cap culture tubes. To avoid excessive age of cultures used to inoculate mice, rapid transfers were made until maximal growth rate of fungi was achieved. To obtain the large number of organisms needed for any single experiment, slants in appropriate number were incubated for from 3 to 5 days to obtain confluent growth. Yeast phase cells removed from slants were suspended initially in 0.85 per cent NaCl solution (saline), gross particles allowed to sediment, and supernatant cells pooled, diluted in saline to the desired concentration (see legends of figures), and enumerated by hemocytometer counts. Monodisperse suspensions of Histoplasma capsulatum and Blastomyces dermatitidis were obtained by careful but rapid trituration of cell suspensions employed for counts. Problems inherent in these procedures can be best appreciated from the publication by Rowley and Huber (19). Sporotrichum Schenckii was consistently troublesome because of its tendency toward transformation to the mycelial phase. This was averted by use of rapidly transferred yeast phase cultures in the log phase of growth.

Bacteria.—The strain of Streptococcus pyogenes, group A, Type 18, and the method for inoculation of mice was described (14). Salmonella typhosa Ty2 was grown at 37°C in brain-heart infusion broth. Serial decimal dilutions in broth of log phase cultures were prepared for inoculation of mice. Density of cell populations inoculated into mice was assayed by standard plate counts on infusion agar for Salmonella typhosa and blood agar plates for Streptococcus pyogenes.

Inoculation of mice.—All infectious agents were injected intraperitoneally, 0.5 ml/mouse. Mice in tests were segregated into groups according to sex. A single pool of stock inoculum of each agent was used for each experiment to minimize variation. Mice after inoculation were inspected daily for a period of not less than 20 days. Animals surviving either bacterial or mycotic infection were often kept under observation for 6-9 months.

Autopsy of mice.—Impression smears were made of splenic tissue from mice infected with mycotic agents. Smears were stained by the Schiff technic (1). Gross pathologic changes in mice infected with mycotic agents were noted as indicated in Figure 1. Response of mice to infection by group A streptococci was unequivocal; death usually occurred in 4-5 days or not at all. Proof that recovered organisms were those used to infect mice has been provided (14). Gross pathologic changes in animals chronically infected by group A streptococci are evident from Figure 2. Response of mice to infection by Salmonella typhosa Ty2 was observed to be either (a) fulminant with either rapid death or quick recovery or (b) progressive illness eventuating either in death or survival with a slow recovery of health. Difficulties common to accurate evaluation of the health or well-being of mice were obviated by recourse to cultural assay of tissues for organisms. Tissues taken aseptically at autopsy from inguinal and axillary lymph nodes and the spleen and liver were triturated in broth and plated on MacConkey’s agar to detect S. typhosa. Presence of S. typhosa in the gastrointestinal tract of mice was detected by aseptic removal of feces from the large bowel and assay
of fecal specimens for organisms as indicated above. Organisms thus isolated were identified serologically as *S. typhosa*.

**RESULTS**

_Preliminary titrations of the relative infectivity of mycotic agents for mice._—Initial studies indicated that, if the concentration of organisms employed to infect mice was not carefully adjusted (2, 4, 9, 19), differences in the innate resistance of the inbred strains of mice to mycotic infection were not as effectively demonstrated. Consequently, it was necessary to determine, for each mycotic agent, the optimal dose of organisms required to kill, in a reasonable period of time, 50 per cent of the animals of the most resistant strain. In these studies the intraperitoneal route of inoculation was selected for reason of convenience, although it was appreciated that the intravenous or intracerebral routes of inoculation have been employed effectively (2, 4, 8-10). To compare the relative immunologic competence of the strains of mice genetically predisposed to a high incidence of spontaneous leukemia with controls, all test animals were from 7 to 10 weeks old when used. Mice of each strain were segregated according to sex, and from ten to twenty mice were allocated to each test group. Mice were observed daily after inoculation and the cumulative per cent mortality recorded as a function of time.

Representative results for the dose-response of Swiss mice to infection by *Histoplasma capsulatum* and *Sporotrichum Schenckii* are recorded in Table 1. At the 10 per cent level (24.8 X 10^8 cells) of *H. capsulatum* 9 days were required for 50 per cent of the animals to die. At concentrations of less than 5 per cent no deaths occurred during 20 days, although mice became sick and died after from 3 to 6 months. Results from infection with *Sporotrichum Schenckii* were similar, viz., the 5 per cent dose level (32.5 X 10^8 cells) of organisms killed 50 per cent of mice in 9 days, whereas the 2 per cent dose level (13.0 X 10^8 cells) was lethal in 18-20 days. At dose levels of *Sporotrichum Schenckii* of less than 1 per cent, mice became ill and often died in from 3 to 9 months after infection. Preliminary titrations such as these permitted selection of the dose of organisms which would kill 50 per cent of the animals within a reasonable time of continuous observation, and which was most effective for emphasizing differences in the innate resistance of "leukemic" (C58 and AKR) and "low-leukemic" mice (BALB, C37BL/6, Swiss) to mycotic infection.

_Innate resistance of "leukemic" and "low-leukemic" mice to infection by Candida albicans._—To assess the relative immunologic competence of strains of mice predisposed to spontaneous leukemia (C58 and AKR strains) to resist mycotic infection, both the "leukemic" and "low-leukemic" strains of mice were divided into groups according to sex and infected with the optimal lethal dose of *Candida albicans*. Twenty mice of each sex of each strain were employed, and each experiment was repeated in triplicate. To assure accuracy in reporting results, data were first analyzed to determine whether the sex of the mice influenced response to infection (8). To assure uniformity in

**TABLE 1**

<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>DOSE OF ORGANISM USED TO INFECT MICE:</th>
<th>TIME IN DAYS FOR 50% DEATH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dose of organism (X10^8)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Per cent suspension</td>
<td></td>
</tr>
<tr>
<td><em>Histoplasma capsulatum</em></td>
<td>10  24.8  9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5        12.4  9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3        6.2   9</td>
<td>&gt;20</td>
</tr>
<tr>
<td></td>
<td>2        4.2   &lt;20</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1        2.1   &gt;20</td>
<td></td>
</tr>
<tr>
<td><em>Sporotrichum Schenckii</em></td>
<td>5        22.5  9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4        26.0  9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3        19.5  12</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2        13.0  19</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1        6.3   19</td>
<td></td>
</tr>
</tbody>
</table>

*Suspensions in 0.85 per cent NaCl solution of *Histoplasma capsulatum* of yeast phase organisms were enumerated by hemocytometer counts and graded doses inoculated intraperitoneally into groups of ten to twenty mice for each dilution of each test organism. Mice were observed daily, and the time for 50 per cent deaths was recorded.*

The data summarized in Chart 1 illustrate that male mice of the BALB strain were relatively more susceptible to infection than the corresponding females. The pattern of susceptibility for AKR mice was the reverse (Chart 2)—i.e., the females were significantly more susceptible to infection. In separate experiments designed to compare the relative resistance of the five strains of mice employed, the data (Chart 3) show that mice (C58 and AKR) predisposed to leukemia were substantially more resistant to infection than the "low-leukemic" BALB and Swiss mice.

_Innate resistance of "leukemic" and "low-leukemic" mice to infection by Blastomyces dermatitidis and Histoplasma capsulatum._—Because of
the marked differences in host-parasite relationships in superficial and systemic mycotic infections, *Blastomyces dermatitidis* and *Histoplasma capsulatum* were employed to characterize the response of "leukemic" and "low-leukemic" mice to infection by mycotic agents which can be intracellular parasites and which significantly involve the RE system (21). The technics indicated above for *Candida albicans* were employed. Twenty mice of each sex of each strain were used for each dose-response assay, and all experiments were repeated in triplicate.

With regard to results with *Blastomyces dermatitidis*, only the males of the C58 strain (Chart 4) were substantially more susceptible to infection than the corresponding females. In contrast to results with *Candida albicans*, AKR and C58 mice were noticeably more susceptible to infection than the "low-leukemic" controls (Chart 5). However, mice of the C57BL/6 strain were essentially as susceptible to infection as the "leukemic" strains of mice.

The influence of sex (Chart 6) on innate re-
sistance of mice to infection by *Histoplasma capsulatum* was more general than with either *Candida albicans* or *Blastomyces dermatitidis*. For AKR mice the influence was marked; for the C57BL/6 strain it was extraordinary. Thus, the observed pronounced effect of sex on resistance of mice to infection by *Histoplasma capsulatum* had the net result of essentially introducing new strains of mice (females) into the experiments. The data (Chart 6) show, however, that the C58 and AKR mice were not unique in their response to infection.

*Innate resistance of "leukemic" and "low-leukemic" mice to infection by *Sporotrichum Schenckii*.—To further characterize the capacity of "leukemic" and "low-leukemic" strains of mice to resist infection by a mycotic agent which causes self-limited disease in man, *Sporotrichum Schenckii* was selected. In this portion of the study only Swiss, BALB, and C58 mice were subjected to infection as noted above. The results with *Sporotrichum Schenckii* (Chart 7) indicated clearly that Swiss mice were more resistant to infection than were either the BALB or C58 mice. Moreover, males and females of each strain were essentially equivalent in susceptibility.

*Innate resistance of "leukemic" and "low-leukemic" mice to bacterial infections.—Studies carried out concurrently (14, 18) with the experiments recorded in this report provided an opportunity to assay the capacity of "leukemic" and "low-leukemic" mice of diverse strains to resist bacterial infection. At this point of the experimental work, however, sufficient evidence had been accumulated to indicate very strongly that the innate resistance of mice to the infectious agents did not depend upon whether mice were predisposed to leukemia, but was determined by other genetic factors characteristic of each strain of mice. Consequently, only two bacterial agents were tested, viz., group A hemolytic streptococci and *Salmonella typhosa* Ty2.
Data for *Streptococcus pyogenes*, Type 18, are recorded in Table 2. The pattern of response of the test inbred strains to infection was essentially similar to those recorded above for the mycotic agents, viz., differences in resistance to infection among strains of mice did not correlate with a genetic predisposition toward spontaneous leukemia. Female mice of the BALB, C58, and Swiss strains were more resistant to infection than were males.

A comprehensive study of the relative capacity of "leukemic" and "low-leukemic" strains of mice to resist infection by *Salmonella typhosa* was clearly beyond the scope of the investigation recorded here. Moreover, such studies have been reported (12), and there was little reason to anticipate significant new findings. Consequently, experimental design was simplified to give a relative measure of the innate resistance to infection by *Salmonella typhosa* Ty2 of the inbred strains of mice readily available. Experiments, repeated in duplicate, employed at least ten mice per test group for each dilution of organisms. \( \text{LD}_{50} \) titers were calculated by the method of Reed and Muench (17). All test animals for each individual experiment were inoculated from a single pool of *S. typhosa* in the log phase of growth.

Because of the limited nature of the experimental work one may only conclude from the data listed (Table 3) that differences in the susceptibility of the inbred strains of mice to infection by *S. typhosa* Ty2, as indicated by \( \text{LD}_{50} \) titers, were not significant. However, subjective observations suggested differences in response of animals to lethal infection. For example, the C58, Swiss, and DBA/2 mice that died succumbed to the acute toxic death characteristic of infection from inoculation of Salmonella spp. in great numbers (12). Moreover, animals that survived infection rapidly recovered and returned to normal patterns of feeding and movement. BALB mice generally did not experience a fulminant toxemic death but became progressively ill and finally expired. Females of the BALB strain were somewhat more resistant to infection than males. Organisms were recoverable from tissues (spleen, liver, lymph nodes) of BALB mice for a more prolonged period of time than for the C58, Swiss, or DBA/2 mice. Generally, there was no correspondence between genetic predisposition to leukemia and resistance to *S. typhosa* infection.

**DISCUSSION**

Strains of inbred mice with a natural high incidence of spontaneous leukemia, for which there is evidence that viruses can be implicated etiologically (7), provide model systems for studies of
the early changes in tissues before the onset of the overt leukemic process. Although a variety of factors, in addition to virus, have been recognized as being important in the pathogenesis of cancer (11) only limited information is available which serves to characterize the initial stages of the leukemic process (8). Epidemiologic (16) and clinical studies (6) of neoplasms in man may provide evidence to suggest some of the factors which may be studied in regard to the evolution of spontaneous malignancies. For example, clinical observations (21) have suggested an association between mycotic infection and neoplastic disease. Similarly, studies (6) of the dysproteinemias have revealed a correlation between intercurrent pyogenic infection and multiple myeloma. The occurrence of bacterial infections in Hodgkin’s disease has been a common clinical finding (6). Thus, such observations suggest either a defect in the function of the reticuloendothelial system (RES) which results from the carcinogenic process, and which predisposes to infection coincidentally, or an initial subnormality of the RES which is causally related to both the initiation of the malignant process and/or inadequacy to resist microbial infection.

The objective of the studies which are the subject of this report was to determine whether young mice of “leukemic” and “low-leukemic” strains predisposed to infection by Candida albicans, Blastomyces dermatitidis, Histoplasma capsulatum, and Sporotrichum Schenckii revealed striking differences in the capacity of mice to resist infection. Valid comparisons of the relative susceptibility to infection of mice of “leukemic” and “low-leukemic” strains could be made only after the dose of infective organisms was carefully adjusted to permit differences to be made clearly evident and when mice in test groups were segregated according to sex. The large numbers of mice used in the study provided convincing evidence that resistance or susceptibility to infection did not correlate generally with genetic predisposition to leukemia. A possible relationship, undoubtedly fortuitous, between predisposition to leukemia and susceptibility to mycotic infection was observed only for Candida albicans and Blastomyces dermatitidis. As one might anticipate, sex differences in response to mycotic infection were most pronounced in the inbred strains. Chronic infection of mice by the mycotic agents was commonly noted, since animals were frequently observed for as long as a year after infection. However, only meager evidence was obtained to suggest correlation between predisposition to leukemia and latent infection by either the mycotic or bacterial agents.

The pattern of response of “leukemic” and “low-leukemic” mice to infection by Streptococcus pyogenes and Salmonella typhosa Ty2 gave the same general results as those obtained for the mycotic agents, viz., sex of mice influenced response to infection to various degrees, and genetic predisposition to spontaneous leukemia did not correlate with relative immunologic capacity to resist experimental infection. Thus, the attempt to assess the relative immunologic competence of the RES as measured by its functional capacity (15) to restrain growth of infectious organisms failed to suggest either an immunologic hyperactivity of the RES of mice predisposed to leukemia or a basic defect in capacity of “leukemic” animals to resist infection. The use of a reasonable number of inbred strains of mice in these studies emphasized the importance of obtaining a more general prospective by use of available host systems. Although no comprehensive study was made of possible alterations in the immunologic capacity of “leukemic” mice to resist infection as they became older and malignancy progressed, such experiments are indicated to provide an additional measure of the capacity of tumor growth to influence (suppress) immunologic response. The nature of the difficulties to be anticipated in such a study have been reviewed in the timely discussion by Old and associates (15), and there can be little doubt that there is the basic need to characterize further the subversion of RES function during tumor progression. Whether characterization of the loss of immunologic competence can best be made by measurement of (a) changes in resistance to infection, (b) capacity of the RES to phagocytize colloidal particles or to produce antibody, or (c) predisposition to latent bacterial or mycotic infection, etc., remains to be determined. In any case, a sufficient spectrum of inbred mice ought to be used to minimize the possibility of obtaining misleading and/or spurious results.

ACKNOWLEDGMENTS

The authors are indebted to Dr. Dennis W. Watson for his many helpful suggestions.
REFERENCES


Fig. 1.—Gross pathologic changes that occurred in mice infected with mycotic agents were characterized by massive involvement of lymphatic and visceral organs (C57 mouse 1 month after infection with Blastomyces dermatitidis).

Fig. 2.—Suppurative lesions in extremities of C57 mouse infected previously (4-6 months) with Streptococcus pyogenes, Type 18.
Relative Immunologic Capacity of Leukemic and Low-leukemic Strains of Mice To Resist Infection

William H. Murphy, Jr. and Jerome T. Syverton


Updated version  
Access the most recent version of this article at:  
http://cancerres.aacrjournals.org/content/21/7/921

E-mail alerts  
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.