GONOCOCCAL INFECTION IN MICE: MICROBIAL AND HOST FACTORS RELATED TO INFECTION

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by

PHILIP REEL STREETER

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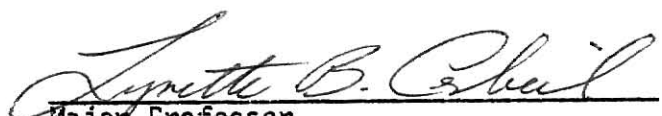
Approved by:



Major Professor

DEDICATION

To my parents, Robert and Gloria, and wife, Jeanne, whose love, support and understanding made my graduate studies worthwhile.

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ABSTRACT . . . . . .

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INTRODUCTION

Uncomplicated gonorrhea is currently epidemic throughout the world. In the United States alone, close to one million cases were reported in 1979. Social acceptance of relaxed sexual mores, changes in contraception methods, and an increase in resistance of the gonococcus to antibiotics have all been implicated as causes of the current epidemic.

Control of this disease is made difficult by a number of factors: (1) The gonococcus is highly infectious. (2) Gonorrhea has a very short incubation period, making it possible to transmit the infection to many individuals before it can be recognized and treated in the initially infected individual. (3) Widespread asymptomatic infection makes diagnosis of this disease extremely difficult.

During the past two decades several factors have been associated with gonococcal virulence in uncomplicated gonorrhea. These factors include colonial type, the presence of pili, and endotoxin. With the exception of gonococcal endotoxin, these characteristics have been associated with infectivity rather than tissue damage.

In approximately 1 to 3% of locally infected individuals, gonococci invade the bloodstream and produce systemic complications. Both microbial and host factors have been associated with this dissemination. Host factors related to increased incidence of disseminated gonococcal infection include pregnancy, menstruation, and the asymptomatic carrier state. Microbial factors include serum resistance, auxotype, antibiotic susceptibility, and light/transparent colony type. As in the mucosal infection, these factors have been associated with infectivity rather than tissue damage.

In the present investigation, genital and systemic mouse models of gonococcal infection were used to study the influence of some of the host and microbial virulence factors on the development of gonococcal infection.

PAPER I

INFLUENCE OF MICROBIAL AND HOST FACTORS ON THE DEVELOPMENT OF GONOCOCCAL BACTEREMIA IN MICE

ABSTRACT

A mouse model of disseminated gonococcal infection (DGI) was used to study microbial and host factors that may be involved in the pathogenesis of gonococcal infection. It was demonstrated that neither stage of growth (log or stationary phase) nor culture media (agar or broth) affected virulence of the gonococcus. Also, nonpiliated type 4 colonies were equally as virulent as piliated type 2 colonies, suggesting that pili are not important virulence factors in producing gonococcal bacteremia. Furthermore, although the median lethal dose (LD_{50}) was not affected by the stage of the estrus cycle, sexual factors may be important in that the LD_{50} for male mice ($10^{7.9}$) was significantly higher than the LD_{50} for female mice ($10^{6.9}$). That finding suggests that inherent differences in defense mechanisms between men and women may account in part for the higher frequency of dissemination observed in women than in men.

INTRODUCTION

Disseminated gonococcal infection (DGI) occurs in approximately 1 to 3% of individuals with gonorrhea (1, 2). Both microbial and host factors have been associated with dissemination of the gonococcus. Host factors related to increased incidence of DGI include pregnancy (3, 4), menstruation (3, 5), and the asymptomatic carrier state (3). Microbial factors include serum resistance (6), auxotype (7), antibiotic susceptibility (8), and light/transparent colony type (9). The role of these factors in producing disease has been difficult to study because of the lack of a convenient animal model that mimics the human disease. A recently developed mouse model of DGI, which progresses from local peritonitis to transient or lethal bacteremia, depending on the dose of gonococci administered, (10) allowed us to investigate the role of some of the factors in the pathogenesis of DGI.

MATERIALS AND METHODS

Mice. Caw:CF#1BR male and female mice (Carworth Division of Charles River Breeding Laboratories, Kingston, N. Y.) 7 to 10 weeks old were used. All mice were fed a commercial laboratory-animal diet and water ad libitum. Mice were anesthetized with ether for any manipulation that induced pain.

Bacteria. Neisseria gonorrhoeae strain N24 (a human genital isolate) was used for all experiments. Gonococci were incubated at 37° C in an atmosphere of 5% CO_2 in air and were grown either on GC agar base supplemented with IsoVitaleX (both from BBL, Division of Becton, Dickinson, and Co., Cockeysville, Maryland) or in GC broth supplemented with IsoVitaleX and 5% heat-inactivated fetal bovine serum (for maintenance of colony type). Unless otherwise specified, cultures of gonococci with greater than 95% type 2 colonies (11) were used because of their association with virulence in human genital infections (12). Before experimental use, gonococci were passed serially through mice for adaptation to that animal species.

Disseminated gonococcal infection. For inoculum preparation, gonococci were either concentrated in GC broth by centrifugation or scraped from plates and suspended in GC broth. The concentration of organisms was determined spectrophotometrically and verified by plate counts (13). Known concentrations of gonococci were diluted in a vehicle of 15% mucin (Porcine Gastric Mucin, Sigma Chemical Co., St. Louis, Missouri) and 4% hemoglobin (Difco Laboratories, Detroit, Michigan), hereafter designated mucin/hemoglobin. Mice were inoculated intraperitoneally with 0.3 ml of the gonococcal suspension as described previously (10).

Statistical analysis. For determination of median lethal dose (LD_{50}) values, deaths were recorded daily for groups of mice that had received half-log dilutions of standard suspensions of gonococci. The LD_{50} values were calculated by the probit-transformation method described by Finney (14).

Student's t test was used to compare two LD_{50} values (15) and a weighted analysis of variance was used when more than two LD_{50} values were compared (16, 17).

RESULTS

Before undertaking studies of host defenses, we studied microbial factors that have been related to virulence. The effects of culture media and phase of the growth cycle were compared by infecting groups of five mice with half-log dilutions of gonococci grown for 12, 16, or 20 hours on agar or 16, 20, or 24 hours in broth. These studies were conducted on the same day and were subsequently repeated to insure reproducibility. In preliminary growth-rate studies, the concentration of gonococci used to inoculate media was standardized so that a shift from log to stationary phase growth occurred at between 17 and 19 hours following the inoculation of both broth and agar. (P. R. Streeter, Appendix, M.S. Thesis, Kansas State University, 1980). No statistical differences were found among stages of growth (log or stationary phase) or culture media (agar or broth) ($P > 0.05$), although the LD_{50}s appeared to be slightly lower when organisms were grown on agar (Table 1). In a subsequent experiment, gonococci grown on agar for 16 hours were found to be no more virulent than those grown for 24 hours on agar ($P > 0.05$), again demonstrating no difference between log and stationary phase growth. In studies of the effect of colony type on development of bacteremia, the LD_{50} values obtained after inoculation with piliated type 2 and nonpiliated type 4 colonies were not significantly different ($P > 0.05$), with the LD_{50} for type 2 colonies being $10^{6.6}$ and for type 4 colonies being $10^{6.5}$.

Because the stage of the sexual cycle is important for survival of gonococci in the genital tracts of both women and mice (18), the effect of the estrus cycle on the LD_{50} was investigated. The stage of the estrus cycle was

determined by conventional vaginal smear techniques (19, 20), and mice in late proestrus, early estrus, early metestrus, or diestrus were injected intraperitoneally with gonococci. The LD_{50} values for these stages of the estrus cycle (Table 2) were not significantly different from one another ($P > 0.05$).

Male and female mice were used to further study sexual effects on DGI. It was found that the LD_{50} of $10^{7.9}$ for males was significantly higher than the LD_{50} of $10^{6.9}$ for females ($P < 0.001$), as shown in Table 2.

DISCUSSION

It was expected that cultural conditions would affect LD_{50}s in the murine DGI model. In that in Miller's original mouse model of gonococcal bacteremia, gonococci grown for more than 20 hours on agar lost virulence for the mouse (21). However, in this investigation neither stage of growth (log or stationary phase) nor culture media (agar or broth) were found to affect the LD_{50}. A number of variables could have been responsible for that observed difference, including the use of different media, different CO_2 concentrations, different mouse strains, and different gonococcal isolates. The mouse and gonococcal strains used by Miller are no longer available, so we could not make a comparison. Parallel studies of the survival of gonococci in the female mouse genital tract also showed no differences between stage of growth and culture media (P. R. Streeter, appendix, M.S. Thesis, Kansas State University, 1980).

Colony type did not appear to be an important factor in producing DGI either, in that the LD_{50} obtained following the inoculation of piliated type 2 colonies was essentially the same as that obtained following the inoculation of nonpiliated type 4 colonies. Because it is thought that the role of pili during infection is to enhance attachment of bacteria to epithelial

cells (22, 23), it is reasonable to conclude that when an epithelial surface is not involved (as in bacteremia following peritoneal inoculation) the presence of pili would not be required for virulence.

It was expected that the stage of the estrus cycle might influence the susceptibility of mice to DGI, since this is such an important determining factor in whether gonococci survive in the murine genital tract (18). Also, the fact that the peripheral neutrophil count is lowest at diestrus and proestrus (18) led to the postulation that the mouse may be more susceptible to DGI at that time. However, the LD_{50}s for the different stages of the cycle were not significantly different (Table 2). In other experiments in which bacteremia or positive peritoneal-gonococcal cultures at 24 hours were used as criteria of DGI (L. B. Corbeil, unpublished data), the same lack of difference between stages was demonstrated. Thus, it appears that the greatest survival of gonococci in the genital tract during late proestrus in mice (18) is probably due to local genital factors rather than to systemic factors. If defense mechanisms in women are similar to the mechanisms in female mice, then it is likely that local changes in the genital tract are more important than systemic changes in predisposing women to DGI at or just after menstruation (3, 5). Possible mechanisms responsible for the increased incidence of dissemination at this time in the cycle include the selection of light/transparent colony type (9), the increase in IgG blocking antibody in genital secretions (18), and/or changes in the normal flora (15).

Not only are changes in the menstrual cycle involved in dissemination but also the incidence of DGI is higher in women than in men (3, 5). It has been postulated that the frequency of DGI is higher in women because women are more often asymptomatic and remain untreated longer than men do, allowing time for selection of organisms able to disseminate (3). The

demonstration that female mice are more susceptible to DGI than are male mice following IP inoculation suggests that there may be an inherent difference between men and women in defense against bacteremia as well as a difference in duration of infection before treatment.

Table 1 The Effects of Culture Media and Stage of Growth on Gonococcal Virulence

Culture media	Log $_{10}$ lethal dose $_{50}$ after different incubation times*			
	12†	16†	20†	24†
GC agar	6.1	5.7	5,8	ND‡
GC Broth	ND‡	6.4	6.4	7.0

* Five groups of five mice each were inoculated with half-log dilutions of gonococci, and the experiment was subsequently repeated to insure reproducibility. No significant differences were found between incubation times or culture media ($P > 0.05$).

† Hours of incubation

‡ ND, Not done

Table 2 The Effect of Sexual Differences on Susceptibility to Gonococcal Bacteremia

Log $_{10}$ lethal dose $_{50}$*					
Sexes compared†		Stages of the estrus cycle compared§			
Male	Female‡	Late proestrus	Early estrus	Early metestrus	Diestrus
7.9	6.9	6.9	6.9	6.6	6.8

* Groups of ten mice were inoculated with half-log dilutions of a standard suspension of gonococci.

† A significant difference was found between sexes ($P < 0.001$).

‡ Stage of estrus was not determined when females were compared with males.

§ No significant differences were found among stages of the estrus cycle ($P > 0.05$).

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PAPER II

GONOCOCCAL INFECTION IN ENDOTOXIN RESISTANT AND ENDOTOXIN SUSCEPTIBLE MICE

ABSTRACT

The role of endotoxin sensitivity in defense against gonococcal infection was studied in endotoxin-resistant (C3H/HeJ) and endotoxin sensitive (C3H/HeN) mice by using a model of disseminated gonococcal infection (DGI) and a model of gonococcal survival in the female genital tract to determine the ability of the mice to eliminate gonococci. The median lethal dose (LD_{50}) in the DGI model was $10^{9.6}$ for C3H/HeJ mice and $10^{5.1}$ for C3H/HeN mice. Levels of bacteremia during infection indicated that C3H/HeJ mice cleared large numbers of gonococci from their peripheral blood by 24 hours post inoculation but that C3H/HeN mice did not. Additionally, the peritoneal leukocyte response following intraperitoneal inoculation of gonococci was greater in C3H/HeJ mice than in C3H/HeN mice, which suggested that the ability to mount an inflammatory response to endotoxin may be important in defense against DGI. Besides being different in susceptibility to DGI, C3H/HeJ mice were found to be more resistant than C3H/HeN mice to genital colonization by gonococci. The resistance of C3H/HeJ mice to genital colonization by gonococci appeared to be due to both the high numbers of PMN leukocytes in the genital secretion and the predominance of inhibitory Gram-negative genital flora in that mouse strain.

INTRODUCTION

During the past two decades many factors including colonial type (9, 10), the presence of pili (22), serum resistance (18), color/opacity colony variants (8), and endotoxin (12) have been associated with gonococcal virulence. Gonococcal endotoxin excepted, these characteristics have been associated with infectivity rather than tissue damage. In that gonococcal endotoxin is cytotoxic in human fallopian tube cultures (12), endotoxin is quite

likely responsible at least in part for cell damage in both gonorrhea and disseminated gonococcal infection (DGI). The fallopian tube system is useful in studying tissue damage; but because host defenses such as inflammatory cells and serum factors are lacking, a whole-animal model is necessary to study host responses to gonococcal endotoxin during infection.

To study this host-parasite interaction, we used two murine models of gonococcal infection: 1) a model of DGI, which progresses from local peritonitis to transient or lethal bacteremia, depending on the dose of gonococci administered (4); and 2) a genital model, to determine survival of gonococci on the mucosal surface (2). With these models, it was possible to investigate the interaction of host-defense mechanisms and microbial virulence factors at both mucosal and systemic levels.

To study the role of resistance to gonococcal endotoxin during infection, we used endotoxin-resistant (C3H/HeJ) and endotoxin sensitive (C3H/HeN) mice. The resistance of CeH/HeJ mice is revealed in an increased inflammatory response to endotoxin (14, 19, 20), macrophages which are resistant to the in vitro cytotoxic effects of endotoxin (7, 16), and lymphocytes which do not respond mitogenically or immunogenically to endotoxin (24). The possibility that resistance to endotoxin might represent resistance to Gram-negative bacterial infection prompted us to study median lethal doses of gonococci, kinetics of bacteremia, peritoneal leukocyte responses, and peripheral blood leukocyte responses during DGI in these mice. To determine if mice resistant to the systemic effects of endotoxin were also resistant to mucosal infection, we studied genital survival of gonococci in the two strains of mice as well.

MATERIALS AND METHODS

Mice. C3H/HeJ (Jackson Laboratories, Bar Harbor, Maine), C3H/HeN, and Caw:CF#1BR (CF#1) female mice (both from Carworth Division of Charles River Breeding Laboratories, Kingston, N.Y.) 7 to 10 weeks old were studied. All mice were fed a commercial laboratory-animal diet and water ad libitum and were maintained in accordance with NIH standards for animal care.

Bacteria. Neisseria gonorrhoeae strain N24 (a human gential isolate) was used for all experiments unless otherwise noted. Before they were used, gonococci were passed serially through mice to adapt them to this animal species. The mouse-passed gonococci were grown on GC agar base supplemented with IsoVitaleX (both from BBL, Division of Becton, Dickinson, and Co., Cockeysville, Maryland) for approximately 22 hours at 37° C in an atmosphere of 5% CO_2 in air. Because of their association with virulence (9), gonococci from plates with greater than 95% type 2 colonies (10) were used throughout the study.

Disseminated gonococcal infection. For inoculum preparation, gonococci were scraped from plates and suspended in GC broth. The concentration of organisms was determined spectrophotometrically and verified by plate counts (13). Known concentrations of gonococci were diluted in a vehicle of 15% mucin (Porcine Gastric Mucin, Sigma Chemical Co., St. Louis, Missouri) and 4% hemoglobin (Difco Laboratories, Detroit, Michigan), hereafter referred to as mucin/hemoglobin. Mice were inoculated intraperitoneally with 0.3 ml of this suspension (4).

For the study of bacteremia and peripheral blood leukocyte responses, blood was collected by cardiac puncture. Bacterial numbers were determined by plate count (13), while total and differential white blood cell (WBC) counts were made on aliquots of blood samples from the same mice.

The peritoneal inflammatory response was determined by withdrawing peritoneal fluid at intervals after inoculating mice with gonococci. The fluid was diluted in a citric acid/crystal violet solution to stain the peritoneal exudate cell (PEC) nuclei. Total and differential WBC counts of the PEC were made with a hemacytometer.

Gonococcal infection in the genital tract. Agar-grown gonococci suspended in broth were diluted to a concentration of 2-4 X 10^8 CFU/ml in a vehicle of 15% mucin containing 3 ug of vancomycin, 7.5 ug of colistin, and 12.5 units of nystatin per ml (VCN) the concentrations of antibiotics in the Thayer-Martin medium (11). Mice in late proestrus or early estrus, as determined by conventional vaginal smear techniques (1, 23), were inoculated locally with gonococci. Mice were anesthetized with ether so that they could be positioned for observation of the cervical os as previously described (3). Then 0.02 ml of gonococcal suspension was inoculated with a blunted 23-gauge needle into the uterine body via the cervical canal. At 24-hour intervals post inoculation (PI), vaginal smears and bacterial cultures of the vaginal contents were obtained by stroking the vaginal epithelium with sterile applicator sticks. Bacteria were isolated by streaking the applicator sticks onto GC agar plates with and without VCN.

Normal aerobic flora in the genital tract. The interaction between gonococci and the normal aerobic flora of the genital tract was studied *in vitro* as described previously (2). Isolates of the normal flora were grown as a single streak on GC agar base plus IsoVitaleX. After 24 hours of growth, the isolates were removed with cotton swabs and any remaining growth was killed with chloroform. Plates were then cross-streaked with two indicator strains of gonococci, N24 and F62 (the laboratory strain of Kellogg) (10), at concentrations of 1 X 10^6 CFU/ml. These plates were incubated for 24 hours at 37° C in 5% CO_2 and examined for growth inhibition of the gonococcus.

Statistical anaylsis. The median lethal doses (LD_{50}), calculated by the probit-transformation method described by Finney (6), were compared by using student's t test. Data on the kinetics of bacteremia, the peritoneal leukocyte responses, and the peripheral blood-leukocyte responses following intraperitoneal inoculation of mice with gonococci were analyzed by using analysis of variance procedures (15, 17). Proportional analysis was used to compare recovery of gonococci, normal flora, and PMN leukocytes from the genital tracts of the three mouse strains (5). Differences were considered significant when the probability (P) was <0.05.

RESULTS

Disseminated gonococcal infection. To calculate the LD_{50} values for DGI in C3H/HeN and CeH/ HeJ mice, we injected groups of 5 animals intraperitoneally with half-log dilutions of a standard suspension of gonococci. All animals to be compared were inoculated on the same day, and the experiment was repeated to insure reproducibility. The LD_{50} value of $10^{5.1}$ for gonococcal infection in C3H/HeN mice was significantly different ($P < 0.001$) from that of $10^{9.6}$ in C3H/HeJ mice.

The nature of this innate resistance to gonococcal infection in C3H/HeJ mice was investigated by determining levels of bacteremia during infection. Groups of mice were injected intraperitoneally with three dilutions of gonococci (10^8, 10^6, and 10^4) in mucin/hemoglobin. At 4, 12, and 24 hours PI, two mice from each group were euthanized and bled for bacterial counts. At the same time, additional groups of mice were inoculated with each of the three doses to determine whether the doses killed mice. The results showed that bacteremia occurred at all dose levels in both sublines of mice (Fig. 1). In mice of the C3H/HeJ subline all three dose levels produced a transient bacteremia, which reached a peak 4 hours PI and subsided by 12 to 24 hours PI.

In contrast, a persistent bacteremia, which reached a peak 12 hours PI, was produced in the C3H/HeN mice that received the two highest doses (10^6 and 10^8). The C3H/HeN mice that received the lowest dose of gonococci (10^4), cleared the gonococci from their peripheral blood by 24 hours PI. Of the groups kept for observation, the C3H/HeN mice receiving 10^6 or 10^8 gonococci died, whereas none of the C3H/HeJ mice died.

To determine the mechanism of gonococcal clearance during DGI, we studied both the peritoneal-leukocyte and the peripheral blood-leukocyte responses from the above mice. At 4 hours PI, the total peritoneal-leukocyte responses of the two strains of mice were similar for all dose levels of gonococci (Fig. 2). At 12 hours PI, however, the peritoneal responses to the two highest doses (10^6 and 10^8) were greater in the C3H/HeJ mouse strain. That difference was even more dramatic at 24 hours, with all three doses producing a greater response in C3H/HeJ mice than in C3H/HeN mice. The total peritoneal-leukocyte response in C3H/HeN mice was dose-dependent, whereas it was not in C3H/HeJ mice. Differential counts revealed that neutrophils were the predominant PEC in all cases except the 10^8 dose in C3H/HeN mice, in which mononuclear cells predominated at 12 hours PI and neither cell type predominated at 24 hours PI. Analysis of total and differential peripheral-blood leukocyte responses did not reveal any significant differences between the two C3H mouse strains with respect to dose of gonococci or time following inoculation (P. R. Streeter, appendix, M. S. Thesis, Kansas State University, 1980). Results might not have been significant because only two observations were made at each dose and time, and variation was considerable. Although the peripheral blood-leukocyte levels of the two C3H sublines were not significantly different, the C3H-HeJ subline appeared to have fewer peripheral-blood leukocytes at 24 hours PI. That trend could have been due to the constant migration of peripheral-blood leukocytes to the peritoneal cavity.

Gonococcal infection in the genital tract. In studies to determine whether mice resistant to the systemic effects of endotoxin would be resistant to mucosal gonococcal infection, we gave intrauterine inoculations of 4-8 X 10^6 gonococci to 30 C3H/HeN, 30 C3H/HeJ and 20 CF#1 mice in late proestrus or early estrus. CF#1 mice were used as positive controls, because most of the earlier work was done with this strain (2). Gonococci were recovered more frequently from C3H/HeN mice than from C3H/HeJ mice ($P < 0.01$) (Table 1) and were recovered even more frequently from CF#1 mice than from either of the C3H sublines ($P < 0.001$). To determine the cause of these differences, we studied both the vaginal PMN leukocyte response and the normal vaginal flora during infection. In comparing the genital PMN-leukocyte responses of the three strains (Table 1), we found no differences between the C3H sublines ($P > 0.05$). Both of the C3H sublines, however, had more mice with PMN leukocytes in vaginal smears 24 hours PI than did the CF#1 strain ($P < 0.005$). The CF#1 strain also had fewer mice with detectable normal genital flora (Table 1) than did either of the C3H sublines ($P < 0.05$). Though at first glance there appeared to be no difference in the normal flora of the two C3H sublines, more C3H/HeJ mice had detectable Gram-negative flora than did C3H/HeN mice ($P < 0.005$), and conversely more C3H/HeN mice had detectable Gram-positive flora than did C3H/HeJ mice ($P < 0.005$).

Aerobic genital flora of uninoculated mice. In that there appeared to be differences in the normal genital flora of C3H/HeN and C3H/HeJ mice, we studied the flora of 45 mice of each strain in detail. The mice had not been inoculated with gonococci. Again, differences between the strains were not apparent until the isolates were divided on the basis of their Gram reaction (Table 2). Then it was obvious that more C3H/HeJ mice had detectable Gram-negative flora than did C3H/HeN mice ($P < 0.005$) and

conversely more C3H/HeN mice had detectable Gram-positive flora than did C3H/HeJ mice ($P < 0.001$). When the semiquantitative estimates of the number of colonies of each bacterial isolate were examined, the data showed that the number of colonies of Gram-negative organisms were usually much higher than the number of Gram-positive colonies in both mouse sublines (P. R. Streeter, appendix, M. S. Thesis, Kansas State University, 1980). Furthermore, the species of aerobic bacteria within the Gram-positive and Gram-negative groups from the two mouse strains were quite different from one another (Table 3), which might be related to the finding that the Gram-negative bacteria isolated from C3H/HeJ mice inhibited the growth of gonococci *in vitro* (Table 4) more frequently than did Gram-negative bacterial isolates from C3H/HeN mice ($P < 0.005$).

DISCUSSION

Endotoxin-resistant C3H/HeJ mice were found to be much more resistant to the lethal effects of gonococcal infection than were C3H/HeN mice. That was similar to findings for *Escherichia coli* infection (21) but opposite to the resistance-pattern of the mice to *Salmonella typhimurium* (25). As suggested by Sultzer and Goodman (21), the intracellular nature of the *S. typhimurium* infection and the extracellular parasitism of *E. coli* quite likely would explain the differences in results. In that *N. gonorrhoea* is probably also an extracellular parasite, it is not surprising that its lethal effect on these mouse strains is similar to that of *E. coli*.

Data for clearance of gonococci from the blood of C3H/HeJ mice help to explain the relative resistance of C3H/HeJ mice to gonococcal bacteremia, because C3H/HeJ mice cleared gonococci from their peripheral blood more efficiently than did C3H/HeN mice, even though the C3H/HeJ mice had a higher level of bacteremia at 4 hours PI. The inability of C3H/HeN mice to clear gonococci was correlated with death of the animals kept for observing the

illness. Thus, it appears that the C3H/HeJ mice resist lethal gonococcal bacteremia because they eliminate the organisms rapidly rather than because of their resistance to the damage caused by prolonged Gram-negative bacteremia.

The peritoneal-leukocyte responses (Figure 2) gave some insight into the mechanism of clearance of gonococci during DGI. Gonococci produced a faster and higher PEC response in the C3H/HeJ subline than in the C3H/HeN subline. Similar peritoneal-leukocyte responses have been observed in mice of these sublines following administration of Salmonella typhosa endotoxin (14). In the DGI model, it is likely that the rapid PEC response in C3H/HeJ mice after intraperitoneal inoculation of gonococci, prevents constant reseeding of the blood stream from the peritoneal cavity.

In the C3H/HeN mouse strain, differential counts of the PEC indicated that the number of neutrophils and mononuclear cells decreased as the dose of gonococci increased. Such a dose-dependent response was not observed in C3H/HeJ mice. Possibly gonococci have a cytotoxic effect on the neutrophilic phagocytes of C3H/HeN mice in similar fashion to their macrophages, which are susceptible to the in vitro cytotoxic effects of endotoxin, whereas macrophages from C3H/HeJ mice are resistant (7, 16). This cytotoxic effect of endotoxin may account in part for the differences observed in the peritoneal leukocyte responses and in sustained dissemination of gonococci from the peritoneal location.

In the genital model, gonococci were recovered at different frequencies from the three mouse strains tested (Table 1). To determine the cause of these different recovery frequencies, we studied genital PMN leukocyte and normal flora numbers during infection (Table 1) because both are thought to influence the survival of gonococci in the mouse's genital tract (2). Apparently, gonococci at a high rate were recovered from CF#1 mice at 24 hours PI because then few of these mice had genital PMN leukocytes or

detectable normal flora. Conversely, gonococci were apparently recovered at a low rate from both C3H sublines at 24 hours PI because then many of those mice had genital PMN leukocytes and detectable normal flora. In that the peritoneal leukocyte responses of the two C3H sublines of mice was different after intraperitoneal inoculation, it was surprising that at 24 hours following intrauterine inoculation the number of C3H/HeJ and C3H/HeN mice with PMN leukocytes in their vaginal smears was not significantly different. Perhaps quantitation of leukocytes per mouse genital tract at 4, 12, and 24 hours PI would have revealed differences.

With respect to the normal genital flora, more mice of the C3H/HeJ than of the C3H/HeN subline were shown to have Gram-negative flora, and the Gram-negative flora of the C3H/HeJ subline inhibited in vitro growth of gonococci more frequently than did the Gram-negative genital flora of C3H/HeN mice. Thus, it is likely that inhibition of gonococcal growth in vitro by the Gram-negative flora of C3H/HeJ mice was at least in part responsible for the difference between the two C3H mouse strains in recovery of gonococci.

In summary, the endotoxin-resistant C3H/HeJ mouse strain was found to be more resistant to both systemic and genital infection with the gonococcus than was the endotoxin-sensitive C3H/HeN mouse strain. Enhanced neutrophil responses of the C3H/HeJ mice appeared to be an important factor in their resistance to both types of infection. In addition, inhibitory Gram-negative flora also appeared to play a role in the resistance of the C3H/HeJ mouse strain to genital infection.

Figure 1. Kinetics of bacteremia in C3H/HeN (A) or in C3H/HeJ (B) mice inoculated intraperitoneally with gonococci. Each point represents the mean of two independent observations. The dilution of blood samples did not allow detection of less than 10^2 gonococci per ml. Additional mice were inoculated and kept for observation; of those, the C3H/HeN mice receiving 10^6 or 10^8 gonococci died, while all C3H/HeJ mice lived. Symbols: ○, 10^4 gonococci; △, 10^6 gonococci; □, 10^8 gonococci.

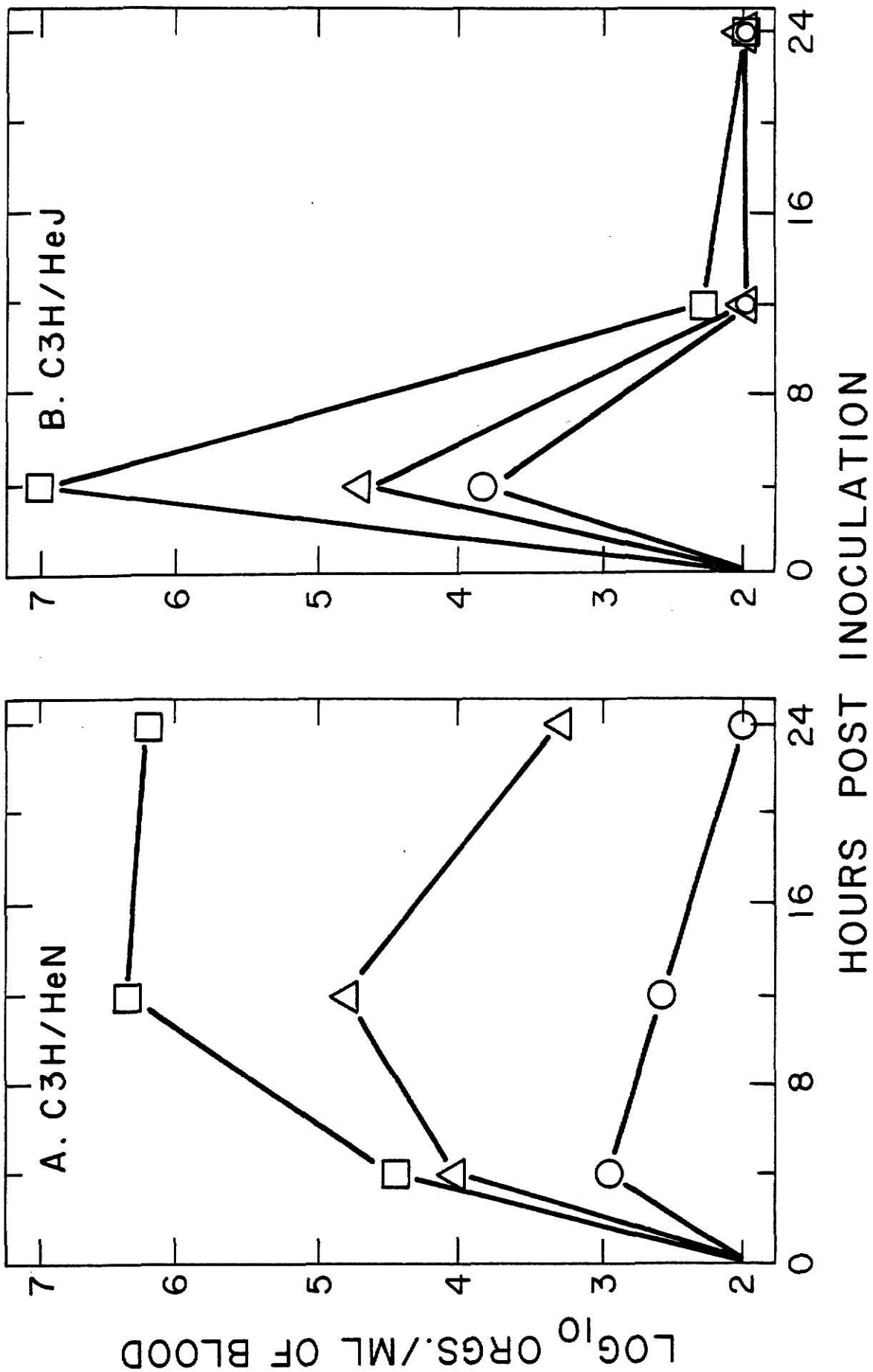
A. C3H/HeN
B. C3H/HeJ
LOG10 ORGS./ML OF BLOOD
HOURS POST INOCULATION
2
3
4
5
6
7
0
8
16
24

Figure 2. Kinetics of total and differential peritoneal leukocyte responses of C3H/HeN and C3H/HeJ mice inoculated intraperitoneally with gonococci. Each point represents the mean of two observations. At 4 hours PI the mucin/hemoglobin vehicle disrupted the peritoneal leukocytes to such an extent that differential counts were not possible. Note that the responses of the C3H/HeN mice were dose-dependent, while the responses of C3H/HeJ mice were not. Symbols: ○, 10^4 gonococci; △, 10^6 gonococci; □, 10^8 gonococci.

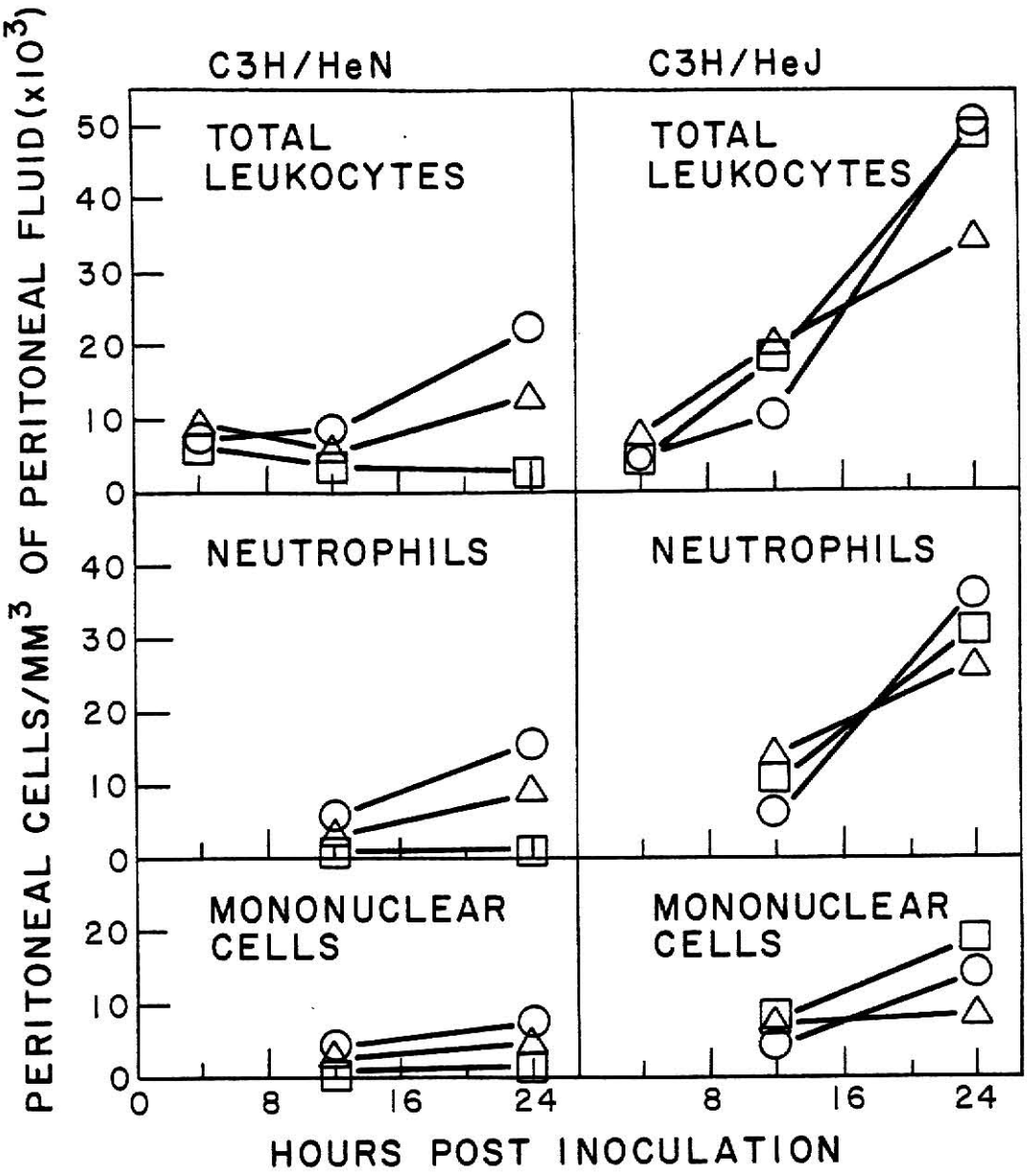
C3H/HeN
C3H/HeJ
TOTAL LEUKOCYTES
TOTAL LEUKOCYTES
NEUTROPHILS
NEUTROPHILS
MONONUCLEAR CELLS
MONONUCLEAR CELLS
PERITONEAL CELLS/MM3 OF PERITONEAL FLUID (x10^3)
HOURS POST INOCULATION
0
8
16
24
10
20
30
40
50

Table 1. Gonococci, Normal Aerobic Flora, and PMN Leukocytes Recovered from Mice 24 Hours After Intrauterin Inoculation with Gonococci

Mouse strain	Number inoculated	Percentage with positive gonococcal cultures [a]	Percentage with detectable normal flora [b]	Percentage with detectable Gram-negative normal flora [c]	Percentage with detectable Gram-positive normal flora [d]	Percentage with PMN leukocytes in vaginal smear [b]
C3H/HeN	30	20	90	57	63	73
C3H/HeJ	30	0	93	90	27	60
CF#1	20	80	65	20	50	20

a All three mouse strains were significantly different.[e]

b The difference between the two C3H strains was not significant; the difference between the CF#1 and either of the C3H strains was significant.

c All three mouse strains were significantly different.

d The difference between the two C3H strains was significant: the difference between the CF#1 and either of the C3H strains was not significant.

e Differences were considered significant if the probability (P) was <0.05.

Table 2. Normal Aerobic Flora Recovered from Uninfected C3H/HeN and C3H/HeJ Mice

Mouse strain	Number cultured	Percentage with detectable flora [a]	Percentage with detectable Gram-negative flora [b]	Percentage with detectable Gram-positive flora [c]
C3H/HeN	45	80	58	47
C3H/HeJ	45	87	84	9

a No significant difference between strains ($P > 0.1$)

b Significant difference between strains ($P < 0.005$)

c Significant difference between strains ($P < 0.001$)

Table 3. Vaginal Isolates from Endotoxin-resistant and Endotoxin-susceptible Mice

Bacterial isolate	Number of isolates	
	C3H/HeN mice	C3H/HeJ mice
Staphylococcus epidermidis	12	0
Staphylococcus aureus	8	0
Corynebacterium pyogenes	3	0
Nonhemolytic streptococcus	0	4
Pasteurella pneumotropica	0	26
Pasteurella haemolytica	5	0
Escherichia coli	10	0
Proteus mirabilis	16	13
Totals	54	43

Table 4. Interaction Between Vaginal Flora and Gonococci *in vitro*

Bacterial isolate	Isolated from	Inhibition		Partial Inhibition		No Effect	
		N24	F62	N24	F62	N24	F62
Staphylococcus epidermidis	C3H/HeN	7	8	3	3	2	1
Staphylococcus aureus	C3H/HeN	3	4	5	4	-	-
Corynebacterium pyogenes	C3H/HeN	-	-	-	-	3	3
Nonhemolytic streptococcus	C3H/HeJ	-	-	-	-	4	4
Pasteurella pneumotropica	C3H/HeJ	22	21	4	5	-	-
Pasteurella haemolytica	C3H/HeN	3	1	2	4	-	-
Escherichia coli	C3H/HeN	-	-	3	3	7	7
Proteus mirabilis	Both sublines	39	39	-	-	-	-

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APPENDIXES

APPENDIX I: ADDITIONAL RESULTS

APPENDIX II: LITERATURE REVIEW

ADDITIONAL RESULTS

The results of paper #1 indicated that neither stage of growth (log or stationary phase) nor culture media (agar or broth) affected gonococcal virulence in murine DGI. Proportional analysis (Dixon and Massey, 1969) of the results of parallel studies on the survival of gonococci in the female mouse genital tract also showed no significant differences (Table 1). To make these comparisons, the concentrations of gonococci used to inoculate media for growth curves were standardized so that a shift from log to stationary phase growth occurred at between 17 and 19 hours following the inoculation of both types of media (Fig. 1). For the growth curve of gonococci in broth, 100 ul of broth containing a total of approximately 5×10^6 broth adapted organisms were inoculated into flasks containing 10 ml of broth. For the growth curve on agar, 20 ul of broth containing approximately 1×10^6 agar adapted organisms were inoculated onto agar plates. Quantitative counts of gonococci were made in triplicate at 4 hour intervals following the inoculation of media. The counts of broth grown gonococci were made by serially diluting aliquots of broth. For the counts of agar grown gonococci, plates were scraped with a rubber policeman and then washed to remove any remaining growth. Organisms were suspended in a final volume of 2 ml of broth, and were counted by serially diluting the broth.

In paper #2 the total and differential peripheral-blood leukocyte responses of C3H/HeN and C3H/HeJ mice inoculated intraperitoneally with gonococci were compared (Fig. 2). For this comparison, groups of mice were infected with three dilutions of gonococci (10^8, 10^6, and 10^4) in mucin/hemoglobin. At 4, 12, and 24 hours PI, two mice from each group were euthanized and bled for study of their peripheral-blood leukocyte responses. Analysis of variance procedures (Mosteller and Turley, 1977; Scheffé, 1949) revealed no significant differences between these two strains of mice with respect to dose of gonococci or time

following inoculation. These results might not have been significant because only two observations were made at each dose and time, and variation was considerable. Although the peripheral-blood leukocyte levels of the two C3H sublines were not significantly different, the C3H/HeJ subline appeared to have fewer peripheral-blood leukocytes at 24 hours PI. That trend could have been due to the constant migration of peripheral-blood leukocytes to the peritoneal cavity.

Characteristics of bacterial isolates recovered from the genital tracts of uninoculated mice were studied in order to understand interactions between gonococci and the normal flora. The semiquantitative counts of the number of colonies formed by each bacterial isolate from the two C3H mouse sublines were compared (Table 2). Statistical analysis of these results (Conover, 1971) indicated that the number of colonies of Gram-negative isolates was usually greater than the number of colonies of Gram-positive isolates in both mouse sublines. These results, considered with the results on the interaction between these bacterial isolates and gonococci in vitro (Paper #2), imply that the Gram-negative flora of the two C3H mouse sublines probably exerts a greater inhibitory effect on the in vivo growth of the gonococcus than do the Gram-positive flora. In addition, a comparison of the two mouse strains indicated that there was no significant difference in the semiquantitative counts of Gram-negative isolates, and that there was a difference in the semiquantitative counts of the Gram-positive isolates in that C3H/HeN mice had more Gram-positive colonies per mouse than did C3H/HeJ mice.

Table 1. Influence of Phase of Growth and Culture Media on the Survival of Gonococci in the Genital Tract

Culture media	No. of mice with positive gonococcal cultures at 24 hours PI [a]	
	14 h [b]	22 h
GC agar	6	3
GC broth	3	5

a Groups of 10 mice were inoculated intrauterinely with gonococci grown for different lengths of time on/in different media. No significant differences were found between incubation time or culture media ($P > 0.05$).

b The length of time gonococci were incubated before inoculation of mice.

Figure 1. Growth Curves for Gonococci Grown in Broth and on Agar.

For the growth curve in broth, 100 ul of broth containing approximately 5 X 10^6 broth adapted organisms were inoculated into flasks containing 10 ml of broth. For the growth curve on agar, 20 ul of broth containing approximately 1 X 10^6 agar adapted organisms were inoculated onto agar plates. Quantitative counts of gonococci were made in triplicate at 4 hour intervals following the inoculation of media. Each point represents the mean of the triplicate counts. The number of organisms used to inoculate media was standardized so that a shift from log to stationary phase growth occurred at between 17 and 19 hours PI for both types of media.

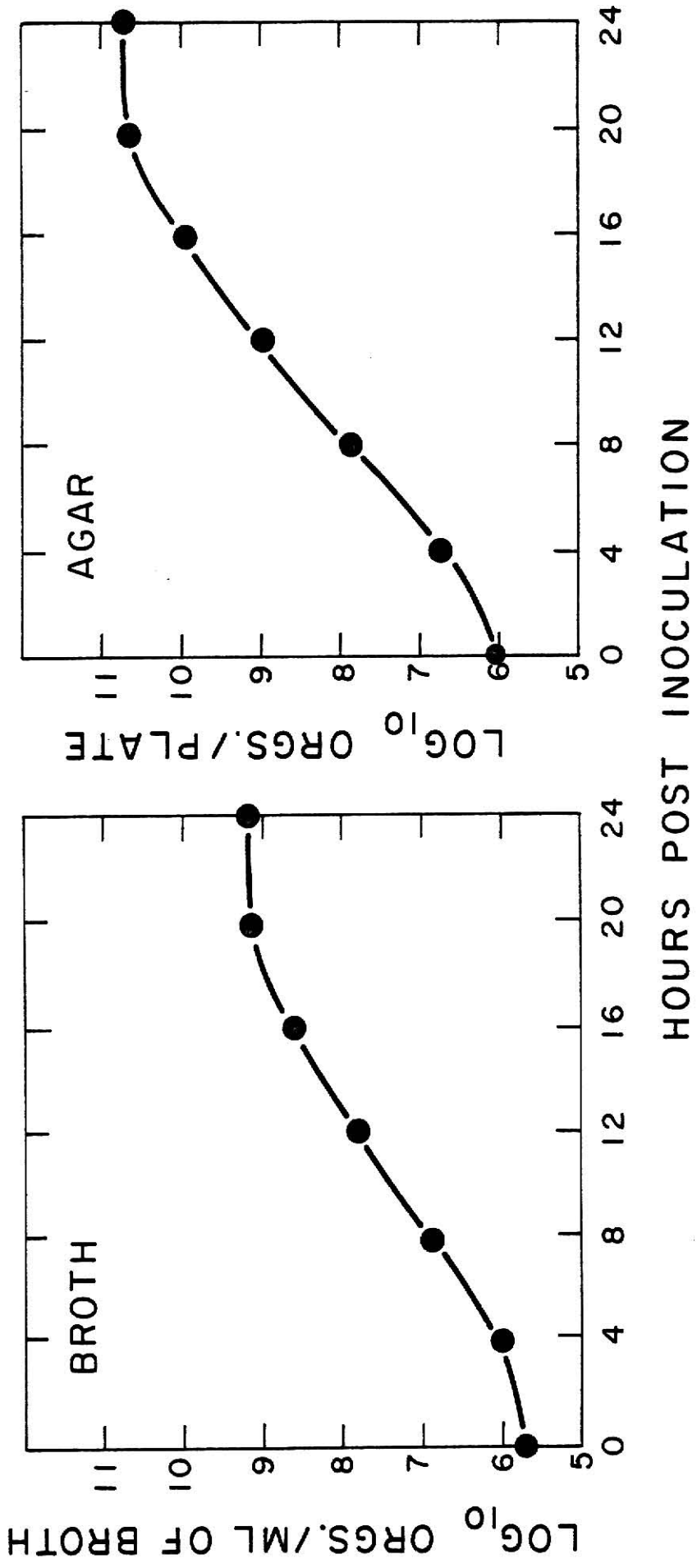
AGAR
BROTH
LOG10 ORGS./PLATE
LOG10 ORGS./ML OF BROTH
HOURS POST INOCULATION
0
4
8
12
16
20
24
5
6
7
8
9
10
11

Figure 2A. Kinetics of total peripheral-blood leukocyte responses of C3H/HeN and C3H/HeJ mice inoculated intraperitoneally with gonococci. Each point represents the mean of two observations. The differences between these two strains of mice with respect to dose of gonococci or time following inoculation were not significant.

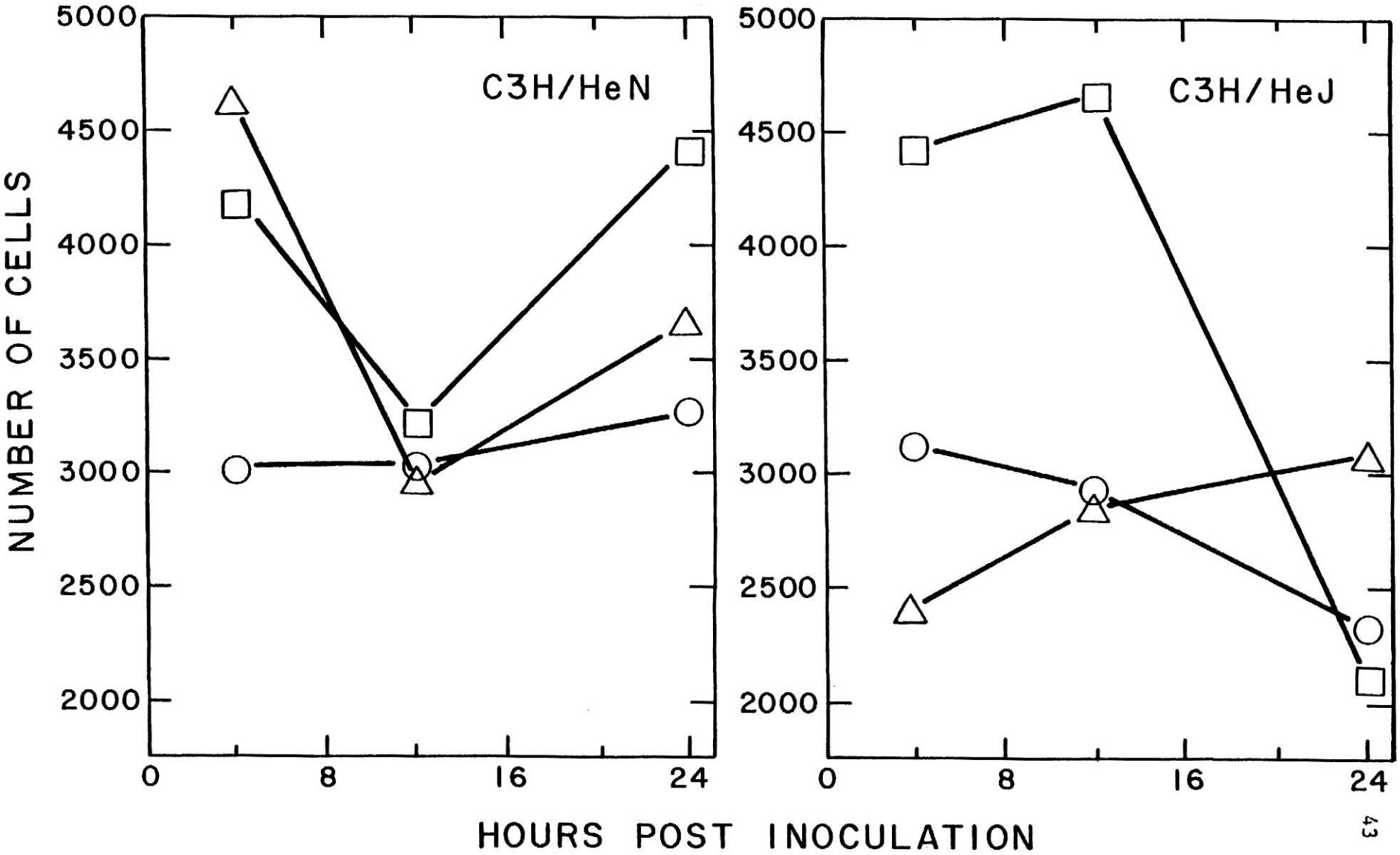
C3H/HeN
C3H/HeJ
NUMBER OF CELLS
5000
4500
4000
3500
3000
2500
2000
0
8
16
24
HOURS POST INOCULATION

Figure 2B. Kinetics of peripheral-blood monocyte responses of C3H/HeN and C3H/HeJ mice inoculated intraperitoneally with gonococci. Each point represents the mean of two observations. The differences between these two strains of mice with respect to dose of gonococci or time following inoculation were not significant.

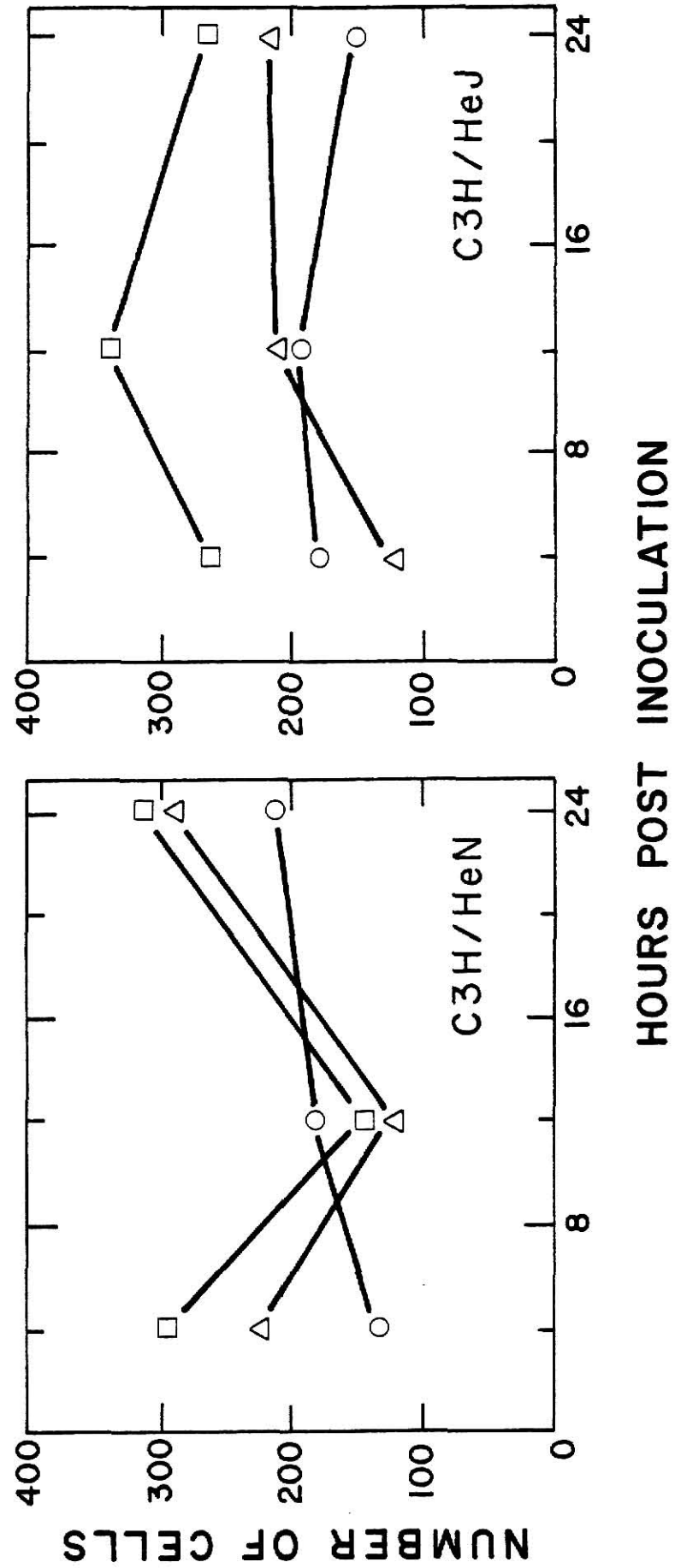
C3H/HeN
C3H/HeJ
NUMBER OF CELLS
HOURS POST INOCULATION
0
100
200
300
400
8
16
24

Figure 2C. Kinetics of peripheral-blood neutrophil responses of C3H/HeN and C3H/HeJ mice inoculated intraperitoneally with gonococci. Each point represents the mean of two observations. The differences between these two strains of mice with respect to dose of gonococci or time following inoculation were not significant.

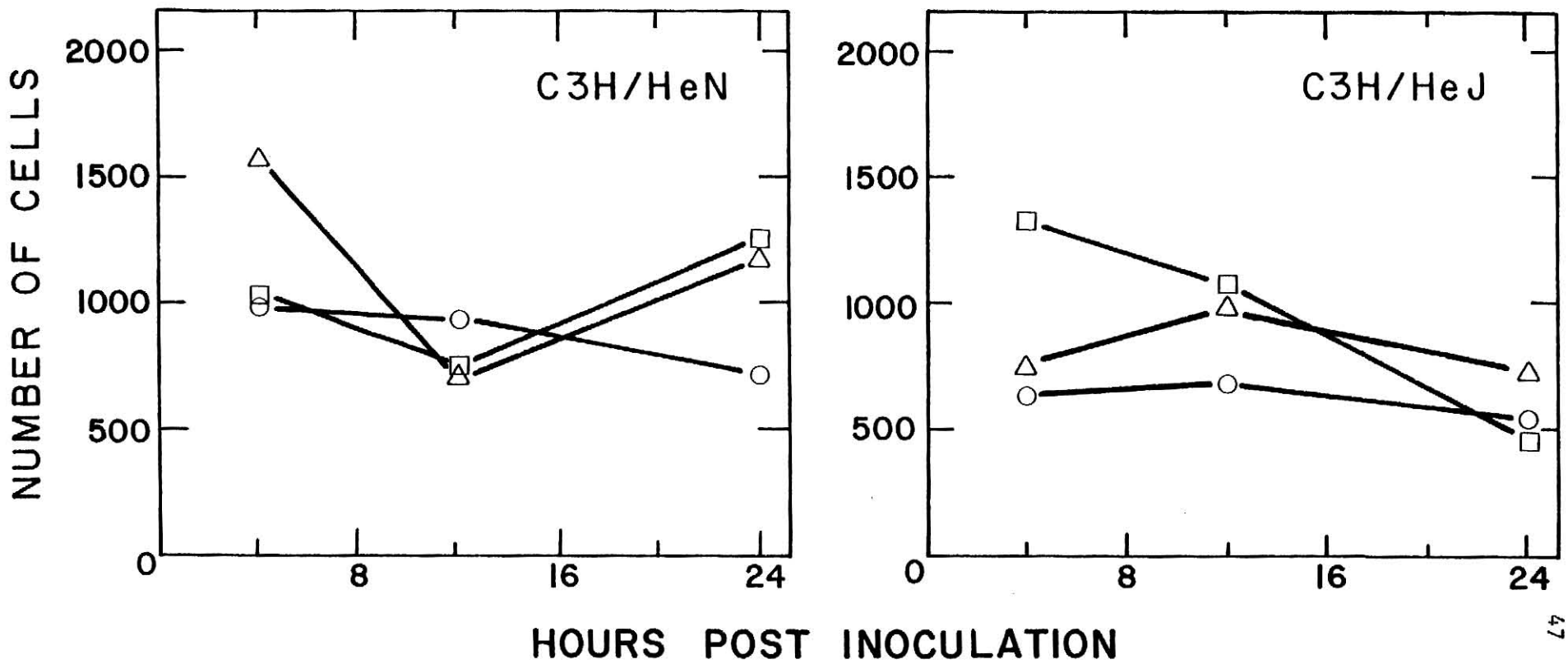
C3H/HeN
C3H/HeJ
NUMBER OF CELLS
2000
1500
1000
500
0
8
16
24
HOURS POST INOCULATION

Figure 2D. Kinetics of peripheral-blood lymphocyte responses of C3H/HeN and C3H/HeJ mice inoculated intraperitoneally with gonococci. Each point represents the mean of two observations. The differences between these two strains of mice with respect to dose of gonococci or time following inoculation were not significant.

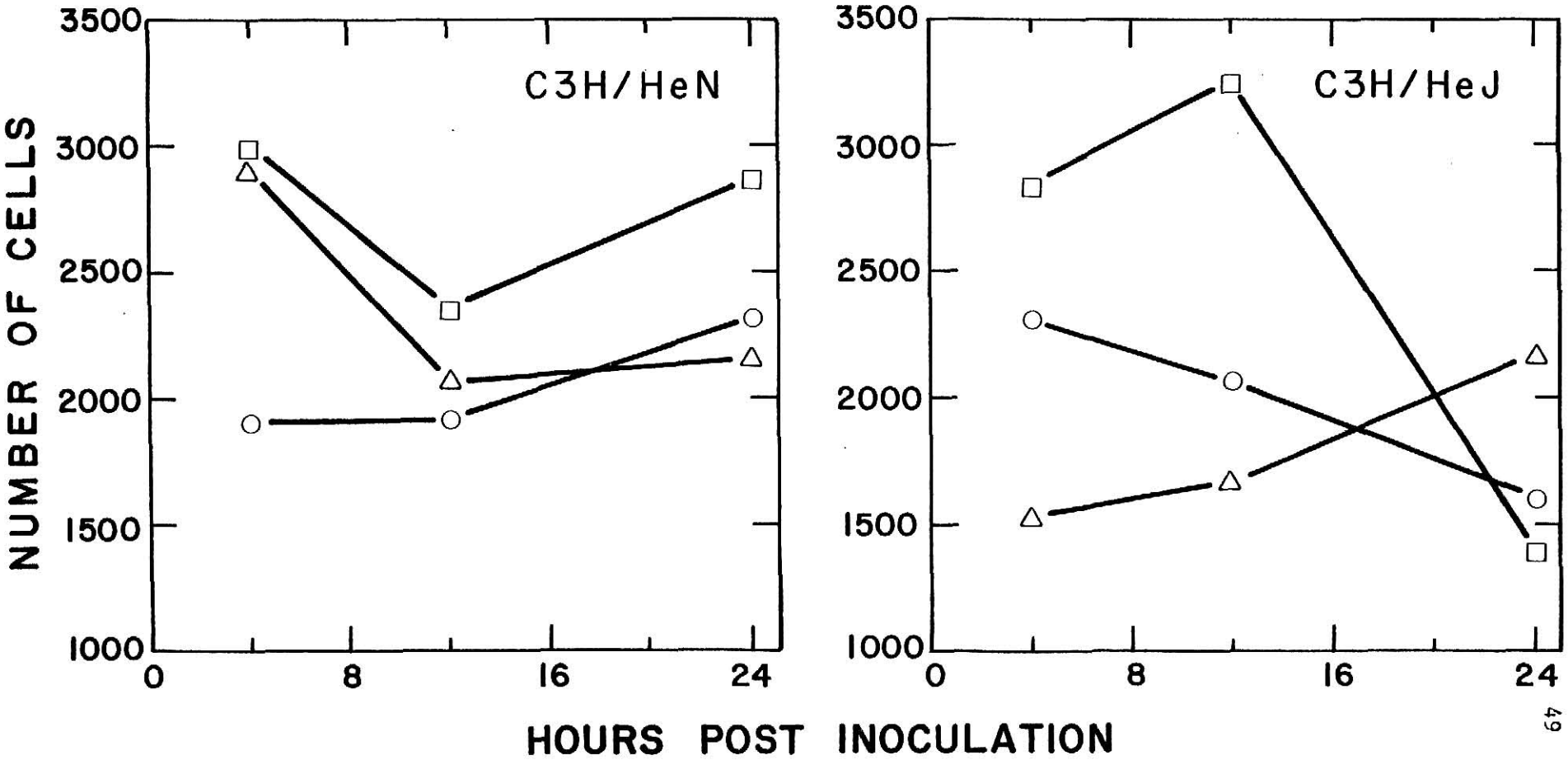
C3H/HeN
C3H/HeJ
NUMBER OF CELLS
3500
3000
2500
2000
1500
1000
0
8
16
24
HOURS POST INOCULATION

Table 2. Semiquantitative Counts of Normal Aerobic Flora Recovered from C3H/HeN and C3H/HeJ Mice

Mouse strain	No. with Gram-negative flora [b]					No. with Gram-positive flora [c]				
	+0[a]	+1	+2	+3	+4	+0	+1	+2	+3	+4
C3H/HeN [d]	19	9	12	4	6	24	16	4	3	0
C3H/HeJ [d]	7	10	11	7	11	41	4	0	0	0

a Semiquantitative indicators of the number of colonies formed by each bacterial isolate:
+0, no colonies;
+1, 1-9 colonies;
+2, 10-99 colonies;
+3, 100-299 colonies;
+4, >300 colonies.

b No significant difference was found between the semiquantitative counts of the Gram-negative flora isolated from C3H/HeN and C3H/HeJ mice.

c A significant difference was found between the semiquantitative counts of the Gram-positive flora isolated from C3H/HeN and C3H/HeJ mice.

d A significant difference was found between the semiquantitative counts of the Gram-negative and Gram-positive flora isolated from either mouse strain.

LITERATURE REVIEW

Neisseria gonorrhoeae, the causative agent of gonorrhea, is a Gram-negative coccus which is generally seen in pairs with adjacent sides flattened (Wilfert and Gutman, 1976). It is a fastidious organism with complex nutritional requirements (Wilfert and Gutman, 1976), and grows best between 36° and 38°C in the presence of 5-10% CO_2 in air (Drutz and Graybill, 1976). Man is the only known reservoir of the gonococcus, and primary infection generally begins on the mucosa of the urogenital tract (Davis et al., 1973). In approximately 1 to 3% of locally infected individuals, gonococci invade the bloodstream and produce systemic complications (Barr and Danielsson, 1975; Holmes et al., 1971 b).

Uncomplicated gonorrhea is currently epidemic throughout the world. In the United States alone, close to 1 million cases were reported in 1979 (Center for Disease Control, 1980). Historically, changes in the incidence of gonococcal infection have been associated with cultural changes and disruptions caused by warfare. During World War II the annual incidence of disease increased from a prewar level of approximately 154 reported cases per 100,000 people to 284 reported cases per 100,000 people (Lucas, 1972). Following World War II the annual incidence decreased through the late 1940s and early 1950s to prewar levels (Lucas, 1972). In the early 1960s the annual incidence of gonococcal infection again began to rise, and since that time has increased more than 300% (Wilfert and Gutman, 1976). Social acceptance of relaxed sexual mores (Lightfoot and Gotschlich, 1971), changes in contraception methods (Rein, 1977), and an increase in resistance of the gonococcus to antibiotics (Martin et al., 1970; Schofield et al., 1971), have all been implicated as causes of the current epidemic.

Control of this disease is made difficult by a number of factors: (1) The gonococcus is highly infectious (Wright and Daunt, 1973). (2) Gonorrhea has a very short incubation period, making it possible to transmit the infection to many individuals before it can be recognized and treated in the initially infected individual (Holmes, 1974). (3) Widespread asymptomatic infection makes diagnosis of this disease extremely difficult (Pariser, 1972; Handsfield et al., 1974).

The urethra is the primary site of urogenital gonococcal infection in the male. Following an incubation period of three to four days (Holmes, 1974), infected individuals generally develop acute anterior urethritis which is characterized by dysuria and a purulent urethral discharge (Drutz and Graybill, 1976). It has been estimated that as few as 2 to 3% (Rein, 1977), or as many as 10% (Pariser, 1972) of infected males remain asymptomatic. Individuals infected asymptomatically, or those with mild symptoms may not seek treatment. In untreated individuals, the primary urethral infection may spread and result in prostatitis, seminal vesiculitis, epididymitis (Holmes, 1974; Dahl and Dans, 1974), and/or disseminated gonococcal infection (Holmes et al., 1971 a).

The endocervix is the primary site of urogenital infection in the female. As in the male, the incidence of asymptomatic infection in the female is unclear, with estimates ranging from 19% (Handsfield et al., 1974) to 90% (Pariser, 1972). When present, symptoms may include vaginal discharge, menstrual irregularity, dysuria, and urinary frequency (Curran et al., 1975; Holmes, 1974). The most common complication of urogenital infection in the female is acute pelvic inflammatory disease (PID) which developes in approximately 10 to 17% of infected women (Eschenbach and Holmes, 1975), due to the spread of gonococci from the endocervix to the fallopian tubes (Rein, 1977). Following PID many women have impared fertility (Eschenbach and Holmes, 1975;

Westrom, 1975) and recurrent episodes of gonococcal and non-gonococcal PID (Eschenbach and Holmes, 1975).

In addition to colonization of the urogenital mucosa, gonococci can infect other body surfaces such as the eyes (Thompson et al., 1974), the rectum (Dans, 1975), and the pharynx (Wiesner et al., 1973 b; Corman et al., 1974). A large proportion of the individuals with pharyngeal (Wiesner et al., 1973 b; Wiesner, 1975) and rectal (Dans, 1975) infections remain asymptomatic.

It has been estimated that disseminated gonococcal infection (DGI) occurs in approximately 1 to 3% of the individuals with mucosal forms of gonorrhea (Barr and Danielsson, 1975; Holmes et al., 1971 b). The clinical features of DGI generally include suppurative arthritis, tenosynovitis, and dermatitis (Holmes et al., 1971 a; Handsfield, 1975). In addition, DGI can occasionally lead to meningitis, endocarditis, or pericarditis (Holmes et al., 1971 a). Both microbial and host factors have been associated with dissemination of the gonococcus. Host factors related to increased incidence of DGI include pregnancy (Holmes et al., 1971 a; Brandt et al., 1974), menstruation (Holmes et al., 1971 a; Keiser et al., 1968), and the asymptomatic carrier state (Holmes et al., 1971 a). Microbial factors include serum resistance (Schoolnik et al., 1976), auxotype (Knapp and Holmes, 1975), antibiotic susceptibility (Wiesner et al., 1973 a), and light/transparent colony type (James and Swanson, 1978).

In order to better understand the pathogenesis and immunobiology of gonococcal infection, different laboratory models have been developed. These models allow for controlled, repeted studies, where a sufficient number of observations can be obtained for statistical analysis. A great deal of valuable information about mucosal gonococcal infection has been obtained through the study of genital infection of the chimpanzee. This model has been used to study local immunity following genital infection (Kraus et al., 1975),

as well as immunity to genital infection following systemic immunization (Arko et al., 1974; Arko et al., 1976). Although genital infection in the chimpanzee is similar to the infection in man, these animals are expensive to maintain and small numbers are available for study. In addition, much information about DGI has been obtained through the study of infections in subcutaneous chambers in animals (Arko, 1972) and through the study of endocarditis in the dog (Drutz, 1978). Although useful, these models are disadvantageous since they require surgery and the diseased produced does not progress from local to disseminated infection. An in vitro model using human fallopian tubes in organ culture has also been used to study gonococcal virulence factors (McGee et al., 1976; McGee et al., 1978). Although valuable, the absence of inflammatory cells and serum factors in this model prevents the study of host responses to gonococcal virulence factors. More recently, two mouse models have been developed for study of the immunobiology of N. gonorrhoeae. The first is a model of DGI which progresses from local peritonitis to transient, or lethal bacteremia, depending on the dose of gonococci administered (Corbeil et al., 1979). In this model, a combination of mucin and hemoglobin is used as a suspending vehicle for gonococci. This vehicle lowers the lethal dose by interfering with host defense mechanisms (Corbeil et al., 1979). The second model is of the early stages of acute genital infection in female mice (Braude et al., 1978). In this model the stage of the sex cycle determines survival of gonococci on the mucosal surface. The amount of normal flora and the number of phagocytes, which vary with stage of the sex cycle, are thought to be the important mechanisms of defense against this infection. These two mouse models are particularly useful for study of the host-parasite relationship during gonococcal infection.

During the past two decades many factors have been associated with gonococcal virulence. Some of these factors include colonial type (Kellogg et al.,

1963; Kellogg et al., 1968), the presence of pili (Swanson et al., 1971), serum resistance (Schoolnik et al., 1976), color/opacity colony variants (James and Swanson, 1978), and endotoxin (McGee et al., 1978). With the exception of gonococcal endotoxin, which has been shown to be cytotoxic in vitro (McGee et al., 1978), these characteristics have only been associated with infectivity. The two mouse models previously mentioned, provide excellent systems for studying the role of gonococcal endotoxin in vivo.

In 1968, while studying intraspecies variation in responses to endotoxin, Sultzer (1968) discovered a mouse strain (C3H/HeJ) which responded to intraperitoneal inoculation of endotoxin differently than all other strains tested. He found that C3H/HeJ mice were much more resistant than the other strains tested to the lethal effects of either Salmonella typhosa endotoxin or Escherischia coli endotoxin. He also demonstrated that 24 hours after inoculation of mice with a low dose of endotoxin from either bacterial species, the number of peritoneal mononuclear cells increased dramatically above normal levels in mice of the C3H/HeJ strain, while the number fell below normal levels in mice of all other strains tested. In a later study, Sultzer (1969) found that 24 hours after inoculation of C3H/HeJ mice with a high dose of endotoxin, the number of peritoneal neutrophils increased well above normal levels, while the number of peritoneal macrophages remained unchanged.

The discovery of the endotoxin resistant C3H/HeJ mouse strain provided a useful tool for analyzing the genetic and cellular aspects of responses to endotoxin. In addition to an altered peritoneal inflammatory response and resistance to endotoxin induced lethality, C3H/HeJ mice appear to respond abnormally to most effects of endotoxin. The lymphocytes of C3H/HeJ mice do not respond mitogenically or immunogenically to endotoxin (Watson and Riblet, 1974), their macrophages are resistant to the in vitro cytotoxic effects of

endotoxin (Glode et al., 1977; Peavy et al., 1978), and an adjuvant effect is not produced by endotoxin (Skidmore et al., 1975). In addition, genetic studies of C3H/HeJ mice indicated that the abnormal responses of this mouse strain to endotoxin are controlled by a single autosomal gene (Watson and Riblet, 1974).

These mice also provide excellent tools for the study of the role of endotoxin and endotoxin responsiveness in disease production by Gram-negative organisms. It has been demonstrated that C3H/HeJ mice are more resistant than control mice to extracellular infection with E. coli (Sultzer and Goodman, 1977), while they are more susceptible than control mice to intracellular infection with Salmonella typhimurium (Vas et al., 1973). From these results Sultzer and Goodman (1977) suggested that the role of endotoxin resistance as a protective mechanism may differ for infection with extracellular and intracellular Gram-negative pathogens.

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GONOCOCCAL INFECTION IN MICE: MICROBIAL AND HOST FACTORS RELATED TO INFECTION

by

PHILIP REEL STREETER

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ABSTRACT

Two mouse models of gonococcal infection were used to study host and microbial factors which may be involved in the pathogenesis of gonococcal infection. The first of these models is of disseminated gonococcal infection (DGI). In this model, infection progresses from local peritonitis to transient or lethal bacteremia, depending on the dose of gonococci administered. The second is a model of gonococcal survival in the female genital tract.

The DGI model was used to study several factors thought to influence virulence. It was demonstrated that neither stage of growth (log or stationary), nor culture media (agar or broth) affected virulence of the gonococcus. Also, nonpiliated type 4 colonies were equally as virulent as piliated type 2 colonies, suggesting that pili are not important virulence factors in producing gonococcal bacteremia. Furthermore, the median lethal dose (LD_{50}) was not affected by the stage of the estrus cycle. However, sexual factors may be important since the LD_{50} for male mice ($10^{7.9}$) was significantly higher than the LD_{50} for female mice ($10^{6.9}$). This finding suggests that inherent differences in defense mechanisms between men and women may account in part for the higher frequency of dissemination observed in women than in men.

The role of endotoxin sensitivity in defense against gonococcal infection was studied in endotoxin resistant (C3H/HeJ) and endotoxin sensitive (C3H/HeN) mice. The ability of these mice to eliminate gonococci was studied in both the DGI model and the model of gonococcal survival in the female genital tract. The median lethal dose (LD_{50}) in the DGI model was $10^{9.6}$ for C3H/HeJ mice and $10^{5.1}$ for C3H/HeN mice. Levels of bacteremia during infection indicated that C3H/HeJ mice cleared large numbers of gonococci from their peripheral blood by 24 hours post inoculation, while C3H/HeN mice did not.

Additionally, the peritoneal leukocyte response, following intraperitoneal inoculation of gonococci was greater in C3H/HeJ mice than in C3H/HeN mice. These findings suggest that the ability to mount an inflammatory response to endotoxin may be important in defense against DGI. Besides being different in susceptibility to DGI, C3H/HeJ mice were found to be more resistant than C3H/HeN mice to genital colonization by gonococci. The resistance of C3H/HeJ mice to genital colonization by gonococci appeared to be due to both the high numbers of PMN leukocytes in the genital secretion and the predominance of inhibitory Gram-negative genital flora in that mouse strain.