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# A mouse intracerebral infection with *Neisseria gonorrhoeae**

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Kellogg, Peacock, Deacon, Brown, and Pirkle (1963) described four colony types of *Neisseria gonorrhoeae*. Subsequently, these authors reported that colony types 1 and 2 (T1, T2) were virulent for human volunteers while T3 and T4 were avirulent. However, studies on the immunology and pathogenesis of *N. gonorrhoeae* colony types have been limited by the lack of an animal model susceptible to *N. gonorrhoeae* infection. Brown, Lucas, and Kuhn (1972) have recently described the experimental infection of the chimpanzee with both fresh gonorrhoeal pus and with T1 colonial type of *N. gonorrhoeae*: the cost and scarcity of this animal, however, severely limit the use of this model. Arko (1972) and Flynn and Waitkins (1973) have reported the *in vivo* cultivation of gonococci in surgically-implanted chambers in laboratory rodents. Buchanan and Gotschlich (1973) and Bumgarner and Finkelstein (1973) have used the chick embryo for the study of the virulence of gonococci; the value of these techniques for immunological studies remains to be defined.

This report describes the intracerebral challenge of the C57 mouse with *N. gonorrhoeae* and its application in the assay of experimental gonococcal vaccines.

## Material and methods

### (A) *N. gonorrhoeae* strains

Those used included No. 188, received from the Ontario Public Health Laboratory, Toronto, Ontario; No. G9, obtained from Dr. H. Schneider, Walter Reed Army Institute of Research, Washington, D.C.; Nos. IN15, IN17, IN21, and IN31 isolated from asymptomatic carriers during a gonococcal vaccine trial in Inuvik, North West Territories, Canada (Greenberg, Diena, Ashton, Wallace, Kenny, Znamirowski, Ferrari, and Atkinson, 1974); Nos. 1630, 1631, 1639, 1646, and 1676 received from the Bacteriological Laboratories of the Ottawa Civic Hospital, Ottawa, Canada.

Colony types T1, T2, T3, and T4 were isolated and maintained by daily passage on GC agar medium (BBL) containing 0·001 per cent. $Fe(NO_3)_3$ final concentration, 2 per cent defined supplement, and 1·5 per cent. Oxoid Agar as recommended by Kellogg and others (1963).

### (B) Challenge

Cultures of *N. gonorrhoeae* grown on GC agar plates for 16 hrs were suspended in Dulbecco phosphate buffer pH 7·2. The inoculum was adjusted to an optical density of 0·5 using a Bausch and Lomb Spectronic 20 at 540 nm. (equivalent to $10^9$ colony-forming units per ml.). Mice were anesthetized and injected intracerebrally with 0.03 ml. of bacterial suspension at different challenge dilutions. $LD_{50}$ values were calculated according to Reed and Muench (1938).

### (C) Vaccines

*N. gonorrhoeae* strain 188, T1 and T4, were grown on GC agar for 16 hrs and harvested in Sorensen's buffered saline pH 7·2. Vaccines were prepared by addition of formalin (final concentration 3 per cent.) to the above suspensions and incubation of the mixtures at room temperature for 30 min. Cells were then sedimented (2,800 G for 20 min.) and re-suspended in Sorensen's buffered saline containing 0·3 per cent. formalin. Vaccines were adjusted to an optical density of 0·5 using a Bausch and Lomb Spectronic 20 at 540 nm. Mice were vaccinated intraperitoneally with a 0·5 ml. dose of vaccine and were challenged intracerebrally 2 weeks later with a dose equivalent to 10 $LD_{50}$s.

### (D) Purified lipopolysaccharide (LPS)

LPS was prepared from *N. gonorrhoeae* T1 strain G9, as described in a recent report (Perry, Daoust, Diena, Ashton, and Wallace, 1974).

## Results and discussion

Preliminary experiments indicated that of the several C57 mouse strains tested, the HPB black strain† was consistently the most susceptible to gonococcal infection. Female mice (16–18 gm.) were used in these studies, since the males were uniformly more resistant to gonococci.

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†Crossbreed of strains C57 B/10 V (Jackson Laboratories, Bar Harbor, Maine) and C57 B/6 (P) (Flow Laboratories, Dublin, Virginia), randomly bred at the Breeding Colony of the Health Protection Branch, Health and Welfare, Ottawa, Canada.

Throughout these experiments, strain 188 was regularly shown to be the most pathogenic, while the Ottawa and Inuvik clinical strains were less virulent. Death occurred from 1 to 6 days after challenge. Representative data in Table I show the pathogenic effects of living and heat-killed *N. gonorrhoeae* strain 188 when injected intracerebrally into mice. It appears from these data that, at the 1 : 5 dose level, T1 gonococci are more pathogenic for mice than the other types in comparison with the heat-killed challenge ($P < 0.01$). Subsequent titrations yielded essentially similar results.

Living gonococci were recovered up to the fifth day from the brain of mice, after intracerebral infection with *N. gonorrhoeae* T1. Organisms recovered 5 days after infection yielded essentially pure culture of T1 colonies, whereas T3 were present in earlier recoveries. Similarly, T1 colonies were obtained up to the fifth day from liver, kidney, and spleen (Table II).

TABLE II *Recovery of* N. gonorrhoeae *from organs of HPB mice infected with* N. gonorrhoeae $T_1$, $T_2$, $T_3$, *or* $T_4$

| | *Colony type* N. gonorrhoeae | |
|---|---|---|
| *Organ* | $T_1$ | $T_2$, $T_3$, $T_4$ |
| Brain | + | + |
| Liver | + | − |
| Kidney | + | − |
| Spleen | + | − |

Recovery of bacteria from brain and other organs having experimental infection with types T2, T3, and T4 was positive from brain tissue only, showing the inability of colony types other than T1 to cause a generalized infection. It is also of interest that, while the colony type of the bacteria recovered was consistent with the type of gonococci used in the challenge, T1 and T2 were occasionally recovered from T4 challenges, showing the possibility of type reversion *in vivo*.

Electron microscopy studies of experimentally-infected mouse brain, sampled 4 days after intracerebral challenge with T1 gonococci will be fully described elsewhere (Ota, Diena, and Ashton, in preparation). Briefly, scattered intracellular bacteria were noted regularly. These seemed to be morphologically intact with complete cell walls, cytoplasmic membranes, and other intracytoplasmic structures. The microscopical examination also suggested that the infection by the gonococci was not localized but rather diffuse in the brain.

These data suggest that *N. gonorrhoeae* cells multiply *in vivo* after challenge of HPB black mice and succeed in producing an infection which may become generalized. Mice die from the combined effect of the bacterial mutiplication and the toxic effect of the released endotoxin.

Results to be published elsewhere (Diena, Ashton, and Wallace, unpublished observations) indicate that a strain of C57 mice deficient in the complement factor C'5* is resistant to this *N. gonorrhoeae* challenge. On the other hand, the same strain of C57 mice, but with full expression of the complement†, is susceptible to gonococcal infection suggesting the role of C'5 released anaphylatoxin in producing a local damage (Gewurz, 1971) enabling the growth and multiplication of the bacteria.

This challenge was used to evaluate the potency of experimental gonococcal vaccines. Groups of mice were vaccinated with formalin-killed vaccines as

*B10 old (Jackson Laboratories, Bar Harbor, Maine).
†B10 new (Jackson Laboratories, Bar Harbor, Maine).

TABLE I *Representative data of pathogenicity of colony types of* N. gonorrhoeae, *strain* 188, *in HPB black mouse*

| *Colony type* | *Bacterial dilution* | *Challenge by live organisms* $\left(\frac{\text{No. of mice surviving}}{\text{No. of mice injected}}\right)$ | *LD50 (in colony-forming units per dose)* | *Challenge by heat-killed organisms*[a] $\left(\frac{\text{No. of mice surviving}}{\text{No. of mice injected}}\right)$ |
|---|---|---|---|---|
| T1 | undiluted | 1/15 ($P<0.01$[c]) | $1.0–2.0 \times 10^6$ | 9/15 |
| | 1: 5 | 2/15 ($P<0.01$) | | 11/15 |
| | 1: 25 | 14/15 | | 15/15 |
| T2 | 1: 1[b] | 1/15 ($P<0.05$) | $3.0–6.0 \times 10^6$ | 7/15 |
| | 1: 5 | 11/15 | | 13/15 |
| | 1: 25 | 14/15 | | 14/14 |
| T3 | 1: 1[b] | 0/15 ($P<0.01$) | $1.5–3.0 \times 10^6$ | 7/15 |
| | 1: 5 | 7/15 | | 10/15 |
| | 1: 25 | 14/15 | | 15/15 |
| T4 | 1: 1[b] | 1/15 ($P<0.01$) | $2.0–4.0 \times 10^6$ | 8/15 |
| | 1: 5 | 8/15 | | 11/15 |
| | 1: 25 | 14/15 | | 15/15 |

[a]Killed at 56°C./30 min.
[b]Viable count per dose of approximately $10–20 \times 10^6$ colony-forming units of gonococci
[c]Significantly more virulent than the heat-killed challenge in the same horizontal line

well as with purified smooth LPS from *N. gonorrhoeae*. Table III shows that good protection was achieved with gonococcal vaccines and with LPS at a concentration of 40 μg. per dose. The mouse intracerebral challenge is at present being evaluated with whole cell and purified homologous and heterologous vaccines.

TABLE III *Mouse protection tests using gonococcal vaccines and purified lipopolysaccharide (LPS)*

| | *Challenged with* 10 $LD_{50}$*'s of strain No.* 188 | | |
|---|---|---|---|
| *Vaccine* | *Test* 1 | *Test* 2 | *Test* 3 |
| Control, no vaccine | 1/15[a] | 0/15 | 1/15 |
| *N. gonorrhoeae* $T_1$ | 10/15 | 10/15[b] | 13/15[b] |
| $T_4$ | 7/15 | 3/15 | 8/15 |
| LPS (μg.) 40.0 | N.T. | N.T. | 9/11 |
| 10.0 | N.T. | N.T. | 6/15 |
| 2·5 | N.T. | N.T. | 1/15 |

[a] Survivors/injected
[b] Significantly better ($P < 0.05$) than the $T_4$ vaccine in the same column

## Summary

An intracerebral challenge of HPB black mice with *Neisseria gonorrhoeae* is described. In this model, the mice died from 1 to 6 days after challenge, and T1 organisms were obtained up to the fifth day from brain, liver, kidney, and spleen. Experimental gonococcal vaccines gave good protection against the challenge.

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## Une infection intracérébrale par *Neisseria gonorrhoeae* chez la souris

SOMMAIRE

On décrit une infection intracérébrale par *Neisseria gonorrhoeae* chez des souris noires HPB. Avec ce modèle, les souris moururent de 1 à 6 jours après l'épreuve, et des organismes T1 furent obtenus jusqu'au sixième jour dans le cerveau, le foie, le rein et la rate. Les vaccins gonococciques expérimentés donnirent une bonne protection contre l'infection.