INFECTION AND IMMUNITY, July 1980, p. 287-289
0019-9567/80/07-0287/03$02.00/0

Vol. 29, No. 1

Cotransformation of a Serum Resistance Phenotype with Genes for Arginine Biosynthesis in *Neisseria gonorrhoeae*

SHARON K. SPRATT,† FRED JONES, THOMAS E. SHOCKLEY, AND JULIUS H. JACKSON

Department of Microbiology, Meharry Medical College, Nashville, Tennessee 37208

Two genes (*arg-3* and *arp-6*) marked by arginine auxotrophy and a gene (*sac-2*) marked by resistance to serum killing were found to be linked by cotransformation. Evidence derived from the linkage of these markers to *spc* (spectinomycin resistance) suggests that *sac-2* may be genetically distinct from a *sac* gene previously described.

Arginine auxotrophs are commonly found among clinical isolates of *Neisseria gonorrhoeae* in frequencies as high as 40% of the total isolates (1-3). Strains of *N. gonorrhoeae* most frequently associated with disseminated gonococcal infection are auxotrophic for arginine (Arg^-), hypoxanthine (Hyx^-), and uracil (Ura^-). These strains are usually highly sensitive to penicillin and tetracycline, ferment sugars weakly, and are resistant to the bactericidal action of normal human serum (7, 12, 13). The roles of auxotrophy and resistance to serum killing are subjects of considerable speculation regarding their contribution to pathogenic mechanisms. Eisenstein et al. (4) have determined that sensitivity to serum killing (Sac^s) and penicillin (Pen^s) are directly correlated with virulence, whereas arginine auxotrophy may enhance the virulence of strains already Pen^s or serum resistant. Auxotrophy for nutrients which are in short supply in the human circulatory system may help these strains to escape from penicillin therapy, which only kills growing cells.

Mayer et al. (9) reported that genetic lesions which cause Arg^-, Hyx^-, and Ura^- in strains they tested were unlinked to a locus responsible for serum resistance. Guymon et al. (5) found that a locus for resistance to normal serum antibody and complement killing (*sac*) cotransformed with *penB* and *spc* (spectinomycin resistance). In a paper presented at the 70th Annual Meeting of the American Society for Microbiology (S. Spratt, F. Jones, T. E. Shockley, and J. H. Jackson, Abstr. Annu. Meet. Am. Soc. Microbiol. 1979, D15, p. 42), we reported the occurrence of *arg* alleles in the region of the chromosome where *sac* was found to lie.

In this study we show that an allele (*sac-2*) which causes Sac^r cotransforms with two alleles for arginine biosynthesis and is probably distinct from the *sac* locus described by Guymon et al. (5) which we designated *sac-1*.

Cultures were routinely grown on GC medium base agar (Difco) containing 1% (vol/vol) defined supplements (6) at 36°C in a humidified atmosphere with 5% CO_2. Clinical isolates were obtained from the division of Laboratory Services, Tennessee Department of Public Health, Nashville, and identified as *N. gonorrhoeae* according to established procedures. A chemically defined medium, GGM minimal, was used to characterize strains according to auxotype (8). Auxotyping was done according to the procedure of Morello et al. (10).

Sensitivity to killing by normal human serum was measured by determining the fraction of cells surviving exposure to pooled human sera for timed intervals. The results were plotted as the logarithm of the surviving fraction versus time. Since the cell killing is described by a negative exponential curve, the equation $\log (N/N_0) = -kt$ describes the curve through the first 15 min of exposure to serum, where N_0 is the initial cell number, N is the final cell number, t is the time of exposure to serum, and $-k$ is the slope of the curve. A value for the constant k, for complement-mediated killing, was determined from the expression: $k = k_e - k_c$, where k_e is the constant measured for a given strain in the presence of unheated serum, and k_c is the constant measured in the presence of serum heated at 56°C for 1 h to inactivate the complement. Routine serum-killing assays were done using a 15-min exposure to serum, and the value of k was calculated for each strain.

From unpublished studies in this laboratory, six genetically distinct classes of arginine auxotrophs have been found in clinical isolates of *N. gonorrhoeae*. Two recombinationally distinct alleles, *arg-3* and *arg-6*, were found to lie in a linkage group with a locus for serum resistance. The *arg-3* allele was shown to be linked to *spc*

† Present address: Department of Molecular Biology and Biochemistry, University of California, Irvine, CA 92664.

TABLE 1. *Cotransformation of arg-3 with spc, rif, and str*

Donor (phenotype)	Recipient (phenotype)	Selected phenotype	Transformants screened	Unselected phenotype	Unselected phenotype appearance	Cotransformation frequency (%)
JN39 (Arg^+ Spc^r)	JN231 (Arg^- Spc^s)	Arg^+	553	Spc^r	7	1.33
JN35 (Arg^+ Rif^r)	JN231 (Arg^- Rif^s)	Arg^+	272	Rif^r	0	0
		Rif^r	272	Arg^+	0	0
JN25 (Arg^+ Str^r)	JN231 (Arg^- Str^s)	Arg^+ Str^r	272	Str^r	0	0

(spectinomycin resistance) by cotransformation, but *arg-3* did not cotransform with *rif* (rifampin resistance) or *str* (streptomycin resistance) (Table 1). Unlinked marker congression, as an explanation for the low linkage, was ruled out by deoxyribonucleic acid dilution experiments (data not shown). The *arg-6* allele did not cotransform with *spc*, *rif*, or *str* when at least 500 transformants were screened.

Sensitivity to serum killing was measured in a series of strains which were characterized according to arginine auxotrophy and sensitivity to serum killing (Table 2). These strains fell into three classes of sensitivity: high ($k \simeq 0.13$ to 0.16), intermediate ($k \simeq 0.05$), and low ($k \simeq 0.005$). Strains with low sensitivity were considered resistant.

Resistance to serum killing, conferred by *sac-2*, cotransformed with *arg-3* and with *arg-6* (Table 3). Owing to the lengthy and tedious method used to measure resistance to serum killing, a small number of Arg^+ transformants were screened for Sac^r (Table 3). We found clear evidence of cotransformation linkage between *arg-3* and *sac-2*, and between *arg-6* and *sac-2*. The average value of $-k$ in the 12 transformants of strain JN240 which received *sac-2* was 0.005 ± an average absolute deviation of 0.0015.

According to published data (11) and from mapping studies in our laboratory, *rif*, *str*, and *spc* form a linkage group in that respective order. Since *arg-3* cotransforms with *spc* at a low frequency, but not at all with *rif* or *str*, a probable gene order is *rif-str-spc-arg-3*. The *sac-2* allele cotransforms with *arg-3* and *arg-6*, but *arg-6* does not cotransform with *rif*, *str*, or *spc*. This suggests a gene order of *rif-str-spc-arg-3-sac-2-arg-6* or *rif-str-spc-arg-3-arg-6-sac-2*, since the positions of *arg-6* and *sac-2* cannot be unequivocally determined from these data.

Guymon et al. (5) reported the *sac-1* locus between *spc* and *penB*, based upon cotransformation frequencies of *sac-1* with *rif* (6%), *spc* (26%), and *penB* (83%). The linkage of *arg-3* to *spc* (1.33%) places *sac-1* between *spc* and *arg-3*. Thus the *sac-2* locus in this study is probably genetically distinct from *sac-1*. Therefore, the probable gene order is either *rif-str-spc-sac-1-arg-3-sac-2-arg-6* or *rif-str-spc-sac-1-arg-3-arg-6-sac-2*.

It is clear from this study that the Sac^r phenotype from *sac-2* is not produced as a consequence of the arginine auxotrophy, since prototrophic transformants were found which were Sac^r.

TABLE 2. *Sensitivity to serum killing*

Strain	Relevant genotype	Relevant phenotype	k ($-min^{-1}$)[a]	Source
JN220	*arg-2 arg-5 sac-2*	Arg^- Sac^r	0.005	This study
JN224	*arg-3*	Arg^- Sac^s	0.050	This study
JN230	*arg-3*	Arg^- Sac^s	0.050	This study
JN231	*arg-3*	Arg^- Sac^s	0.160	This study
JN240	*arg-6*	Arg^- Sac^s	0.130	This study

[a] This constant for serum killing (k) is defined in the text by the expression $-k = \frac{1}{t} \log (N/N_0)$, where k has units of $-min^{-1}$. The value of the constant increases with increasing sensitivity to serum killing.

TABLE 3. *Cotransformation of arg-3 and arg-6 with sac-2*

Donor (relevant genotype)	Recipient (relevant genotype)	Selected marker	Transformants screened	Unselected marker	Unselected marker appearance	Cotransformation frequency (%)
	JN224 (*arg-3*)	*arg-3*$^+$	12	*sac-2*	3	8.3
	JN230 (*arg-3*)	*arg-3*$^+$	12	*sac-2*	0	
	JN231 (*arg-3*)	*arg-3*$^+$	12	*sac-2*	0	
JN220 (*arg-2 arg-5 sac-2*)	JN240 (*arg-6*)	*arg-6*$^+$	15	*sac-2*	12	80

This research was supported by Public Health Service Minority Biomedical Support Grant RR08037 from the National Institutes of Health and by grant SER77-04625 from the National Science Foundation.

One of the authors, J.H.J., is a recipient of Research Career Development Award CA00533 from the National Cancer Institute.

LITERATURE CITED

1. **Carifo, K., and B. W. Catlin.** 1973. *Neisseria gonorrhoeae* auxotyping: differentiation of clinical isolates based on growth responses on chemically defined media. Appl. Microbiol. **26**:223-230.
2. **Catlin, B. W.** 1973. Nutritional profiles of *Neisseria gonorrhoeae*, *Neisseria meningitidis* and *Neisseria lactamica* in chemically defined media and the use of growth requirements for gonococcal typing. J. Infect. Dis. **128**:178-194.
3. **Catlin, B. W.** 1978. Characteristics and auxotyping of *Neisseria gonorrhoeae*, p. 345. *In* T. Bergan, J. R. Novis (ed.), Methods in microbiology, vol. 10. Academic Press Inc., London.
4. **Eisenstein, B. I., T. J. Lee, and P. F. Sparling.** 1977. Penicillin sensitivity and serum resistance are independent attributes of strains of *Neisseria gonorrhoeae* causing disseminated gonococcal infection. Infect. Immun. **15**:834-841.
5. **Guymon, L. F., T. J. Lee, D. Walstad, A. Schmoyer, and P. F. Sparling.** 1978. Altered outer membrane components in serum-sensitive and serum-resistant strains of *Neisseria gonorrhoeae*, p. 139-141. *In* G. F. Brooks, E. C. Gotschlich, F. E. Young, W. D. Sawyers, and K. K. Holmes (ed.), Immunobiology of *Neisseria gonorrhoeae*. American Society for Microbiology, Washington, D.C.
6. **Kellogg, D. S., Jr., W. T. Peacock, Jr., W. E. Deacon, L. Brown, and C. I. Pirkle.** 1973. *Neisseria gonorrhoeae*. I. Virulence genetically linked to colonial variation. J. Bacteriol. **85**:1274-1279.
7. **Knapp, J. S., and K. K. Holmes.** 1975. Disseminated gonococcal infections caused by *Neisseria gonorrhoeae* with unique nutritional requirements. J. Infect. Dis. **132**:204-208.
8. **La Scolea, L. J., Jr., and F. E. Young.** 1974. Development of a defined minimal medium for the growth of *Neisseria gonorrhoeae*. Appl. Microbiol. **28**:70-76.
9. **Mayer, L. W., G. K. Schoolnik, and S. Falkow.** 1977. Genetic studies on *Neisseria gonorrhoeae* from disseminated gonococcal infections. Infect. Immun. **18**:165-172.
10. **Morello, J. A., S. A. Lerner, and M. Bohnhoff.** 1976. Characteristics of atypical *Neisseria gonorrhoeae* from disseminated and localized infections. Infect. Immun. **13**:1510-1516.
11. **Sarrubbi, F. A., Jr., E. Blackman, and P. F. Sparling.** 1974. Genetic mapping of linked antibiotic resistance loci in *Neisseria gonorrhoeae*. J. Bacteriol. **120**:1284-1292.
12. **Schoolnik, G. K., T. M. Buchanan, and K. K. Holmes.** 1976. Gonococci causing disseminated gonococcal infection are resistant to bactericidal action of normal human serum. J. Clin. Invest. **58**:1163-1173.
13. **Wiesner, P. J., H. H. Handsfield, and K. K. Holmes.** 1973. Low antibiotic resistance of gonococci causing disseminated infection. N. Eng. J. Med. **288**:1221-1222.