INFECTION AND IMMUNITY, Mar. 1975, p. 453-459

Vol. 11, No. 3
Printed in U.S.A.

Studies on Gonococcus Infection

VIII. ^{125}Iodine Labeling of Gonococci and Studies on Their In Vitro Interactions with Eukaryotic Cells

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Received for publication 4 November 1974

Intact gonococci (GC) have been labeled with ^{125}iodine by the lactoperoxidase plus hydrogen peroxide procedure. The specific activities of types 2, 4, and 4* GC have been determined and are found to show small differences as follows: T4* $\geq$ T2 > T4. ^{125}I-labeled GC have been studied for their associations with both leukocytes and tissue culture cells. ^{125}I-labeled GC show the following relative order of association with the leukocytes: T2 = T4* $\gg$ T4. This contrasts with the relative degree of interaction between the GC and tissue culture cells, which follows the relative order: T2 > T4 = T4*. Trypsin pretreatment of GC markedly reduces the association of all three types (T2, T4, and T4*) with leukocytes but does not alter the level of attachment of any of the gonococcal types with tissue culture cells.

Virulence factors of *Neisseria gonorrhoeae* are incompletely elucidated but are the topics of recent studies from this and other research groups. Much interest has been aroused by correlating virulence of gonococci (GC) for man with specific colony types (3) and with further correlation of virulent colony types with pilation of GC comprising these colonies (2, 9). Extensions of those correlations have centered on possible biological phenomena that involve GC and are influenced by pilation of the organisms. Numerous reports indicate that pilated GC stick to several types of eukaryotic cells more avidly than nonpilated GC (1, 6, 7, 13). This difference has been attributed to the pili. Other investigations have questioned whether pilation can be correlated with interactions of GC with leukocytes (WBC). Although two groups suggest that the presence of pili reduce phagocytosis of GC by human WBC (5, 12), our studies are not in accord with this suggestion. We have found that association (attachment and phagocytosis) of GC with human WBC involves another gonococcal surface component, distinct from pili, which is trypsin, chymotrypsin, and heat labile and which may be present or absent from nonpilated organisms (10).

In the present report we have investigated the ^{125}iodination of intact, viable GC and use of the radiolabeled GC in studies on their interactions with both WBC and tissue culture cells of epithelial origin. Differences in ^{125}I labeling of various colony types, the differing interactions of diverse colony types of ^{125}I-labeled GC, and the influence of tryptic digestion of GC prior to their incubation with these cells are the topics of the present report.

MATERIALS AND METHODS

GC. Strains F62 and MS11 of *N. gonorrhoeae* were used throughout, and the derivation, identification, and propagation of these organisms have been described previously in detail (7, 9). Each of the strains was propagated as essentially "pure" cultures of types 2 and 4 colony forms by subculture of single colonies every 24 h on GC agar (GC agar base plus 1% IsoVitalex, Baltimore Biological Laboratories, Baltimore, Md.) and incubation at 36 C in a 5% CO_2 incubator. Identification of the T4 and T4* forms of these two strains has also been described previously (10).

^{125}I labeling of GC. GC were removed from GC agar with Dacron-tipped swabs after 21 to 24 h of growth of relatively discrete, separated colonies and were suspended in phosphate-buffered saline (PBS) containing 1 mM $CaCl_2$ to yield 20 ml of suspension with an opacity of 80 Klett units (blue filter, Klett colorimeter). Each of the T2, T4, and T4* suspensions was then centrifuged, and their pellets were resuspended in 1 ml of PBS and ^{125}I labeled by slight modification of the method of Marchalonis et al. (4) by addition of 100 μl of lactoperoxidase (0.8 mg/ml) (Sigma Chemical Co., St. Louis, Mo., or Calbiochem, LaJolla, Calif.) and 100 μl of carrier-free ^{125}I as [^{125}I]Na (1.5 mCi/ml) (New England Nuclear Corp., Boston, Mass., or Amersham-Searle Corp., Des Plaines, Ill.). This mixture was thoroughly mixed in polycarbonate Oak Ridge-type tubes and was warmed to 30 C. A 100-μl amount of a 0.01 M H_2O_2 solution (J.

T. Baker Chemical Co., Phillipsburg, N.J.; obtained as 30% H_2O_2) was added at 0 time 2.5, 5, and 7.5 min during the total incubation period of 10 min carried out at 30 C. At the end of this time, 30 ml of ice-cold PBS plus 5 mM cysteine was added and, after thorough mixing, the mixture was centrifuged (15,000 × *g*, 10 min). The pelleted, ^{125}I-labeled GC were then washed three times with 30 ml (each wash) of cold PBS. After the final wash, the labeled organisms were resuspended in 5 ml of PBS and kept in an ice bath until used further.

Determining specific labeling of GC. The resuspended, ^{125}I-labeled GC of all three types (T2, T4, and T4*) were diluted in Medium 199 (M199) plus 0.01% bovine serum albumin to yield approximately 3×10^7 GC/ml (as determined for each sample by direct counting with Petroff-Hausser chambers). Aliquots of these suspensions were taken for determination of radioactivity while approximately 5-ml aliquots were recentrifuged. The supernatant from this recentrifuged sample was carefully removed (GC-free supernatant), and aliquots were taken for determining radioactivity. After determining the number of organisms of each type and the radioactivities in both GC suspensions and GC-free supernatants, the following calculation was utilized: specific activity (counts per minute of GC) = (counts per minute/ml in GC suspension) − (counts per minute of GC-free supernant per ml)/number of GC per ml.

Incubation of ^{125}I-labeled GC with monolayers of WBC of tissue culture cells. Duplicate plates of either WBC or tissue culture cell monolayers were utilized for incubation with each ^{125}I-labeled T2, T4, and T4* suspension and with the GC-free supernatants derived from each ^{125}I-labeled GC suspension. The WBC or tissue culture cell monolayers were overlain with 2 ml of either suspension or supernatant and were incubated at 36 C for 15 (WBC) or 60 min (tissue culture cells) on a rotating platform at approximately 80 rpm in a 5% CO_2 atmosphere. At the end of the incubation periods, the fluid overlays were decanted and each monolayer was washed vigorously with 10 ml of M199. The monolayers plus associated ^{125}I-labeled GC (or GC-free supernatant containing ^{125}I) were solubilized with either 5 N NaOH (1 h, room temperature) or 0.1% sodium dodecyl sulfate (variable periods of 5 to 30 min at room temperature). Aliquots of the solubilized monolayers that had been incubated with both GC suspensions and GC-free supernatants were then assayed for radioactivity in a Beckman BioGamma gamma counter equipped with a preset window for ^{125}I.

In a few instances, tissue culture cells and WBC were individually attached to glass cover slips (11 by 22 mm) which were placed in the bottom of petri dishes (35 by 10 mm) for incubation with GC suspensions and GC-free supernatants. After incubation with the gonococcal preparations, the cover slips were thoroughly washed and allowed to air dry. They were then placed directly into counting vials for determination of radioactivity.

Microscopic evaluation of GC-WBC association. ^{125}I-labeled GC were suspended in PBS to an optical opacity of 50 Klett units. These organisms were then utilized for incubation with human peripheral blood WBC monolayers as previously described in detail (10). Assessment of interactions between the labeled GC and neutrophils of the monolayer was accomplished through light microscopic evaluation of Giemsa-stained monolayers, and the percentage of neutrophils with attached or ingested GC was determined.

Trypsin treatment of ^{125}I-labeled GC. T2, T4, and T4* GC were radioiodinated, as described above. The washed, ^{125}I-labeled organisms were suspended in PBS (pH 7.4) to an optical opacity of 50 Klett units. These suspensions of T2, T4, and T4* GC were divided into two 4.5-ml aliquots. To one aliquot for each gonococcal type was added 0.5 ml of 1% trypsin (Worthington Biochemical Corp., Freehold, N.J.) and to the other was added 0.5 ml of PBS. These were mixed and both were incubated for 15 min at 36 C. The GC were then pelleted by centrifugation, washed thrice, and resuspended in 5 ml of PBS. The specific labeling of these trypsin-treated and control GC was determined as described above. After enumeration of the organisms in Petroff-Hausser chambers, the organisms were diluted for use with WBC or tissue culture cell monolayers.

Attachment of unlabeled GC to HeLa cells. HeLa cell suspensions were prepared by ethylenediaminetetraacetate plus trypsin removal of the cells from the flask and were washed three times in trypsin and ethylenediaminetetraacetate-free minimal essential medium (Grand Island Biological Co., Grand Island, N.Y.). These cells were resuspended to a concentration of 10^6/ml. GC (T2, T4, and T4*) were suspended in PBS and incubated either with 0.1% trypsin or in PBS for 15 min at 37 C. The GC were then washed three times in PBS and were finally resuspended in PBS to an optical opacity of 50 Klett units. These organisms were diluted 1:10 for incubation with WBC monolayers (to monitor effectiveness of trypsinization) or 1:10,000 for incubation with HeLa cells. These final dilutions were made in M199 containing 2% heat-inactivated fetal calf serum, which maintains viability without allowing excessive multiplication of the GC (8). A 0.1-ml amount of the gonococcal suspension (approximately 2×10^3 GC) was added to 0.9 ml of the HeLa suspension (approximately 10^6 HeLa cells) in M199 plus 2% fetal calf serum, and this mixture was rotated in snap-top polypropylene tubes at 12 rpm for 30 min at 37 C. Controls consisted of the same volume of gonococcal suspensions rotated in the absence of HeLa cells in the same M199 plus 2% fetal calf serum medium. At the end of the incubation the suspensions were centrifuged (45 × *g*, 5 min), and the supernatant was measured and removed. For controls the same volume of supernatant removed from the HeLa cell-containing mixture was aspirated. The HeLa cell pellet was resuspended in the original volume, as was the amount left in the control tubes. Aliquots were taken for plating on GC agar plates which were incubated overnight at 36 C. Colony-forming units were enumerated the following day. All incubation mixtures were duplicates, and duplicate aliquots for plating were taken from each.

RESULTS

Comparative ^{125}I labeling of T2, T4, and T4* GC. Parallel radiolabeling incubations

were carried out with pilated, colony type 2 organisms and with two forms of nonpilated, colony type 4 GC from strain MS11. The nonpilated GC were classified as T4 or as T4* by virtue of their respective low and high levels of association with human neutrophils in vitro, as previously described (10). Specific labeling of each type of organism (T2, T4, and T4*) was determined as outlined in Materials and Methods. Considerable variation in the extent of ^{125}I labeling of each type occurred on different days. These variations could usually be accounted for by use of "old" hydrogen peroxide or use of excessive numbers of organisms, both of which produced reductions in the extent to which individual GC were ^{125}I labeled. For the latter reason, considerable care was taken to insure use of quite similar numbers of the three types of organisms (as determined by direct counting Petroff-Hausser chambers) in each labeling incubation. The comparative levels of ^{125}I labeling are tabulated in Table 1. Both the results of individual experiments and the statistical evaluations of compiled data are shown.

T2 GC consistently label with more ^{125}I than do T4 organisms. In contrast, T2 and T4* GC exhibit quite similar specific activities (counts per minute per gonococcus), with T4* usually labeling more extensively than T2. The differences in labeling of T2, T4, and T4* GC are not sufficiently large to yield statistically different values if all the experiments carried out on different days are compared, as shown by the overlapping standard deviations. However, when the specific activities of the T2, T4, and T4* GC are compared on the basis of each experiment (Table 1: T2/T4, T4*/T4, and T4*/T2), it is evident that T2 labeled more extensively than T4 in every experiment, and T4* labeled more extensively than T2 in the majority of incubations (7 of 10). On this basis, the comparative ^{125}I labeling of the three types of GC follows the following pattern: T4* $\geq$ T2 > T4.

Light microscopically evaluated association of ^{125}I-labeled GC with WBC monolayers. T2, T4, and T4* GC that had been labeled with ^{125}I were utilized as such or were trypsin treated and then used for incubations with WBC monolayers. Association of the bacteria with neutrophils was evaluated by light microscopic examination. The relative association (attachment-ingestion) of these ^{125}I-labeled GC was the same as previously demonstrated for unlabeled organisms, as follows: T4* > T2 $\gg$ T4. Similarly, trypsin treatment of ^{125}I-labeled GC effected the same marked reduction in association levels, as compared to controls, in T2 and T4* GC as has been previously described for unlabeled organisms. These studies were carried out to determine whether radioiodination altered the reactivity of GC with WBC, as evaluated by methods with which considerable experience had been previously obtained.

Comparative association of ^{125}I-labeled T2, T4, and T4* GC with WBC and with tissue culture cells. All three gonococcal types (T2, T4, and T4*) were labeled in parallel incubations and were exposed to either WBC or tissue culture cells monolayers (human amnion cells, human foreskin cells, or HeLa cells) as described in Materials and Methods. Comparisons were then made of the ^{125}I-labeled GC that associated with each cell type.

T2 and T4* GC clearly associate with WBC monolayers to a greater extent than do T4 organisms (Table 2). This can be seen either from inspection of the respective mean percentages and standard deviations of input counts

TABLE 1. *^{125}I labeling of T2, T4, and T4* GC*[a]

Date	GC			Comparative sp act		
	T2	T4	T4*	T2/T4	T4*/T4	T4*/T2
1-16	1.7412×10^{-3}	6.114×10^{-4}	1.78×10^{-3}	2.85	2.9	1.02
1-17	8.583×10^{-4}	8.413×10^{-4}	1.179×10^{-3}	1.02	1.4	1.37
1-18	1.754×10^{-3}	1.0289×10^{-3}	1.393×10^{-3}	1.70	1.35	0.79
1-24	3.368×10^{-3}	1.774×10^{-3}	4.50×10^{-3}	1.89	2.5	1.33
1-25	3.08×10^{-3}	1.78×10^{-3}	3.38×10^{-3}	1.73	1.89	1.09
2-1	2.31×10^{-3}	1.64×10^{-3}	2.83×10^{-3}	1.41	1.73	1.22
4-3	1.67×10^{-3}	1.267×10^{-3}	2.72×10^{-3}	1.32	2.15	1.62
5-2	1.73×10^{-3}	1.42×10^{-3}	2.92×10^{-3}	1.22	2.05	1.68
5-16	3.76×10^{-3}	2.1×10^{-3}	3.56×10^{-3}	1.79	1.69	0.94
$\bar{x} \pm$ SD	2.25×10^{-3} $\pm 9.5 \times 10^{-4}$	1.38×10^{-3} $\pm 4.89 \times 10^{-4}$	2.69×10^{-3} $\pm 1.09 \times 10^{-3}$	1.66 $\pm$ 0.53	1.96 $\pm$ 0.50	1.22 $\pm$ 0.30

[a] Counts per minute per gonococcus. SD, Standard deviation.

TABLE 2. *Association of ^{125}I-labeled GC with eukaryotic cells*

Date	Cell type[b]	% ^{125}I-labeled GC associated with cells[a]					
		T2	T4	T4*	T2/T4	T4*/T4	T4*/T2
1-16	WBC	3.8	1.85	2.5	2.05	1.35	0.66
1-18	WBC	3.9	1.5	3.8	2.6	2.53	0.97
2-5	WBC	4.5	1.9	5.4	2.37	2.84	1.2
	$\bar{x} \pm$ SD WBC	4.07 ± 0.38	1.75 ± 0.22	3.9 ± 1.45	2.34 ± 0.28	2.24 ± 0.79	0.94 ±0.27
1-16	Hu Am	1.8	0.8	0.6	2.25	0.75	0.33
1-25	Hu Am	1.8	0.9	0.75	2.0	0.83	0.42
2-1	Hu Am	1.2	0.6	0.9	2.0	1.5	0.75
4-3	Hu Am	1.16	0.89	0.96	1.3	1.08	0.83
1-24	Hu FS	1.1	0.9	1.2	1.22	1.33	1.09
5-2	Hu FS	1.34	1.01	0.71	1.33	0.7	0.53
1-31	Hu FS	2.61	1.34	0.58	1.95	0.43	0.22
5-16	HeLa	3.48	2.18	1.9	1.6	0.87	0.55
	$\bar{x} \pm$ SD TCC[c]	1.81 ± 0.84	1.08 ± 0.49	0.95 ± 0.44	1.71 ± 0.39	0.936 ± 0.35	0.59 ± 0.29

[a] Based on input counts per minute as 100%.

[b] WBC, human leukocytes; Hu Am, human amnion cells; Hu FS, human foreskin cells. SD, Standard deviation.

[c] TCC, All tissue culture cells (Hu Am C, Hu FS C, and HeLa).

per minute associated with the WBC monolayers or from the T2-T4, T4*-T4 ratios of cell-associated radioactivity. T2 and T4* GC appear to associate with leukocyte monolayers to a similar extent. Thus, the following comparative association of GC with human WBC monolayers obtains: T2 = T4* > T4.

Comparisons of the association of ^{125}I-labeled T2, T4, and T4* GC with tissue culture cells is somewhat more difficult due to the rather low levels of tissue culture cell-associated radioactivity found. The mean values derived from compiled data obtained on several days are not significantly different for T2, T4, and T4* organisms. However, determining the ratios of T2/T4, T4*/T4, and T4*/T2 radioactivity associated with the tissue culture cells and analyzed according to individual experiments shows the following: T2 consistently (nine of nine experiments) shows greater association with tissue culture cells than T4/T2 exhibits greater association with the cells than does T4* in all but one experiment (eight of nine). T4 and T4* GC associate with tissue culture cells to a similar degree. On these bases, the comparative association of these three gonococcal types with tissue culture cells is T2 > T4 = T4*.

Effect of trypsin pretreatment on GC on their interactions with human WBC and tissue culture cells. T2, T4, and T4* GC were labeled with ^{125}I and were then either incubated with trypsin or in trypsin-free medium. After washing, the GC were incubated with WBC or with tissue culture cells, and the number of organisms that associated with the eukaryotic cells was determined. The data shown in Table 3 summarizes these experiments and is presented as the relative association of trypsin-treated GC versus non-trypsin-treated organisms of the same type. Incubations of such trypsin-treated and control organisms with human amnion cells, human foreskin cells, and HeLa cells are combined, as no differences in the results were noted with the various tissue culture cell types. On incubation with WBC, trypsin treatment resulted in diminished association of all three (T2, T4, and T4*) gonococcal types with the WBC. The ratios of association of trypsinized-association of control GC are similar for all three gonococcal types (T2 = 0.66; T4 = 0.5; T4* = 0.46). This reduction in GC plus WBC association by trypsin contrasts with the lack of effect of trypsin pretreatment on interactions between GC and tissue culture cells. The number of cell-associated GC is approximately the same (ratio of trypsinized to control is approximately 1.0) for both the trypsin-treated and control ^{125}I-labeled GC of all three types. Certainly T2 and T4* show no real differences between trypsinized and control values; the apparent reduction in attachment of T4 GC to tissue culture cells is small and of questionable significance.

The results outlined above regarding relative association of T2, T4, and T4* GC with tissue culture cells and also the effect of trypsin

treatment on association of the organisms with such cells were corroborated through use of nonlabeled GC. The data from such an experiment are shown in Table 4. The GC which are sedimented but which are not associated with HeLa cells were enumerated through use of control, HeLa cell-free specimens. The number of organisms that associate with the HeLa cells can be determined by correcting the apparent number of GC that sediment with the HeLa cells by the number that sediment in the absence of such cells in the incubation mixture. The summary of the data shows that T2 associate with HeLa cells to a much greater extent than do T4 or T4* GC. This is found both with control, nontrypsinized, and trypsin-pretreated organisms. Trypsin pretreatment does not appear to alter the extents to which T2, T4, or T4* GC adhere to HeLa cells.

TABLE 3. *Effect of trypsin pretreatment on ^{125}I gonococcal association with leukocytes or tissue culture cells*

Eukaryotic cells	Trypsin-treated GC/control GC, cell associated[a]		
	T2	T4	T4*
WBC	0.66 ± 0.07	0.50 ± 0.03	0.46 ± 0.17
TCC[b]	1.19 ± 0.22	0.82 ± 0.16	0.94 ± 0.2

[a] Based on counts per minute (total recovered counts per minute = 100%) associated with cell monolayers.

[b] TCC include human amnion, human foreskin, and HeLa cells.

DISCUSSION

We have previously inquired into the interactions between GC and eukaryotic cells of diverse kinds. The first study dealt with attachment of GC to tissue culture cells (human amnion cells) and suggested that such attachment was enhanced or, perhaps, mediated by gonococcal pili. That study focused on differential attachment of pilated and nonpilated GC, as have subsequent publications documenting a similar, enhanced attachment of pilated GC to human sperm (1), human Fallopian tubal epithelium (13), buccal mucosal cells (6), and other tissue culture cell types (7). More recently we have examined the interactions between GC and human WBC in vitro (8, 10, 11). Our findings in those experiments suggest that pili play little or no role as determiners of attachment, phagocytosis, or killing of GC by the phagocytes. In the course of those studies, however, nonpilated organisms of two different biological behavior patterns were found. One of these (type 4) showed little interaction with WBC, whereas the other (type T4*) exhibited higher degrees of association with WBC than did either pilated, type 2 GC or the other form of nonpilated gonococcus (T4). Those studies suggested that interactions between GC and WBC were primarily mediated by a nonpilus surface material on GC and that this material was trypsin, chymotrypsin, heat, and glutaraldehyde sensi-

TABLE 4. *Effect of trypsin treatment on attachment of GC to HeLa cells*

GC	Trypsin treated	HeLa cells	GC colony-forming units			
			Sediment	Supernatant	Total	Sediment/total[a]
T2	−	−	3.1×10^2	2.2×10^3	2.51×10^3	0.12
T2	+	−	9.0×10^1	8.1×10^2	9.0×10^2	0.10
T2	−	+	1.2×10^3	1.7×10^3	2.9×10^3	0.41
T2	+	+	6.3×10^2	1.0×10^3	1.63×10^3	0.39
T4	−	−	7.4×10^2	2.1×10^3	2.84×10^3	0.26
T4	+	−	2.6×10^2	1.1×10^3	1.36×10^3	0.19
T4	−	+	5.2×10^2	1.6×10^3	2.12×10^3	0.25
T4	+	+	6.2×10^2	1.9×10^3	2.52×10^3	0.25
T4*	−	−	4.5×10^2	1.5×10^3	1.95×10^3	0.23
T4*	+	−	4.6×10^2	1.2×10^3	1.66×10^3	0.28
T4*	−	+	3.7×10^2	1.5×10^3	1.87×10^3	0.20
T4*	+	+	5.3×10^2	1.2×10^3	1.73×10^3	0.31

[a] Sediment/total (HeLa specimen − control): T2 (−trypsin), +0.29; T2 (+trypsin), +0.28; T4 (−trypsin), −0.01; T4 (+trypsin), +0.06; T4* (−trypsin), −0.03; T4* (+trypsin), +0.03.

tive. These findings prompted a reappraisal of interactions of GC (T2, T4, and newly designated T4*) with tissue culture cells to determine the influence of the "WBC-association-mediating surface factor" on interactions of GC with tissue culture cells. For these studies and as a preliminary step to studying the gonococcal surface factor we chose surface labeling of GC with ^{125}I by the lactoperoxidase, hydrogen peroxide-catalyzed reaction.

The slightly differing specific activities of the three gonococcal forms (T4* $\geq$ T2 $>$ T4) is somewhat suggestive, but by no means conclusive evidence, of the presence of more tyrosyl residues on the surfaces of T2 and T4* GC than are present on T4 organisms. Additional information is necessary before one can determine whether these small differences are, in fact, real and why pilated GC do not display levels of ^{125}I labeling higher than those for T4* by virtue of the proteinaceous pili that cover the former and are absent from the latter. It is perhaps interesting that T4* GC constitute the majority of nonpilated derivatives which arise in T2 cultures. Type 4 organisms then appear after variable numbers of passages of the T4* cultures on artificial medium (J. Swanson, E. Sparks, D. Young, M. Blake, and G. King, unpublished data). This would suggest that T2 and T4* organisms are more closely related, though the mechanisms of change are not well understood, than are T2 and T4 GC.

Striking differences in the interactions of GC with eukaryotic cells are found in experiments utilizing these different gonococcal types and WBC or tissue culture cells. Pilated, type 2 and nonpilated, type 4* GC associate with WBC to a fairly similar level. This level is much higher than that found with nonpilated, type 4 organisms of the same strain. The results found in the present study with ^{125}I-labeled GC parallel those presented previously with the following differences. Association of T4* GC with WBC is slightly greater than for T2 organisms, as evaluated through light microscopy, or with ^{14}C-labeled GC (10). Also, T4* are killed to a greater extent than T2 by human WBC (8). In both these previous studies, however, T4 was decidedly lower in its association with WBC than T2 or T4* organisms and, as an apparent consequence, the T4 organisms were much less susceptible to killing by human WBC than the other two gonococcal forms.

In spite of their differing behaviors with WBC, types 4 and 4* GC exhibit similar levels of association with tissue culture cells. Both T4 and T4* organisms attach to human amnion cells, human foreskin cells, and HeLa cells less readily than do pilated, type 2 organisms. This is similar to the reactivities of the three gonococcal forms in their association with human sperm (A. N. James-Holmquest, and J. Swanson, unpublished data) as well as with buccal mucosal cells (G. King, and J. Swanson, unpublished data).

Iodination of the gonococcus' surface does not appear to alter the reactivity of the organisms with either WBC or tissue culture cells. This can be deduced from several bits of information. First, T2, T4, and T4* ^{125}I-labeled GC were used for incubation with WBC, and their association with the WBC was scored by light microscopy. The ^{125}I-labeled GC exhibited the same levels of interaction with WBC as did unlabeled organisms. Second, similar comparative results were obtained in experiments utilizing ^{125}I-labeled GC and unlabeled GC and assessing attachment of the organisms to tissue culture cells.

Trypsin pretreatment of GC markedly reduces the association of the organisms with WBC but does not appear to change the level of attachment of the bacteria to tissue culture cells. Our previous observations have shown that, under the conditions of trypsinization we have utilized, pili remain visible by electron microscopic examination of trypsin-treated type 2 GC (10). These data support our proposals that (i) gonococcal attachment to tissue culture cells is enhanced or mediated by pili, and (ii) association of GC with WBC in vitro is mediated by a trypsin-sensitive, nonpilus surface material on the organisms.

Utilization of ^{125}I labeling for intact, whole GC appears to offer some promise for future experiments but is beset with certain drawbacks and limitations. It should be pointed out that the interactions of GC with eukaryotic cells do not appear to be greatly modified by the iodination procedure nor the presence of ^{125}I on their surfaces. We have been unable to label GC to the heightened specific activities which we would need for use of the ^{125}I-labeled GC in several types of experiments. However, it is hoped that the opportunity to label the surfaces of viable organisms will be helpful in characterization of the components comprising those surfaces and for determining the biological functions of the surface constituents.

ACKNOWLEDGMENTS

We appreciate Becky Felton's help in the preparation of this manuscript.

This work was supported by contract DADA 17 72 2146 of the U.S. Army Medical Research and Development Command and by Public Health Service research grant AI-11073 from the National Institute of Allergy and Infectious Diseases.

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