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Changes in Surface Hydrophobicity and Charge in *Neisseria meningitidis* and Their Correlation with the Association to Phagocytic Cells

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Pairwise correlations among surface charge, relative hydrophobicity and association with phagocytes were studied in two strains of *Neisseria meningitidis* both under normal conditions and after different surface modifying treatments. The variations in the values of the three variables depended on both strains and treatment, and correlations were highly significant, although the biological significance of these correlations is questionable.

Key words: Hydrophobicity, Phagocytic cells, *Neisseria meningitidis.*

Surface hydrophobicity is a non-specific adhesion factor that is important in the proliferation of micro-organisms on solid surfaces, and consequently in their adherence to epithelial cells, phagocytes and other cells (2, 4, 6). Other physico-chemical surface properties, such as charge, are also involved in the binding (8). STJERNSTROM *et al.* (14) and MAGNUSSON *et al.* (7) found that although the physico-chemical consequences of opsonization during phagocytosis depend on the type of particles bound to the phagocyte, phagocytic assimilation is mainly affected by hydrophobic interactions and surface charge.

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We have studied the modification of surface charge and surface hydrophobicity, by treatments which alter molecules and/or structures in the bacterial surface layer, in order to investigate the role of these properties in the association of two *Neisseria meningitidis* strains (one from a carrier and the other invasive) to phagocytic cells.

Materials and Methods

Organisms and culture conditions. — *Neisseria meningitidis* strain Lab 1 (serogroup A) was obtained from a carrier in our laboratory. Strain DS2 (serogroup B), originally isolated from the cerebrospinal

fluid of a patient, was obtained from the collection of the «Dirección de Salud (La Coruña, Spain)». Both strains had less than three subcultures after isolation and were maintained at −30° C as described in a previous paper (3). For each experiment, the bacteria were cultured directly from the frozen stocks onto Choc-Iso agar plates (13) at 37° C for 18-24 h in a 5 % CO_2 atmosphere and then subcultured in the same conditions for 14 h in order to obtain enough growth for the experiments. For the surface charge and phagocyte-association assays, the culture plates were sprayed prior to inoculation with 2 µCi of [1 − ^{14}C] acetic acid (sodium salt, 57 mCi/mmol, Amersham) into 100 µl of Tryptone Soya Broth (TSB, BBL). For the assays, bacteria were initially suspended in Balanced Salt Solution (BSS) (9) (4 ml per plate), washed twice in the same buffer and finally suspended as explained below.

Measurement of negative surface charge. — Negative surface charge was determined by batch ion-exchange chromatography on Dowex 1X8 resin (100-200 mesh, chloride form) using a modification of the technique describen by PEDERSEN (10). Briefly, the bacteria were suspended in NSS buffer (pH 8.2) to a density of 5×10^8 bacteria/ml and 0.5 ml of this suspension was mixed with 0.5 ml of resin (1 g/ml in the same buffer). After 10 min incubation at room temperature with constant mild shaking, the resin was allowed to settle for 5 min and the supernatant was prepared for liquid scintillation counting. Total count controls consisted of 0.5 ml of the initial bacterial suspension. Negative charge values are expressed as the percentage of radioactivity cleared from the bacterial suspension after incubation with the resin.

Measurement of surface hydrophobicity. — Surface hydrophobicity was determined by adherence to p-xylene in a water-hydrocarbon two phase system (11). Briefly, the bacteria (suspended in 2 M ammonium sulfate/phosphate buffer, pH 7) were mixed with p-xylene and the percentage of the bacteria associated to the p-xylene phase was calculated as the difference between the absorbance of the aqueous phase at 540 nm before and after the assay (12).

Measurement of association with phagocytes. — Human peripheral phagocytic cells were obtained by the VAN FURTH and VAN ZWET'S (16) procedure from 50 ml of human blood drawn not more than 30 min previously. Briefly, the blood was mixed with dextran-500 and allowed to stand for 30 min at 37° C for the erythrocytes to settle. The supernatant was then centrifuged at 110 × *g* and the red cells remaining in the pellet were lysed with 0.85 % ammonium chloride for 10 min. The phagocytes were then washed twice in calcium- and magnesium-free BSS (pH 7.2-7.4) with 10 mM EDTA and resuspended to a density of 10^7 cell/ml in normal BSS containing gelatin (1 mg/ml). Bacteria were suspended at the same concentration in gelatin-BSS. For the assay, 0.5 ml of each suspension were mixed, and 0.1 ml of human inactivated serum was added to each assay tube. After 30 min incubation at 4 rpm constant shaking and 37° C, phagocyte association was stopped by mixing 0.5 ml of the assay suspension with 1.5 ml of ice-cold gelatin-BSS. Bacteria and phagocytes were then separated by centrifugation at 110 × *g* for 4 min in a swinging-out rotor, and radioactivity in 0.5 ml of the supernatant was measured by liquid scintillation. Total count controls consisted of exactly the same assays performed at zero time, just after mixing bacteria and phagocytes. The phagocyte association index used was the percentage of bacteria cleared from the suspension after incubation.

Treatments. — Before the assays, the bacterial surface was modified by treating

bacterial suspensions with 3 % glutaraldehyde (30 min/25° C), sodium metaperiodate (15), or formaldehyde and/or (1-ethyl)-3-(dimethylaminopropyl)-carbodiimide (EDC-methylamine) (5). After treatment, the bacteria were washed three times in BSS and resuspended for the assays. Bacterial integrity was checked microscopically.

Statistical analysis. — Due to the heteroscedasticity of the data, non-parametric statistical tests (1) were used. The Mann-Whitney U test was used in lieu of analysis of variance and Kendall's Tau or Chi-squared independence tests were used to assess correlations.

Results

Table I shows that most of the changes in phagocyte association index, relative hydrophobicity and negative surface charge caused by the different treatments were significant and strain dependent. All the effects on strain Lab 1 were statistically significant except for those of formaldehyde + EDC-methylamine on phagocytosis and on surface charge. In strain DS2, phagocytosis was only affected by metaperiodate and formaldehyde + EDC-methylamine, but surface charge and surface hydrophobicity were significantly modified by all the treatments.

Kendall's Tau test indicates statistically significant correlations between surface hydrophobicity and charge (Kendall's Tau B = 0.38; p = 0.0008), phagocytosis and surface hydrophobicity (Kendall's Tau B = 0.38; p = 0.0010) and phagocytosis and surface charge (Kendall's Tau B = 0.27, p = 0.0139), though the values of this coefficient are relatively low, especially for the correlation between phagocytosis and charge.

Discussion

Glutaraldehyde is used in the investigation of many intercellular interaction

Table I. *Variations in the phagocyte association index, relative hydrophobicity and negative surface charge induced in* Neisseria meningitidis *by treatment with different chemicals.*
Data (means from three experiments) are expressed as percentages of variation. Standard errors were always less than 5 % of the mean. Only figures of significant ($p < 0.05$) variations are given.

Treatment	Phagocyte		Negative hydrophobicity	Charge
	Strain	Association index		
Glutaraldehyde	Labl	−95.00	1000.00	−22.03
	DS2	ns.*	516.67	−20.00
Metaperiodate	Labl	−96.95	−100.00	−22.03
	DS2	−77.83	−100.00	−60.00
Formaldehyde	Labl	−95.00	−100.00	−22.03
	DS2	ns.	−100.00	−16.67
EDC-Methylamine	Labl	−95.00	2300.00	30.51
	DS2	ns.	966.67	170.00
Formaldehyde + EDC-Methylamine	Labl	ns.	2566.67	ns.
	DS2	233.33	966.67	43.33

* No significant change ($p > 0.05$).

phenomena. Treatment of our bacteria with this compound increased hydrophobicity and decreased surface charge significantly. This behaviour is in keeping with the theory of the physicochemical properties of particles in aqueous media (18). Curiously, association with phagocytes decreased in both strains indicating that the bacterial surface probably contains molecules whose mobility within it is relevant for phagocytosis.

Metaperiodate treatment of biological materials cleaves bonds between vicinal carbon atoms with free hydroxyl radicals in sugar molecules, which in *Neisseria meningitidis* could a priori destroy the bacterial capsule and thus enhance resistance to phagocytosis. The fact that phagocytosis increased while hydrophobicity decreased indicates that hydrophobicity plays a role in phagocytic processes, while suggesting that the treatment applied affected the capsule as well as the hydrophobic molecules on the bacterial surface.

HECKELS *et al.* (5) found both that formaldehyde treatment increased the negative charge of *Neisseria gonorrhoea* by blocking free protonated amino-groups and that treatment with EDC-methylamine inverted the overall charge by blocking carboxyl-groups. In our strains negative charge was significantly decreased by formaldehyde and increased by EDC-methylamine treatment. It seems likely that formaldehyde and EDC-methylamine not only produce charge neutralization but may also bring about other alterations affecting molecules involved in the measuring procedures.

The global correlations found between surface charge and hydrophobicity are in keeping with the physico-chemical principles governing the interactions of particles in aqueous media as the non-polarity of hydrophobic molecules in increasing numbers causes a corresponding reduction in the net surface charge of the bacteria. The importance of charge and hydrophobicity for association with phagocytes is shown by highly significant correlations, although the biological significance of these correlations is questionable due to the relatively low correlation indexes obtained (Kendall's Tab B = 0.38 in both cases).

Resumen

Se estudian las correlaciones mutuas entre la carga de superficie, la hidrofobicidad relativa y la unión a células fagocíticas en dos cepas de *Neisseria meningitidis* tanto en condiciones normales como tras la aplicación de diferentes tratamientos que modifican la superficie de las cepas. Las variaciones en los valores de las tres variables dependen tanto de la cepa como del tratamiento, y las correlaciones entre ellas son altamente significativas, aunque la significación biológica de estas correlaciones es cuestionable.

Palabras clave: Hidrofobicidad, Células fagocíticas, *Neisseria meningitidis.*

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