

Loss of Transferrin Receptor Activity in *Neisseria meningitidis* Correlates with Inability To Use Transferrin as an Iron Source

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Although *Neisseria meningitidis* does not produce siderophores, it is able to obtain iron from human transferrin. We observed saturable specific binding of ¹²⁵I-labeled human transferrin to meningococci. Human lactoferrin and mouse transferrin did not compete with human transferrin for binding, whereas human apotransferrin and 100% iron-saturated transferrin competed equally well. Meningococci thus have a specific receptor for human transferrin. Scatchard analysis yielded a relatively low K_d of 0.7 μ M and an apparent copy number of 2,900 receptors per CFU. Receptor activity was iron-regulated. A meningococcal transformant specifically unable to utilize transferrin as an iron source had decreased transferrin receptor activity. These data are consistent with the hypothesis that receptor-mediated binding of transferrin is a rate-limiting step in meningococcal iron uptake from transferrin.

Neisseria meningitidis is a pathogen for which humans are the only host. Most of the available iron (Fe) in the body is stored in iron-binding proteins; in serum the principal Fe source is transferrin (TF), and on mucosal surfaces the principal Fe source is lactoferrin (LF) (5, 17). Successful pathogens have evolved mechanisms by which they obtain Fe from TF and LF (32). Most bacteria accomplish this by production of iron chelators, termed siderophores (20, 21, 25). The pathogenic *Neisseria* species, however, are unusual in their inability to produce siderophores (22, 28, 33). Nevertheless, meningococci can use TF in vitro as the sole Fe source (19). Virulence in mice is enhanced by subjecting meningococci to Fe-limited conditions prior to infection (4) and by injecting mice with Fe-TF prior to infection (11, 12). These data suggest that the ability to use Fe-TF is necessary for growth in vivo.

The mechanism by which meningococci scavenge Fe from TF is unknown but may be similar to receptor-mediated mechanisms postulated for a few other human pathogens (28). The binding of LF but not TF to the surface of *Trichomonas vaginalis* suggests the presence of a specific LF receptor (23). *Bordetella pertussis* binds LF and TF in a manner consistent with the presence of specific receptors (24). An LF receptor of *Mycoplasma pneumoniae* has been partially characterized (29). Evidence for a TF receptor on meningococci includes the observation that meningococci cannot utilize TF as an Fe source when TF is sequestered in a dialysis bag; contact appears to be necessary (1, 28). Simonson et al. (28) demonstrated that human TF would bind to meningococci but did not demonstrate the saturable specific binding characteristic of a receptor. Recently, Schryvers and Morris (27) showed that horseradish peroxidase-conjugated human TF (HRP-TF) bound to meningococci immobilized on nitrocellulose in a solid-phase assay; binding was both saturable and specific.

We have used a classical receptor-binding assay to evaluate the affinity, specificity, and copy number of the meningo-

coccal TF receptor. We also demonstrate the biological importance of receptor function through the use of mutants specifically unable to use TF for growth.

MATERIALS AND METHODS

Strains, media, and growth conditions. *N. meningitidis* FAM30 is a nalidixic acid-resistant (Nal^r) spectinomycin-resistant (Spc^r) derivative of the wild-type serogroup C serotype 2a parent strain FAM18 (8). FAM30 is wild type in its ability to use Fe from ferric dicitrate complexes, TF, LF, heme, and hemoglobin. FAM38 is a *fud-6 hga-1* transformant of FAM30; FAM38 is specifically unable to use TF as the sole source of required Fe for growth (TF⁻) and lacks 85- and 95-kilodalton (kDa) outer membrane FeRPs (Fe-repressible proteins) produced by FAM30. FAM41 is a related transformant of FAM30 that carries only the *fud-6* mutation; FAM41 also is TF⁻ but has wild-type outer membrane protein profiles. These strains were described recently (8). Strain M986-NCV-1 is a nonencapsulated derivative of serogroup B *N. meningitidis* M986 (10); it was provided by C. Frasch and is designated FAM2 in this paper. FAM11 is a mutant of FAM2 unable to use TF, LF, ferric dicitrate, or aerobactin as Fe sources and lacks a 70-kDa FeRP. FAM63 is a revertant of FAM11 that has partially recovered its ability to use TF without regaining the 70-kDa FeRP (9).

Meningococci were routinely cultured on GCB agar (Difco Laboratories, Detroit, Mich.) containing Kellogg supplements I and II (14) at 37°C in a 5% CO₂ atmosphere. To Fe-deplete cells, we used a low-Fe, Chelex-treated defined medium (CDM) (34). Control (Fe-sufficient) cells were obtained from cultures in CDM supplemented with 10 μ M ferric nitrate. Cell growth was measured by optical density with a Klett-Summerson colorimeter with a green filter. All glassware was acid-washed to remove contaminating Fe (19).

Iron sources. Human TF purchased from Sigma Chemical Co., St. Louis, Mo., was at least 98% pure by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and significantly Fe-free, according to the supplier. Human LF was the gift of S. V. Pizzo (Duke University Medical Center) and was approximately 95% pure as determined by SDS-PAGE. Mouse TF was obtained from Chem-

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icon International, El Segundo, Calif. Preparation of Fe- and ^{55}Fe -loaded forms of these proteins has been described elsewhere (19). All Fe-binding proteins used in these experiments were 100% Fe saturated except apotransferrin (apoTF). HRP-TF was obtained from Jackson Immunoresearch Laboratories, Avondale, Pa.

ApoTF was made by dialyzing TF overnight against two changes of 50 mM sodium acetate–150 mM sodium chloride (pH 5.0) buffer containing 100 μM desferrioxamine mesylate (Desferal; Ciba Pharmaceutical). The TF was renatured by dialysis overnight versus 40 mM Tris–20 mM sodium bicarbonate–150 mM sodium chloride (pH 7.4) containing 10 g of Chelex-100 per liter (Bio-Rad Laboratories, Richmond, Calif.). ^{125}I -TF was made from 100% Fe-saturated human TF by the method of Markwell (16). A 500- μg sample of TF was added to 1 mCi of carrier-free Na^{125}I (Amersham Corp., Arlington Heights, Ill.) and two Iodo-beads (*N*-chloro-benzenesulfonamide [sodium salt]-derivatized uniform nonporous polystyrene beads; Pierce Chemical Co., Rockford, Ill.) in 500 μl of 100 mM sodium phosphate buffer (pH 7.0). The reaction was allowed to proceed for 5 min at room temperature with agitation. Unincorporated ^{125}I was removed by passing the ^{125}I -TF down a Sephadex G-25 column (Pharmacia, Inc., Piscataway, N.J.) preblocked with bovine serum albumin (BSA) and equilibrated with 40 mM Tris–20 mM sodium bicarbonate–150 mM sodium chloride (pH 7.4). The ability of ^{125}I -TF to donate Fe and support growth was tested by growing FAM30 in CDM plus 1 μM ^{125}I -TF, 1 μM unlabeled TF, or no added iron source. Purity of ^{125}I -TF was assessed by SDS-PAGE on 10% Laemmli gels (15) and autoradiography.

Transferrin-binding assays. Fe-replete cells grown overnight on GCB agar plates were suspended in CDM broth and grown for two mass doublings; these cells were diluted into CDM at 37°C and grown for another two mass doublings to mid-log phase to afford relative Fe depletion. Fe-stressed cells were harvested at an optical density of 80 Klett units, pelleted by centrifugation, and suspended in a 0.01 \times volume of CDM containing 1% BSA (CDM-BSA) in BSA-coated 1.5-ml polypropylene Microfuge tubes (Sarstedt, Inc., Princeton, N.J.). An equal volume of CDM-BSA was added, containing ^{125}I -TF and competing ligands where appropriate. Concentrations of ^{125}I -TF and competitors are indicated below. Each reaction mixture contained a final volume of 200 μl and about 10^9 CFU of bacteria. After 20 min, the cells were pelleted by centrifugation and washed twice by resuspension and centrifugation for 1 min in 100 μl of CDM-BSA. After the final resuspension, the cells were transferred to polystyrene tubes (12 by 75 mm); the amount of cell-associated ^{125}I -TF was determined by using a Minoxi γ auto 5000 series gamma counter (Packard Instrument Co., Inc., Rockville, Md.). All steps were performed at room temperature to prevent cold-induced cell lysis. Microtubes were coated with BSA by filling with 2% BSA and agitating for 1 h at room temperature before removing the BSA solution and drying overnight at 37°C. Specific binding was defined as the difference between ^{125}I -TF bound in the presence and absence of a 100 \times concentration of cold TF. ^{125}I -TF used in specific binding experiments was adjusted to an activity of 500 cpm/pmol. The effect of Fe limitation on binding was examined similarly by using cells harvested at growth intervals in CDM; by the use of larger culture volumes for early-phase cultures, cell densities in the binding assays were constant.

Binding of HRP-TF to cells in a solid-phase dot enzyme assay described by Schryvers and Morris (27) was examined

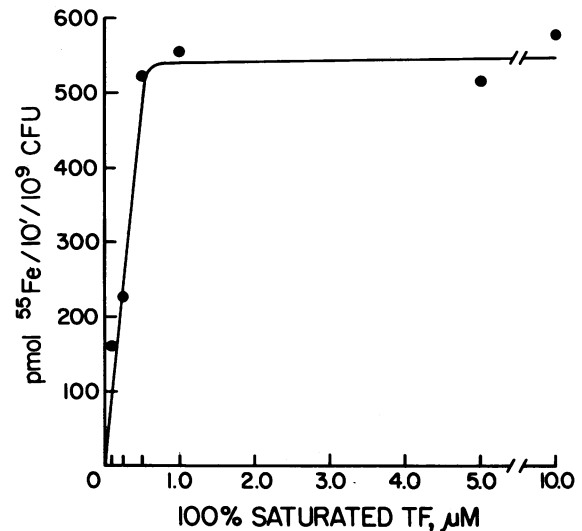


FIG. 1. Rate of ^{55}Fe uptake (measured after 10 min) from TF by Fe-starved FAM30 as a function of TF concentration. Measurement of uptake after 30 min revealed higher absolute uptake, but saturation occurred at the same TF concentration. Results depict the average of two experiments.

by a modified procedure. Cells were harvested after overnight growth on CDM agar (8) and were suspended to a density of 30 Klett units in CDM broth. A 50- μl sample of each suspension was filtered onto nitrocellulose paper (0.45- μm BA-85; Millipore Corp., Bedford, Mass.) by using a 96-well filter manifold (Schleicher & Schuell, Inc., Keene, N.H.). The paper was dried (37°C, 20 min), blocked, probed with serial dilutions of HRP-TF, washed, and developed with a chloronaphthol-hydrogen peroxide substrate mixture (HRP reagent; Bio-Rad) as described previously (27), except that all incubations were done at room temperature. Developed blots were scanned by using an ACD-15 densitometer (Gelman Sciences, Inc., Ann Arbor, Mich.) absorbing at 565 nm.

Western blot assays with HRP-TF were done with modifications of a previously described procedure (27). Cells were harvested from mid-late-log-phase CDM cultures, and whole membranes were prepared as described elsewhere (33). Solubilized membrane proteins (100 μg) were separated by SDS-PAGE on 7.5% Laemmli gels (15) and transferred to nitrocellulose (3). The binding of HRP-TF to proteins on the Western blot was examined as described for the dot enzyme assay.

^{55}Fe uptake assays. Log-phase Fe-starved meningococci were tested for their ability to accumulate ^{55}Fe from ^{55}Fe -TF by using methods previously described (8).

RESULTS

Concentration dependence of Fe uptake from TF. The ability of log-phase Fe-starved FAM30 to internalize ^{55}Fe from 100% Fe-saturated human TF was assayed as a function of TF concentration (Fig. 1). Uptake of ^{55}Fe increased linearly up to 1 μM TF; little further increase in ^{55}Fe uptake was noted at TF concentrations above 1 μM . These results were similar to those noted recently for gonococci (18). They differ, however, from the saturation of Fe uptake from TF at 30 μM observed in meningococcal strain SDIC (28). Reasons for the discrepancy in results with FAM30 and SDIC are unclear.

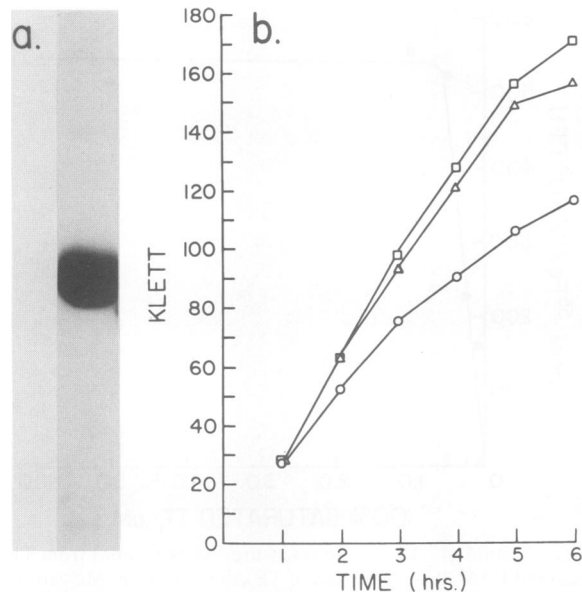


FIG. 2. (a) Autoradiogram of ¹²⁵I-TF labeled by the Iodo-bead method and subjected to SDS-PAGE. (b) Growth of FAM30 in CDM containing either 1 μ M 100% Fe-saturated human TF (\square), 1 μ M ¹²⁵I-TF (Δ), or no added Fe source (\circ).

Quality of ¹²⁵I-TF. We labeled TF with ¹²⁵I by using chloramine-T (13), lactoperoxidase-glucose oxidase (6), and Iodo-beads (16). Analysis by SDS-PAGE and autoradiography showed that the Iodo-bead method produced the least aggregation and fragmentation of TF (Fig. 2a and data not shown). Few impurities were detected in the Iodo-bead-labeled ¹²⁵I-TF (Fig. 2a). Iodinated TF was fully able to donate Fe to growing meningococci, since FAM30 utilized 1 μ M ¹²⁵I-TF as well as unlabeled TF for growth under Fe-limiting conditions (Fig. 2b). To further limit radiation-induced damage, ¹²⁵I-TF was used within 2 weeks of labeling.

Binding of ¹²⁵I-TF. Meningococci bound ¹²⁵I-TF in a saturable specific manner (Fig. 3). Saturation occurred at approximately 1 μ M for both FAM30 and FAM38; however, FAM30 bound four times more TF at this concentration than did the TF⁻ transformant FAM38. Specific binding data were subjected to Scatchard analysis (26), which yielded a K_d of 0.7 μ M and a copy number of 2,900 receptors per CFU for FAM30. FAM38 had a similar K_d of 0.4 μ M but a reduced apparent copy number of 560 (Fig. 4). The correlation between the inability of FAM38 to use TF as an Fe source and the reduced binding of ¹²⁵I-TF strongly suggests that the observed TF-binding activity represents a functional receptor. In control experiments, *E. coli* HB101 did not bind ¹²⁵I-TF (data not shown).

Specificity of the meningococcal TF receptor. Competition experiments were performed to test the specificity of the TF receptor. Fe-stressed FAM30 cells were incubated with a constant, saturating concentration of ¹²⁵I-TF (1 μ M) and increasing concentrations of mouse TF, human LF, 100% Fe-saturated human TF, or apoTF. Neither mouse TF nor human LF displaced the ¹²⁵I-TF even at 20 times the concentration of radioligand, although human TF competed strongly. The ability of apoTF to displace ¹²⁵I-TF did not differ significantly from that of 100% Fe-saturated human TF under these conditions (Fig. 5).

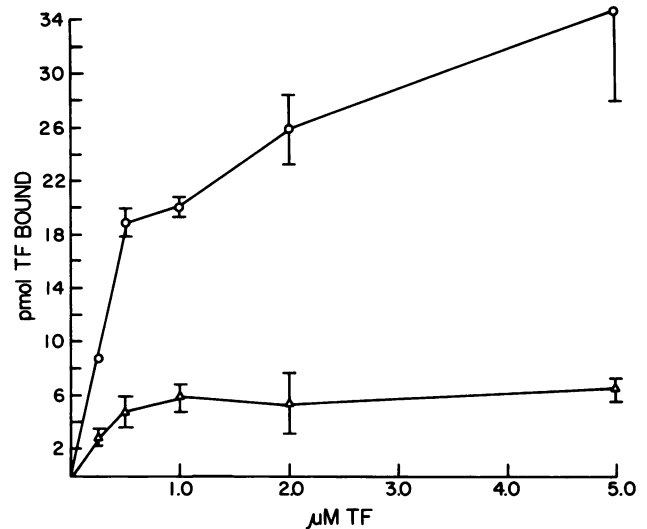


FIG. 3. Specific binding of ¹²⁵I-TF by FAM30 (\circ) and FAM38 (Δ). Specific binding is defined as the difference in the amount of ¹²⁵I-TF bound in the presence and absence of 100 \times 100% Fe-saturated human TF. Results depict the mean and standard deviations for three experiments.

In contrast with our results, a recent report (27) indicates that apoTF does not compete as effectively as Fe-saturated TF with HRP-TF for binding to meningococci in a solid-phase dot enzyme assay. This discrepancy could have arisen from our use of saturating (1 μ M) concentrations of TF, as the previous study employed very low concentrations of HRP-TF (0 to 4 nM). Furthermore, we could not rule out the possibility that Fe contamination of BSA used to block nonspecific binding of ¹²⁵I-TF to Microfuge tubes and meningococcal cells afforded ferration of apoTF. We repeated the competition experiments using radioligand concentrations of 0.25 K_d (0.17 μ M) in the presence of 50 mM

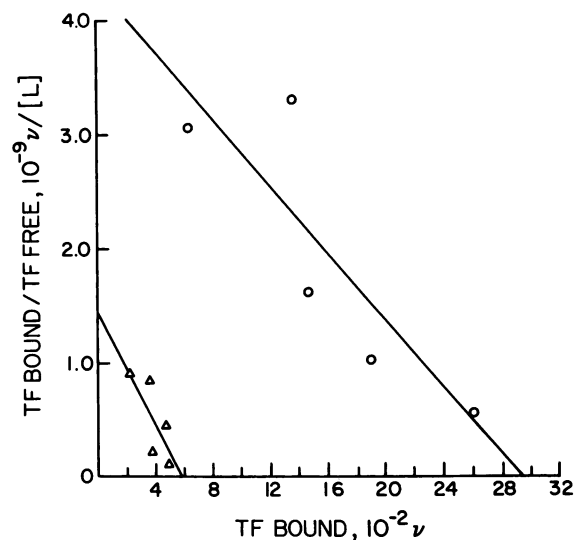


FIG. 4. Scatchard plot of ¹²⁵I-TF specific binding to FAM30 (\circ) and FAM38 (Δ) from data shown in Fig. 3. Lines were fitted by linear regression. Correlation coefficients were -0.845 for FAM30 and -0.701 for FAM38.

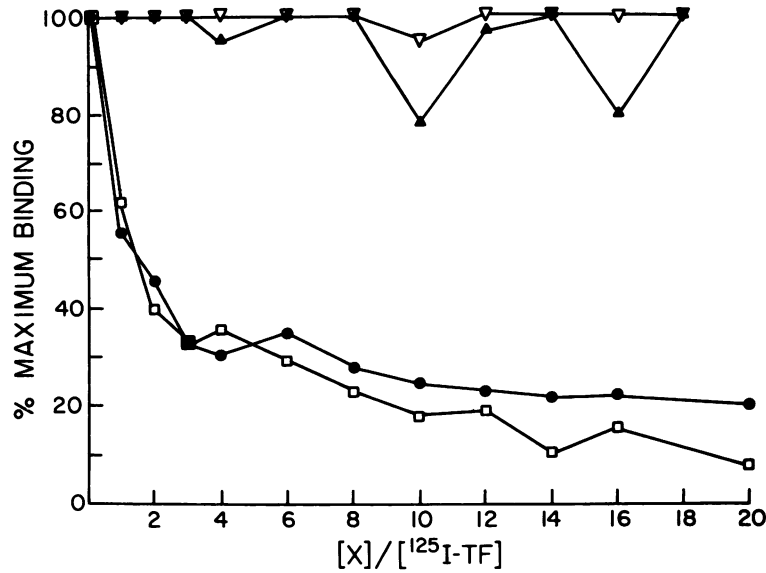


FIG. 5. Binding of ¹²⁵I-TF (1 μM) in the presence of increasing concentrations of 100% Fe-saturated human TF (□), apoTF (●), mouse TF (▽), and LF (▲). Maximum binding is defined as the amount of ¹²⁵I-TF associated with FAM30 in the absence of competing ligands. Data shown are the average of two experiments done in duplicate. [X], Concentration of competitor.

desferroxamine. The results were unchanged (Fig. 6) and suggested that the meningococcal TF receptor bound TF and apoTF with equal affinity. The solid-phase dot enzyme assay for HRP-TF binding suggested a diminished ability of apoTF to compete compared with that of 100% Fe-saturated TF (data not shown), as reported previously (27). Reasons for the discrepancy with the two assays are unknown.

Fe-regulated expression of binding. Preliminary binding studies suggested that receptor activity increased with progressive Fe starvation (data not shown). Therefore, ¹²⁵I-TF bound to FAM30 and the isogenic TF⁻ strains FAM38 and FAM41 after each mass doubling in Fe-limited CDM was

assayed. Receptor activity of FAM30 dramatically increased with Fe starvation, whereas FAM38 and FAM41 bound less TF at all time points (Fig. 7). After six cell doublings, expression of TF receptor activity continued to increase. Routine use of cells after four mass doublings in CDM thus somewhat underestimated maximal TF binding. Even after six mass doublings, however, the TF⁻ derivatives of FAM30 still showed little TF binding. We concluded that the TF receptor activity was Fe repressible and that the differences

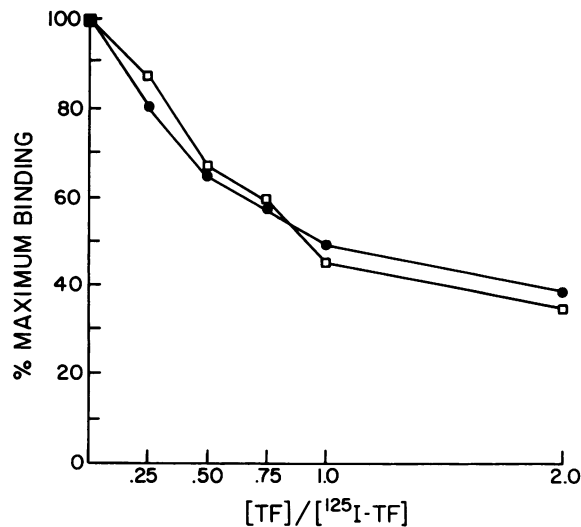


FIG. 6. Binding of 175 nM (0.25 K_d) ¹²⁵I-TF in the presence of increasing concentrations of 100% Fe-saturated human TF (□) and apoTF (●), and 50 μM desferroxamine to bind any free Fe. Desferroxamine was added prior to addition of ligand or competitors. Maximum binding was calculated as shown in Fig. 5. Data shown are the average values for two experiments done in duplicate.

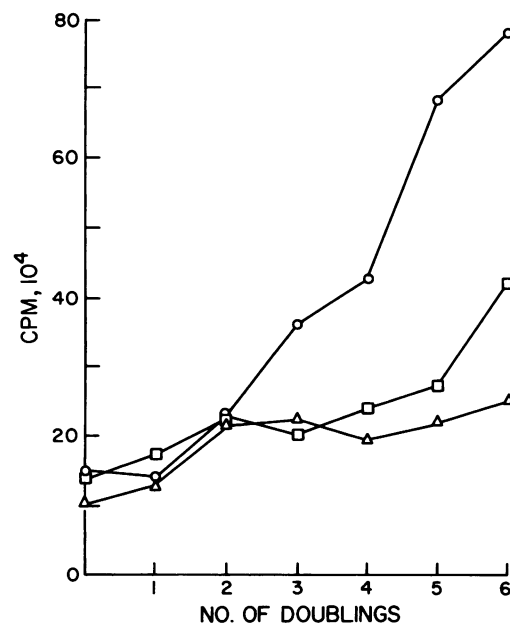


FIG. 7. Binding of ¹²⁵I-TF as a function of iron starvation. The amount of ¹²⁵I-TF bound to FAM30 (○), FAM38 (△), and FAM41 (□) was assayed after growth in Fe-depleted CDM; binding was measured after each doubling in cell mass. Results depict the average of two experiments done in duplicate.

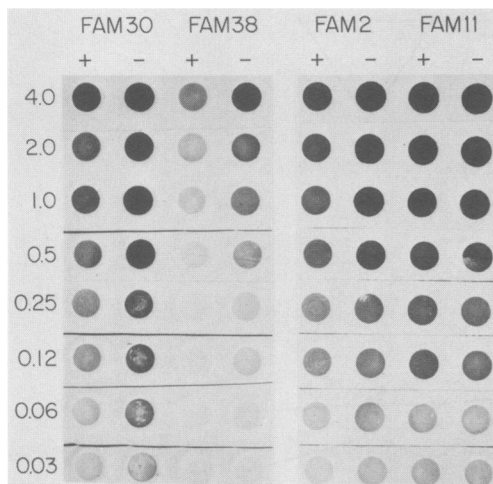


FIG. 8. Dot enzyme assay. Binding of serial twofold dilutions of HRP-TF to Fe-sufficient (+) and Fe-starved (-) FAM30, FAM38, FAM2, and FAM11. Concentrations of HRP-TF are shown on the left (μM).

between FAM30 and either FAM38 or FAM41 were not the result of delayed expression of receptor activity in FAM38 or FAM41. Results were essentially the same in FAM38 and FAM41 in these and other experiments (data not shown).

HRP-TF dot assays. Solid-phase assays with HRP-TF as ligand were also performed to compare FAM30 with FAM38 (Fig. 8). The results were qualitatively similar to those of the equilibrium-phase assay using ^{125}I -TF: FAM38 bound less TF than did FAM30, and in both strains TF receptor activity was increased by Fe starvation. In the same manner, we also examined the binding of TF to FAM2 and its pleiotropic Fe-transport mutant FAM11. The inability of FAM11 to internalize Fe bound to TF, LF, citrate, or aerobactin (9) suggests that FAM11 probably is altered in a common step involved in Fe transport, subsequent to or in addition to specific binding of TF to a receptor. Results showed that Fe-starved and Fe-sufficient FAM11 bound TF equally and to the same extent as Fe-starved FAM2 (Fig. 8). The constitutive expression of TF receptor activity in FAM11 was consistent with earlier evidence that FAM11 is functionally Fe starved even when grown in the presence of Fe because of its inability to internalize Fe (9). Specific binding of ^{125}I -TF to FAM11 also was equivalent to that observed with Fe-starved FAM2 in the equilibrium-phase assay (data not shown). Loss of TF receptor activity thus correlated very well with the phenotypes of the meningococcal Fe mutants. Only mutants such as FAM38 that were specifically unable to use TF were altered in TF receptor activity.

Western blots. Recently, an Fe-regulated meningococcal membrane protein (about 71 kDa in some strains, 85 kDa in others) which bound HRP-TF on a Western blot was reported (27), along with the suggestion that this TF-binding protein (TBP) might be part of a meningococcal TF receptor. We examined this possibility by determining whether TBP activity on the Western blot correlated with the results of the TF-binding assays. Strain FAM30 produced an FeRP of ca. 65 kDa that bound HRP-TF following Western transfer (Fig. 9). Strains FAM38 (Fig. 9) and FAM41 (data not shown) produced quantities of 65-kDa TBP similar to that in FAM30, although other assays (Fig. 3, 7, and 8) showed that these TF⁻ transformants bound significantly less TF to whole cells. In contrast, FAM11 and FAM63, both of which

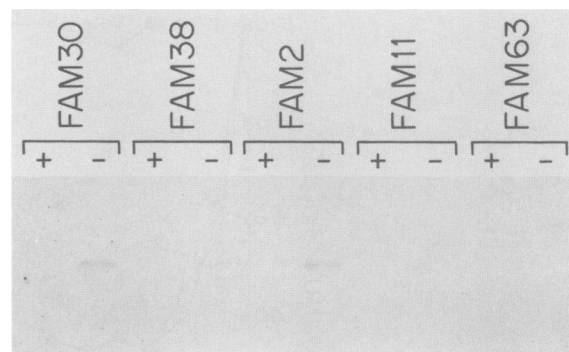


FIG. 9. Western blot. Binding of HRP-TF to total membrane proteins of Fe-sufficient (+) and Fe-starved (-) FAM30, FAM38, FAM2, FAM11, and FAM63. The HRP-TF-binding protein in FAM30, FAM38, and FAM2 is about 65 kDa.

bound TF normally to whole cells (Fig. 8 and data not shown), failed to produce significant amounts of 65-kDa TBP (Fig. 9). These data suggest that 65-kDa TBP is not necessary for functional TF receptor activity.

DISCUSSION

Concentration-dependent saturability and ligand specificity are two criteria for a biological receptor. Well-characterized eucaryotic receptors recognize only their legitimate ligand and perhaps those very closely related in structure (2, 7). Specifically bound ligand and should be displaced by an excess of competing ligand at equilibrium, while nonspecifically bound ligand should be less readily displaced (2, 7). Our data confirmed the existence of a receptor for human TF on the meningococcus, as saturable specific binding and specificity for human TF were demonstrated for *N. meningitidis* FAM30. Saturation occurred at 1 μM TF, and Scatchard analysis showed a K_d of 0.7 μM and a copy number of 2,900 receptors per CFU. The conclusion that these binding data represent a functional receptor was strengthened by the demonstration that FAM38, a TF⁻ transformant of FAM30, was deficient in receptor activity. Scatchard analysis suggested that FAM38 had a receptor of approximately normal affinity but in reduced apparent copy number. Our data do not allow determination of whether the TF⁻ allele (*fud-6*) in FAM38 altered the TF receptor or altered the cell surface framework in which the TF receptor is found. The reduction in TF receptor activity in FAM38 may reflect primary alteration in other cell surface components, with resulting steric effects on the TF receptor.

In addition to confirming the existence of a receptor, these data also suggest a possible mechanism for receptor-mediated Fe acquisition by the meningococcus. While the K_d of 0.7 μM is low relative to values established for eucaryotic receptors (2, 7), it is typical of some receptors in lower organisms (29). Since TF is present physiologically at 30 μM , a concentration far higher than the 1 μM saturation point for both binding and uptake, high-affinity binding of TF may be unnecessary for efficient Fe uptake from TF. Our data suggest that meningococci bind human TF regardless of whether TF contains Fe; low binding affinity results in high turnover of TF at the cell surface. This may allow the meningococcus to sort through the available TF and remove Fe from ferrated molecules. If the receptor bound Fe-TF with high affinity, as do human (31) and mouse (30) TF receptors, efficient use of the receptor for further uptake of

Fe would require preferential release of apoTF. This would be reflected in an attenuated affinity of meningococci for apoTF, which was not observed. Unlike the situation with eucaryotic cells, however, TF is not internalized after binding to pathogenic *Neisseria* species (1, 18, 28). The correlation between concentrations of Fe-uptake saturation and binding of TF (1 μ M) suggests that receptor-mediated binding of TF may be the rate-limiting step in Fe uptake from Fe-TF. This idea is supported by the phenotype of FAM38; an approximately fivefold reduction in apparent receptor copy number is responsible for at least a 95% reduction in Fe uptake from TF (8).

Care must be taken in interpreting the 125 I-TF binding data. At infinite dilution, about 10% of bound ligand is shed after 0.15 times the necessary binding time (2). The washes performed after binding required significantly longer than 0.15 times the 20-min binding time (which is probably greater than the necessary binding time). Affinities and copy numbers may thus be underestimated. The estimates, however, describe the relative differences between strains. Moreover, the existence of reproducible specific binding after the substantial interval at infinite dilution is a compelling argument for the presence of a receptor.

Although the dot enzyme assay measures total (including nonspecific) binding and gives only rough qualitative answers, the similarity in results from the equilibrium 125 I-TF-binding assay suggests that the relatively simple dot enzyme assay may be a rapid method to screen meningococcal mutants for TF-binding ability or to screen recombinant libraries for TF receptor activity. A significant difference in results with the two binding assays concerned the apparent affinity of apoTF binding to the receptor. Schryvers and Morris (27) demonstrated a significant difference between the abilities of apoTF and 100% Fe-saturated TF to compete with the binding of HRP-TF, but our 125 I-TF assay showed no difference. This discrepancy was not due to the presence of contaminating Fe in the 125 I-TF assay. TF may be modified by the bulky HRP in such a way that binding is, for unknown reasons, less sensitive to competition by apoTF. Alternatively, the 125 I-TF assay may be complicated because of oxidative damage to TF during iodination. However, damage from iodination was undetectable by SDS-PAGE and autoradiography, and 1 μ M (barely saturating) 125 I-TF supported normal growth of meningococci, indistinguishable from that with unmodified TF.

Time course experiments showed greater receptor activity with increasing Fe starvation. This suggests that the receptor is an FeRP or at least includes an Fe-regulated surface component. The identity of the receptor is unknown. The receptor cannot be the 85- or the 95-kDa FeRP, since FAM38 and FAM41 were equally deficient in receptor activity despite their differences in expression of these FeRPs. The 70-kDa FeRP is likely unimportant to the TF receptor, since FAM11, which lacks the common 70-kDa FeRP by chemical and immunological criteria (9), demonstrated wild-type TF receptor activity. Recently, Schryvers and Morris used TF-affinity chromatography to identify several meningococcal proteins with apparent specificity for TF; one of these was about 65 kDa (27a). Our results showed a 65-kDa protein that bound HRP-TF in a Western blot, but this protein probably is not necessary for TF receptor function, since it was much reduced in a mutant (FAM11) that exhibited normal TF receptor activity. Further experiments are required to establish the identity of the TF receptor.

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