created in the culture medium. It was shown previously [3] that colony-stimulating activity of bone marrow feeder cells cannot replace serum growth factors, especially PDGF, and that the formation of CFU-colonies is made possible by their combined action on bone marrow stromal stem cells. The fact that the colony-stimulating action of feeder cells is transmitted through the culture medium makes it possible to identify substances activating resting CFU-f.

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ROLE OF FIBRONECTIN IN THE PATHOGENESIS OF MENINGOCOCCAL INFECTION

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Among the factors determining resistance of the nasopharyngeal epithelium (the portals of entry for meningococcal and certain other infections) to colonization by Gram-negative flora, much attention has been paid to the fibronectin layer covering these cells – this is a high-molecular-weight glycoprotein which performs widely different functions in the body [9, 11]. Gram-negative bacteria as a whole are known to differ from Gram-positive in that they bind only weakly with fibronectin and, according to some authorities, the weak colonization of the nasopharyngeal epithelium by the Gram-negative flora under normal conditions can be explained by blocking of the cell receptors for these bacteria by fibronectin [10], although there is evidence [8] that fibronectin promotes adhesion and colonization of certain Gram-negative bacteria also [7, 10], and for that reason in each concrete case, when the role of fibronectin in the pathogenesis of a particular infection is being evaluated, a special investigation is required.

In this investigation, in experiments in vitro we studied binding of various strains of Neisseria meningitidis with fibronectin and the effect of cell surface structures [pili; bacterial adhesins) on the character of this interaction, with a view to explaining the role of fibronectin in adhesion of the pathogen to cells of the host's nasopharyngeal epithelium.

EXPERIMENTAL METHOD

Experiments were carried out with 20 strains of N. meningitidis, whose characteristics are given in Table 1. Meningococcal pili were detected by electron microscopy of negatively stained preparations by the method in [6]. Freeze-dried strains were cultured for 18 h on Hottinger's agar with the addition of 20% horse serum at 37°C in an atmosphere with 5% CO_2, and then transferred into 30 ml of semisynthetic medium [1], pH 7.4 (in some experiments the pH of the medium was adjusted to 6.6) and cultured for 8-10 h at 37°C with continuous mixing.

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TABLE 1. Characteristics of Test Strains

Strain	Serogroup	Source	Hemagglutinating activity	Hyaluronidase activity	Piliated cells, percent	Cells with aggregated pili (per cent)	Binding with fibronectin
611	W135	Carrier	—	—	0	6,5	19,9
1080	Not grouped	»	n.d.	+	0	0	24,0
86	W135	»	n.d.	—	0	2,6	10,5
638	C	»	n.d.	—	0	1	10,9
B 15	B	CSF		—	0	17,6	12,3
1867	A	CSF	+	+	0	0	21,2
1092	A	Carrier	+	+	1,5	17	13,5
1013	Not grouped	»	+	+	2,6	0	11,4
1645	A	CSF	+	+	3	30	10,0
1073	A	»	+	+	5	20	7,3
168	E29	Carrier	+	—	5	50	7,8
255	A	CSF	—	—	6,3	9	6,6
1635	A	CSF	+	+	10,0	70	4,4
1084	A	CSF	+	±	10,0	20	4,5
BC 59	B	CSF	n.d.	—	15	5	5,5
427	Z	CSF	+	+	16	1,2	2,1
1091	Not grouped	CSF	n.d.	+	16	0	4,5
630	C	Carrier	n.d.	—	27	0	2,4
286	W135	»	+	—	30	6,5	1,1
4358	A	CSF	±	—	76	0	0

Note. CSF) Cerebrospinal fluid; n.d.) not determined.

The meningococci were labeled by the addition of 100 μCi of ^{14}C-protein hydrolysate or 50 μCi of ^{3}H-lysine to the medium. After incubation the cells were washed three times with PBS with 1 mM $CaCl_2$ and their concentration adjusted to $1 \cdot 10^7$ cells/ml with this same buffer.

Fibronectin was obtained from human plasma by the method in [3]. Experiments were carfied out in 24-well planchets ("Costar"). After sensitization of the wells with 0.2 ml of fibronectin solution in a concentration of 0-100 μg/ml, 0.2 ml of cell suspension ($1 \cdot 10^7$ cells/ml) was added to each well and the samples were incubated for 30 min at 37°C with constant mixing. Unbound cells were washed three times with PBS, the pooled supernatants were transferred to flasks for scintillation counting, and 0.2 ml of trypsin solution (1 mg/ml) was added to the wells for 10 min at 37°C, and washed twice with the same solution; the pooled solutions also were counted on a "Beckman LS 6800" scintillation system. In the control, instead of fibronectin, BSA was added to the wells in the same concentrations. The degree of binding of the meningococcus with fibronectin was estimated from the ratio of bound radioactivity to added radioactivity (deducting nonspecific binding with BSA, in percent).

EXPERIMENTAL RESULTS

A series of preliminary experiments on strains 1635 and 1867 showed that binding of both strains with fibronectin exceeded that with BSA, although to a different degree in the two strains, and it was dependent on the dose of fibronectin added to the well (maximal binding was observed with fibronectin in a concentration >70 μg/ml, on that basis the dose of fibronectin used in the subsequent experiments was 70 μg/ml).

Table 1 gives the general characteristics of the test strains and values of binding of the meningococcus with fibronectin. Clearly, adhesion of the meningococcus to fibronectin is independent of the serologic group of the strains, the source from which they were isolated, their hemagglutinating capacity and hyaluronidase activity, and was determined only by the degree of piliation of the bacteria, and under these circumstances, interaction between meningococcus and fibronectin is influenced only by the free-lying pili, and those which are aggregated cause virtually no change in the character of bacterial adhesion. As Fig. 1 shows, appreciable binding of cells with fibronectin is observed only in nonpiliated or weakly piliated strains, when with an increase in the degree of piliation there was a sharp decrease in adhesiveness of the cells, and strains with 8-10% of piliated cells virtually did not bind with fibronectin. That the observed effect was in fact due to pili and not to any other components of the cell wall, differing from strain to strain, was proved in experiments on two well piliated strains, grown in culture at the ordinary pH values and also at pH 6.6 (at pH 6.6 the degree of piliation of the meningococcus is sharply reduced due to dissociation of pilin subunits [2], and we confirmed disappearance of the pili by electron microscopy). The results in Table 2 show that reduction of the degree of piliation of the test strains leads

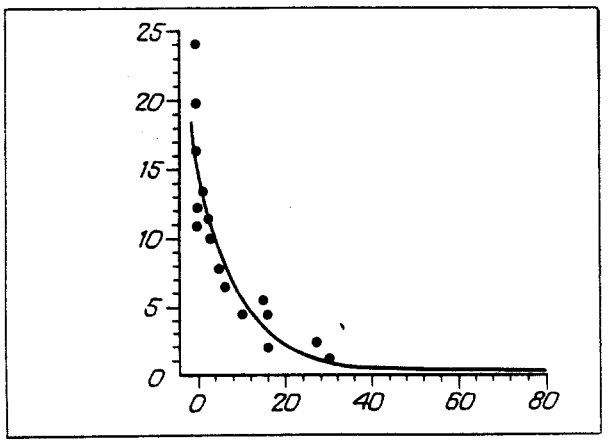

Fig. 1

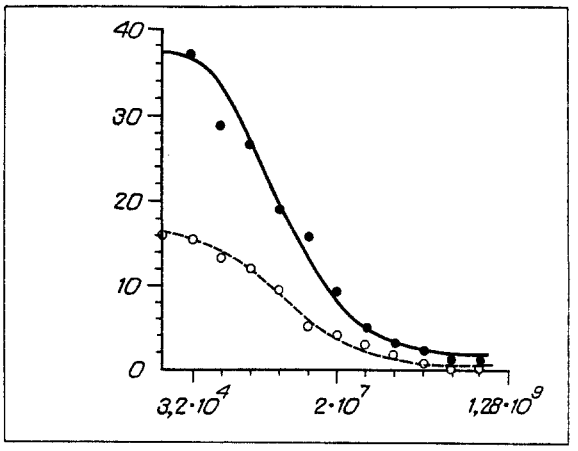

Fig. 2

Fig. 1. Adhesion of different strains of N. meningitidis to fibronectin. Each point on the curve (here and in Fig. 2, mean value of three experiments) corresponds to one strain of N. meningitidis listed in Table 1. Abscissa, binding (in percent); ordinary, piliated cells (in percent).

Fig. 2. Kinetics of binding of N. meningitidis with fibronectin. Abscissa, binding (in percent); ordinate, number of cells in 1 ml.

TABLE 2. Effect of Pili on Binding of N. meningitidis with Fibronectin

Strain	Number of cells in 1 ml	Binding with fibronectin, percent
286p$^+$	$2\cdot10^6$	2,45±0,83 (3)
	$1\cdot10^6$	3,11±1,04 (3)
286p$^-$	$2\cdot10^6$	11,20±1,22 (3)
	$1\cdot10^6$	14,50±0,20 (3)
1084p$^+$	$2\cdot10^6$	6,2 (1)
1084p$^-$	$2\cdot10^6$	19,5 (1)

Note. p$^+$ and p$^-$) Piliated and nonpiliated strains, respectively.

to a marked increase in binding of the meningococcus with fibronectin.

In the next experiments the kinetics of binding of the meningococcus with fibronectin were studied. For this purpose, serial twofold dilutions of piliated and nonpiliated cells were introduced into wells with fibronectin and the values obtained for binding of the cells with the substrate (Fig. 2) were analyzed by Scatchard plot. The number of binding sites of these cells with protein was about equal ($2\cdot10^6$ cells/cm^2), but the value of the binding constant with fibronectin for the piliated cells (21.9 cells/ml) was considerably lower, and the dissociation constant ($6\cdot10^6$ ml/ml) higher than those same parameters for nonpiliated cells (143.2 cells/ml and 10^6 ml/ml, respectively). These data show that the presence of pili on the meningococcus, while not affecting the cell receptors for fibronectin proper, modified only the kinetic parameters of association and dissociation during interaction of the meningococcuc with fibronectin.

The results show that virtually all strains of N. meningitidis studied can bind, although to different degrees, with fibronectin. The fact that the intensity of interaction of the meningococcus with fibronectin was determined purely by the degree of piliation of the bacteria, and was independent on their serological group, or other characteristics, means that, first, the general role of interrelations between bacterial cell and fibronectin in the pathogenesis of meningococcal infection can be evaluated, and second, new approaches can be outlined to its prevention. It can be tentatively suggested that normally, i.e., when the nasopharyngeal epithelium is protected by fibronectin, absence of adhesion of the meningococcus to the target cells can be expected, for in vivo most nasopharyngeal strains are well piliated [4]. Under these circumstances any reduction in the quantity of fibronectin bound with the epithelial cells will lead to increased risk of the development of this infectious

disease. We know that the quantity of fibronectin is not only subject to individual variations, but also differs in different age groups (in children (a particularly vulnerable contingent as regards meaningococcal infection) the fibronectin level is significantly lower than in adults [5]). On the other hand, the results point to a common type of fibronectin receptor in miningococci of different strains. This receptor is evidently one of the common meningococcal antigens, interest in which is explained by the search for preparations capable of exerting a protective effect against meningococcal infection of whatever specificity. Thus elucidation of the concrete mechanism of ligand-receptor interactions in the chain of processes leading to adhesion of the menigococcus to epithelial cells requires further study.

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EFFECT OF PLATINUM PREPARATION ON PHAGOCYTIC ACTIVITY OF MOUSE PERITONEAL EXUDATE MACROPHAGES

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A wide range of effect of cells is involved in the realization of antitumor immunity: natural killer cells, cytotoxic T lymphocytes, and macrophages. The last of these are the most interesting. Macrophages can cause lysis of various types of tumor cells, without damaging normal cells of the same histogenesis [4, 6, 8]. Normal "unarmed," unactivated macrophages can interact with tumor cells at the stage of their appearance and during the initial stage of their development. Cytostatics used in the chemotherapy of neoplasms influence the immune system of the host and, in particular, damage the mononuclear system. The effect of various classes of cytostatics on function of the macrophagal component of immunity has been studied in sufficient depth [5]. However, data on the character of the effect of a new class of antitumor compounds, namely coordination compounds of platinum, on macrophages could not be found in the accessible literature. The aim of this investigation was to determine the action of platinum preparations on phagocytic activity of peritoneal exudate macrophages.

EXPERIMENTAL METHOD

Oxoplatinum (cis-dichlorodiamino-trans-dihydroxoplatinum IV, produced by "Lachema," Czechoslovakia) and cycloplatam [cyclopeptidylamino-S-malatoplatinum (II) amine], produced in the USSR, were generously provided for the study by Professor A. B. Syrkin M.D. (All-Union Oncologic Scientific Center, Academy of Medical Scientific Center, Academy of Medical Sciences of the USSR). The preparations were made up when required in 5% glucose solution to give doses of between 0.01 and 1.0 MAD (LD_{10}) in experiments in vivo, and in complete medium RPMI-1640 to concentrations of 0.09-90.0, or 0.46-46.0 μg/ml for oxoplatinum and cycloplatam,

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