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Neisseria spp. and AIDS

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***Neisseria meningitidis* from various serogroups and two commensal neisseriae (*N. sicca* and *N. perflava*) were isolated from 15 patients at various stages of human immunodeficiency virus infection in this clinical and bacteriological study. The cases were grouped into the following three classes: (i) infections with an *N. meningitidis* strain of a serogroup known to be pathogenic (A, B, or C) and apparently independent of the human immunodeficiency virus infection, (ii) infections with a *N. meningitidis* strain of a serogroup which is normally either commensal or poorly pathogenic (serogroups Y, X, Z, and Z,29E), (iii) pulmonary and disseminated infections occurring in the course of the clinical evolutionary stage of AIDS, in two cases of which commensal neisseriae (*N. sicca* and *N. perflava*) were isolated from blood cultures.**

Since the beginning of the 1980s, an increased incidence of AIDS has coincided with an increased incidence of a variety of diseases, such as tuberculosis and pneumocystosis.

Most patients with AIDS suffer from secondary infections at some stage (16, 18, 32). Secondary infections due to *Salmonella* spp. (12, 14, 18, 30) and *Mycobacterium* spp. (15) are commonly used to classify infections by the human immunodeficiency virus (HIV) (6).

The association of neisseriae and HIV infection is rare. Only one case of meningococcemia secondary to an HIV infection has been described. The causative strain was of *Neisseria meningitidis* serogroup B (2). *N. gonorrhoeae* infections, urethritis, and arthritis are more common (19). Transmission is sexual, as in cases of AIDS-associated syphilis (3). A few cases of infection with *Branhamella catarrhalis*, in the nomenclature of Catlin (4), associated with AIDS have also been reported (16, 18, 33).

Among the strains sent to the National Reference Center for Meningococci and Related *Neisseria* (NRCM) between March 1988 and January 1991, there were 15 accompanying reports indicating that the patients had HIV infections. These infections were at various stages of development, from seropositivity to the confirmed clinical stage of AIDS. The strains were mainly *N. meningitidis* from various serogroups and two commensal neisseriae: *N. sicca* and *N. perflava*. The purpose of this work was to determine whether there was a relationship between *Neisseria* infection and HIV infection.

MATERIALS AND METHODS

Clinical studies. Regarding epidemiological surveys of meningococcal disease in France, two modes are used in parallel: one is obligatory notification of the Ministry of Health by physicians; the second is monitoring of strains submitted to the NRCM.

Strains of *N. meningitidis* are systematically sent to the NRCM for epidemiological studies and serosubtyping. Atypical strains of *Neisseria* spp. more or less related to *N. meningitidis* are handled likewise. During the reference period (March 1988 to January 1991), 2,304 strains were submitted to the NRCM (1,541 were *N. meningitidis*, and 763 were *Neisseria* spp.). Among the clinical inquiries accompaning the strains were 15 reports of various stages of AIDS (18a).

Three main groups of patients were formed on the basis of symptoms of *Neisseria* infection: (i) characteristic *Neisseria* infections. (ii) miscellaneous infections, especially pulmonary infection as defined by clinical symptoms, X-ray examinations, and laboratory identification of the strains isolated. (iii) carriage without symptoms (or poorly symptomatic infections).

Classification of AIDS. Four groups were formed on the basis of the Centers for Disease Control description of 1987 (6). Group I was acute primary infection. Group II was seropositivity without symptoms. Group III was generalized chronic lymphadenopathy. Group IV was subdivided into subgroups IVA, IVB, IVC, IVD, and IVE. The main clinical symptoms at stage IVA were fever for more than 1 month, loss of weight (more than 10%), and diarrhea for more than 1 month. Secondary infections were typical at stage IVC. A special pediatric classification (P2) was used for case 9 (a 7-month-old child).

Risk factors. The risk factors for the cases included in the study were as follows: drug abuse (10 cases), multiple partners (4 cases), homosexuality (1 case), and mother-child transmission (1 case). One case presented two risk factors: multiple partners and drug abuse. There were no cases of infection following blood transfusion.

Bacterial identification. Bacterial strains were grown on the previously described medium for gonococci and meningococci (25) (Diagnostics Pasteur, Marne-la-Coquette, France). It contained (i) a base medium with meat extract and yeast extract and (ii) supplement G with horse plasma, yeast extract, and glucose. For convenience, the medium was designated G medium. Addition of VCF (vancomycin, 3.4 μg/ml; colimycin, 7.5 μg/ml; fungizone, 2.0 μg/ml) made it selective for some *Neisseria* spp. (*N. gonorrhoeae*, *N. meningitidis*, and others). The selective medium was designated G + AB.

After growth, strains were identified as previously described (26, 27). The identification procedures comprised inspection of colony morphology and microscopic analysis after gram staining; assessment of growth under various conditions, i.e., at 22 and 37°C and on selective medium (G + AB) or other media for neisseriae; acidification of six substrates (glucose, maltose, fructose, sucrose, mannitol,

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and lactose) as indicated on cystine tryptic agar (Difco); nitrate and nitrite reductase activities tested on appropriate media (27); and γ-glutamyltransferase activity tested as previously described (26).

Strains were stored both in glycerol at −70°C and lyophilized.

Susceptibility to antibiotics. G medium was used to study antibiotic susceptibility. Petri dishes (12 by 12 cm) containing 60 ml of G medium were inoculated with bacterial suspensions adjusted to an optical density of 50 Klett units, which is equivalent to 1 McFarland unit. These suspensions were made in phosphate-buffered saline (0.15 M, pH 7.2). The filter paper disks used (Diagnostics Pasteur) contained the following quantities of antibiotics: penicillin G, 10 μg; ampicillin, 10 μg; amoxicillin, 25 μg; amoxicillin and clavulanic acid, 10 μg; cefotaxime, 30 μg; ceftriaxone, 30 μg; streptomycin, 10 μg; spectinomycin, 100 μg; tetracycline, 30 μg; minocycline, 30 μg; erythromycin, 15 μg; chloramphenicol, 30 μg; spiramycin, 100 μg; rifampin, 30 μg; pefloxacin, 5 μg; sulfonamides, 200 μg. Readings were performed after 24 and 48 h of growth, and the diameters of the inhibition zones were measured. Under these conditions, strains were considered susceptible with a diameter larger than 20 mm, intermediately susceptible with a diameter between 19 and 16 mm, and resistant with a diameter smaller than 16 mm.

Serogrouping. When the bacteriological analysis suggested diagnosis of *N. meningitidis* after 18 to 24 h of culture on Mueller-Hinton medium (Difco), serogroups were determined by the method described by Devine and Hagerman (10). Agglutination was tested by using the 12 immune sera described to date: A, B, C, X, Y, Z, 29E, W135, H, I, K, and L (26). The immune sera were prepared by the NRCM after intravenous injection with live meningococci as described by Vedros (31). New Zealand rabbits were used for immunization.

Serotyping and subtyping. Isolates were serotyped by the whole-cell enzyme-linked immunosorbent assay technique of Abdillahi and Poolman (1, 22), with minor modifications (28).

Reference strains of *N. meningitidis* used for serosubtyping. The antigenic formula of reference strains was written in accordance with the scheme proposed by Frasch et al. (13), i.e., serogroup-serotype-serosubtype. The strains used were M1080 (B:1:P1.1,7), B16B6 (B:2a:P1.2), 2996 (B:2b:P1.2), M981 (B:4:P1.−), M982 (B:9:P1.9), S3446 (B:14:P1.6), H355 (B:15:P1.15), 60E (C:16:P1.1,7), and 444/76 (B:15:P1.7,16).

Monoclonal antibodies. Monoclonal antibodies against outer membrane proteins of *N. meningitidis* were designated as described by Poolman and Abdillahi (22). The monoclonal antibodies used were specific for the following serotypes: MN3C6B, 1; MN2C3F, 2a; MN2C3B, 2b; MN14C21.17, 4; MN5C8C, 14; MN15A14H6, 15; 93E9, 16. They were specific for the following subtypes: MN14C2.3, P1.1; MN16C13F4, P1.2; MN14C11.6, P1.7; DG-13, P1.6; MN15A10F, P1.9; MN3C5C, P1.15; MN5C11G, P1.16.

The bacteria were cultured for 18 h on medium G under 8 to 10% CO_2. *o*-Phenylenediamine (Sigma) was used as the substrate for the peroxidase reaction. Reactions were monitored at 492 nm by using an LP 200 spectrometer (Diagnostics Pasteur). Optical densities greater than 0.5 were scored as positive.

RESULTS

Clinical results. (i) General characteristics of patients. Table 1 shows demographic, clinical, and biological details of 15 HIV cases associated with recovery of neisseriae. Of the 15 patients, 13 were men and 2 were women. The patients were young adults between 18 and 41 years old, with the exception of a 7-month-old child of a seropositive mother. Secondary infection by *Neisseria* spp. occurred at the following time periods after seroconversion: 15 to 20 days (cases 5 and 14); 2, 6, and 7 months (cases 1, 7, and 9); and 3 to 5 years (cases 8, 10, 13, and 15). In cases 2, 3, 4, 6, 11, and 12, it was not possible to determine the time of infection. During the study period, there were 15 known cases of HIV infection in France, ranging from seropositivity (HIV^+) to clinically manifest AIDS, from which neisseriae were isolated. These strains made up 0.5% of the strains of neisseriae registered with the NRCM (including all species) from March 1988 to January 1991. The notifications of AIDS and meningococcal meningitis in France were as follows: from March 1988 to the end of 1988, 2,622 cases of AIDS and 430 cases of meningitis; in 1989, 3,679 cases of AIDS and 510 cases of meningitis; in 1990, 3,679 cases of AIDS and 426 cases of meningitis (10a).

Table 1 also lists stages of HIV infection based on the Centers for Disease Control classification. The 15 cases included in the study were classified as P2 (1 pediatric case, no. 9), II (cases 1, 2, 3, and 5), IVC1 (cases 6, 12, 13, and 14), IVC2 (cases 7 and 15), IVD (cases 8 and 10), and 2 cases (4 and 11) in which the stage could not be determined.

Neisseria spp. were isolated from cerebrospinal fluid or cerebrospinal fluid and blood (cases 1 and 2), blood (cases 3, 4, 14, and 15), bronchoalveolar fluid (cases 5 and 6), bronchial aspirate (cases 7 and 8), and the rhinopharynx (cases 9, 10, 11, 12, and 13). The clinical results are shown in Tables 1 and 2. Pulmonary infections and bacteremia were the most frequent forms of infection observed in this study.

(ii) Classification of patients by neisserial infections and association with other symptoms. Typical neisserial infections (group I in Table 2) were observed in cases 1 and 2. These two patients were diagnosed with characteristic meningitis and recovered. *N. meningitidis* (A:4:P1.9) was isolated in both cases. Cases 3 and 4 were diagnosed with clinical symptoms of meningococcemia. Two strains of meningococci were isolated; both were of serogroup C and serotype 2a or 2b and were nonsubtypeable. Miscellaneous symptoms (groups IIA and IIB, Table 2) were observed in eight cases. Cases 5 to 8 were diagnosed with pulmonary infections. Case 5 showed aspects of bronchitis upon fibroscopic examination, with isolation of *N. meningitidis* and *Pneumocystis carinii* from bronchoalveolar fluid. Case 6 had a cough and dyspnea, and *N. meningitidis* was isolated from bronchoalveolar liquid. *P. carinii* had been isolated previously. Cases 7 and 8 were similar, with clinical and X-ray aspects of pulmonary infection, and *N. meningitidis* was isolated from bronchial aspirates.

Patients 9 to 13 had various symptoms. In these patients, *N. meningitidis* was isolated from the pharynx. The main symptoms included pharyngitis with fever in case 9 (a 7-month-old child) and angina with a skin rash and candidiasis in case 10. Case 11 had pulmonary signs, including a cough and dyspnea, but *N. meningitidis* was isolated from the throat rather than from the lower pulmonary tract. The clinical signs in cases 12 and 13 appeared to be pneumocystosis related. Bacteremia was observed in cases 14 and 15 (group III, Table 2). Patient 14 had a fever and thoracic pain, and X-ray examination revealed alveolar opacities. *N. perflava* was isolated three times from blood cultures. Patient 15 had a clinical history that included herpesvirus infection, pneumocystosis, toxoplasmosis, and candidiasis. At the stage at which *N. sicca* was isolated, there where mainly symptoms of bacteremia and pulmonary infection. The pa-

TABLE 1. Clinical, biological, and bacteriological results of HIV cases associated with recovery of *Neisseria* spp.

Case no.	Age (yr)	Sex[a]	RF[b]	AIDS			*Neisseria* infection	
				CDC[c] group	T CD4 lymphocyte count (per mm^3) or T4/T8 ratio (<1)[d]	Clinical aspect(s)	Culture source[e]	*N. meningitidis* antigenic formula
1	40	M	MP	II	184	Meningitis	CSF	A:4:P1.9
2	18	F	DA	II	230	Meningitis	CSF, blood	A:4:P1.9
3	26	M	DA	II	?	Meningococcemia Phlebitis	Blood	C:2b:–[f]
4	41	M	DA MP	?	?	Meningococcemia Lung infection	Blood	C:2a:–[f]
5	20	M	MP	II	0.79	Lung infection Treponematosis	BAL Chancroid	Z:NT[g]:–[f]
6	30	M	DA	IVC1	22	Lung infection	BAL	Z,29E
7	29	M	DA	IVC2	70	Lung infection	BA	Y:NT:P1.1,7
8	37	M	DA	IVD	399	Lung infection	BA	Y:NT:P1.14
9	0.6	F	Mother HIV$^+$	P2B[h]	?	Pharyngitis	Throat	Y:ND[i]:ND
10	25	M	Homosexual	IVD	119	Angina, eruption, diarrhea, oral candidiasis	Throat	B:ND:ND
11	32	M	DA	?	36	Cough, fever	Throat	B:ND:ND
12	33	M	DA	IVC1	150	Pneumocystosis	Throat	X:NT:P1.10
13	27	M	DA	IVC1	24	Tonsil lesion	Throat	X:ND:ND
14	31	M	MP	IVC1	?	Lung infection, pneumocystosis	Blood	NA[j] (*N. perflava*)
15	24	M	DA	IVC2	0.09 7	Cough, fever	Blood	NA (*N. sicca*)

[a] M, male; F, female.
[b] RF, risk factor; MP, multiple partners; DA, drug abuse.
[c] CDC, Center for Disease Control.
[d] The normal ranges are as follows: T CD4, 800 to 1,500/mm^3; T CD8, 250 to 1,000/mm^3; T CD4/T CD8 ratio, 1.8 to 2.2.
[e] CSF, cerebrospinal fluid; BAL, bronchoalveolar liquid; BA, bronchial aspirate.
[f] Strain not subtypeable.
[g] NT, nontypeable.
[h] Child.
[i] ND, not done.
[j] NA, not applicable.

tient was hospitalized outside of France and died before a second blood culture could be performed.

Bacteriological analysis. All isolates were gram-negative diplococci, oxidase positive, catalase positive, and strict aerobes.

Thirteen of the isolates were classified as *N. meningitidis* on the basis of growth characteristics on media selective for neisseriae, acidification of glucose and maltose, and γ-glutamyltransferase activity. Two isolates acidified four sugars (glucose, maltose, fructose, and sucrose) and reduced nitrites but not nitrates. One strain was identified as *N. sicca* and was differentiated from *N. perflava* on the basis of colony morphology and agglutination in 0.8 NaCl. The remaining strain was classified as *N. perflava*.

Susceptibility to antibiotics. All strains were susceptible to all of the antibiotics evaluated except streptomycin.

Serogrouping. The serogroups found among the 13 *N. meningitidis* strains were A (two strains), B (two strains), C (two strains), Y (three strains), X (two strains), Z (one strain), and Z,29E (one strain) (Tables 1 and 2).

Serotyping and subtyping. The serogroup A strains (Table 1) had the antigenic formula A:4:P1.9. It was not possible to determine the antigenic formulas for the serogroup B strains and one strain each of serogroups X and Y because they could not be maintained in culture. The two serogroup C strains were of serotypes 2b and 2a and not subtypeable (C:2b:– and C:2a:–).

DISCUSSION

Certain bacterial infections are considered to be of critical value in establishing a diagnosis of AIDS. They include atypical mycobacterial infections (8, 15, 34) and infections due to "*Salmonella* minor" (12, 14, 18). In all cases, these infections are disseminated (20, 21). Tuberculosis, now found in many countries, is also considered a diagnostic criterion (5, 7).

Other bacterial infections associated with HIV infection may be caused by a variety of species, including but not limited to *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Listeria monocytogenes*, *Campylobacter jejuni*, *Pseudomonas aeruginosa*, *Shigella* spp., *Nocardia asteroides*, *Legionella* spp., *Haemophilus influenzae*, *Klebsiella pneumoniae*, and *Bordetella pertussis* (18, 23, 30). Among the *Neisseriaceae* and the *Branhamaceae* isolated from patients with AIDS, *N. gonorrhoeae* is frequently found as a sexually transmitted agent (19). There has been one case of *N. meningitidis* (serogroup B) infection (2) and a few cases of *Branhamella catarrhalis* (syn. *Moraxella catarrhalis*) infections (16, 33).

In our study of 15 cases, the patients were grouped into three classes on the basis of the relationship between HIV and *Neisseria* sp. infections (Table 2).

Group I includes patients from whom *N. meningitidis* strains of serogroup A or C were isolated. These serogroups are commonly pathogenic in immunocompetent subjects,

TABLE 2. Clinical diagnoses, *N. meningitidis* serogroups, and other *Neisseria* spp. isolated

Group and diagnosis	Species isolated (serogroup)	Case no.
I		
Cerebrospinal meningitis[a]	*N. meningitidis* (A)	1
	N. meningitidis (A)	2
Meningococcemia	*N. meningitidis* (C)	3
	N. meningitidis (C)	4
II		
A, pulmonary infection	*N. meningitidis* (Z)	5
	N. meningitidis (Z,29E)	6
	N. meningitidis (Y)	7
	N. meningitidis (Y)	8
B, miscellaneous symptoms[b]	*N. meningitidis* (B)	10
	N. meningitidis (B)	11
	N. meningitidis (X)	12
	N. meningitidis (X)	13
C, carriage	*N. meningitidis* (Y)	9
III: bacteremia, pulmonary[c] infection	*N. perflava*	14
	N. sicca	15

[a] Cerebrospinal meningitis and meningococcemia were associated (case 2).
[b] Cough, fever, pneumocystosis.
[c] Pulmonary infections and bacteremia were associated (cases 14 and 15).

and infections with such strains are therefore not surprising in immunodeficient patients. The *N. meningitidis* serogroup A strains had the same antigenic formula (A:4:P1.9) as those previously described in France (24) and in many other countries since the outbreak in Mecca. Most of these strains were found to be pathogenic. The presence of serogroup C and serotype 2a or 2b was shown to be correlated with virulence in *N. meningitidis* strains (28). However, it is unclear whether HIV infection favors secondary infection with *N. meningitidis*. As serum bactericidal activity was maintained in these patients, we tentatively conclude that these secondary infections are largely independent of the lymphocyte immunodeficiency (9, 29).

The second group of strains, as defined in Table 2, comprises three types of cases which need to be discussed separately. Four cases (no. 5 to 8) had pulmonary infections as demonstrated by clinical symptoms and X-ray examinations, and strains of *N. meningitidis* normally poorly pathogenic, such as serogroups Z (cases 5 and 6) and Y (cases 7 and 8), were isolated. Strains were isolated from bronchoalveolar lavages in cases 5 and 6 and from bronchial aspirates in cases 7 and 8. We have shown that *N. meningitidis* can often be isolated from pulmonary infections of immunodeficient but not HIV-infected patients. In a study performed in our laboratory, over 38 cases of various infections were due to *N. meningitidis* serogroup Y and one-third of the patients showed complement deficiency (17). *N. meningitidis* serogroups Z and Z,29E (cases 5 and 6) are rarely pathogenic under normal conditions. HIV-infected patients are particularly susceptible to pulmonary infections (21), and involvement of *N. meningitidis* in this type of infection is probably underestimated, with or without immunodeficiency.

The T CD4 lymphocyte count is an indicator of the immune status of HIV-positive patients, and depression of this count suggests a state of lowered immunocompetency. It is very likely that the immunodeficiency caused by HIV facilitated the *N. meningitidis* infections caused by strains of not commonly pathogenic serogroups.

Chemoprophylaxis with rifampin or vaccination (although only anti-A and anti-C vaccines are available in France) seems to be indicated for cases in which, for whatever reason, *N. meningitidis* is isolated from the throat of a seropositive patient, as in cases 9 to 13.

The third group includes patients with *N. perflava* and *N. sicca* infections (cases 14 and 15). These commensal, nonpathogenic species were isolated from blood cultures. In case 14, *N. perflava* was isolated three consecutive times. In this case, a pathogenic role of commensal neisseriae can be assumed, in accordance with Feder and Garibaldi (11), in that isolation of the organism occurred more than twice. In case 15, in which *N. sicca* was isolated only once, the pathogenic role of the isolate is questionable. The clinical signs of pneumopathy and the site of isolation support such a role.

Both subjects were severely immunodeficient, with a T CD4 lymphocyte count of 7/mm^3 and a CD4/CD8 T-lymphocyte ratio of 0.09 in case 15. These values were the lowest of the 15 cases studied.

Infections of HIV-positive patients with commensal *Neisseria* spp. have not been described previously. It seems probable that the effects of HIV infection allowed secondary infection with these species. Such patients should be considered to be at the AIDS stage of HIV infection.

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