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Transmission of *Neisseria meningitidis* among asymptomatic military recruits and antibody analysis

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SUMMARY

Following the occurrence of a case of systemic meningococcal disease in a military camp in Norway, throat cultures and blood samples were collected from 33 healthy individuals belonging to the same troop as the patient (troop A) and from 29 individuals from a different troop (troop B) in the same camp. Serological studies showed that 91% of the recruits had bactericidal antibodies against the disease-causing strain. The isolates of *Neisseria meningitidis* recovered from the throat cultures were serogrouped, serotyped, and assigned to a clone on the basis of an analysis of the electrophoretic mobilities of 14 metabolic enzymes. None of the 23 carriers in troop A harboured the clone responsible for the case of disease, but 6 carried isolates of the same electrophoretic type, ET-7, which was not identified in any of the 19 carriers of troop B. Individuals in troop A were resampled 2 and 17 weeks after the meningococcal disease episode. Five of the carriers had acquired different clones and one of them changed clone twice in that period. Four of the six newly acquired clones had previously been identified in other carriers of troop A, demonstrating transmission of clones among individuals living and working in close proximity.

INTRODUCTION

The normal and exclusive habitat of *Neisseria meningitidis* is the upper respiratory tract of man. The proportion of asymptomatic carriers in populations of healthy individuals is highly variable and carriage rates from 5–60% have been reported [1]. Some characteristics of the populations studied, such as age and living conditions of the individuals, have been shown to influence the prevalence of carriage [2–6]. Military recruits, in particular, are population-groups with high meningococcal carriage rates [7–9].

It is not fully understood why, in some individuals, *N. meningitidis* may invade and grow in the blood stream, and cause systemic disease. Since most patients

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with meningococcal disease have not had demonstrable contact with another case, it must be presumed that asymptomatic carriers are the major source of transmission.

When a case of meningococcal meningitis occurred in a military camp in Norway in 1986, a survey of meningococcal carriage was undertaken to determine the prevalence of the infecting strain in close-contacts of the case and to evaluate transmission of strains among healthy carriers sharing living quarters.

Because of quantitative variation in expression of surface antigens of the organisms [3], conventional methods of typing are generally unsatisfactory for characterizing *N. meningitidis* strains from carriers. Consequently, multilocus enzyme electrophoresis was employed to identify the meningococcal clones [10]. Blood samples were also taken to evaluate the serum antibody status of the individuals in contact with the patient.

MATERIALS AND METHODS

Human population studied

On 30 January, 1986 a case of meningitis occurred in a 19-year-old military recruit, in a camp in Oslo, Norway, and *N. meningitidis* was subsequently cultured from the blood of the patient. The day following hospitalization of the recruit, throat cultures and blood samples were taken from the 30 other recruits and the 3 officers of the troop in which the case occurred (designated as troop A). Two and 17 weeks later, throat cultures and blood samples were again taken from all available individuals of troop A: on 13 February, 27 recruits and 2 officers were sampled, and on 10 June, 25 recruits were sampled. Troop A was formed in October 1985. Officers were born between 1959 and 1964 and recruits between 1964 and 1966. Recruits in troop A lived in four apartments (designated by roman numerals I–IV) on the same floor of a building. There were usually 8 men per apartment, distributed in 2 bedrooms (noted A and B, respectively) for 4 men each (Table 1).

In addition, throat and blood samples were taken on 13 February from 28 recruits belonging to another troop (troop B) of the same camp. Troop B was selected as the troop within the camp that had least contact with troop A. For example, recruits in troop A and troop B were never on guard together. Troop B was formed in July 1985 and recruits were born between 1963 and 1966.

Bacterial isolates

Under guidance of vision, the tonsillar region on one side, including the crypts and the posterior pharyngeal wall, was scrubbed vigorously with a charcoal-impregnated swab. Tonsillopharyngeal samples were placed in modified Stuarts transport medium [11] and plated within 4 h on chocolate agar with colistin 7·5 mg/l, lincomycin 0·5 mg/l, amphotericin B 1·0 mg/l, and trimethoprim 5·0 mg/l. Plates were incubated at 33 °C for 2 days and meningococci were identified by standard methods.

Semi-quantitative evaluation of the number of colony-forming units (c.f.u.) per primary plate was performed. Usually, one colony from each throat culture was subcultured twice, each time from a new single colony, and preserved at −70 °C

until further analysis. From five primary cultures, two morphologically different colonies were subcultured twice, stored and further examined.

Electrophoresis of enzymes

Methods of protein-extract preparation, starch-gel electrophoresis and selective enzyme staining were similar to those described by Selander et al [12]. The 14 enzymes assayed were malic enzyme (ME), glucose 6-phosphate dehydrogenase (G6P), peptidase (PEP), isocitrate dehydrogenase (IDH), aconitase (ACO), NADP-linked glutamate dehydrogenase (GD1), NAD-linked glutamate dehydrogenase (GD2), alcohol dehydrogenase (ADH), fumarase (FUM), alkaline phosphatase (ALK), two indophenol oxidases (IP1 and IP2), adenylate kinase (ADK), and an unknown dehydrogenase (UDH).

Electromorphs of each enzyme, equated with alleles at the corresponding structural gene locus, were related with those previously recorded in *N. meningitidis* [13]. Each isolate was characterized by its combination of alleles at the 14 enzyme loci. Distinctive multilocus genotypes were designated as electrophoretic types, ETs, and equated with clones. ETs were numbered sequentially according to their position in the dendrogram (Fig. 1). With the exception of ET-5 [14], ET numbers are not cognate with those used in other studies.

Statistical analyses

Mean genetic diversity over loci among ETs was calculated as previously indicated [13]. Genetic distance between pairs of ETs was expressed as the proportion of enzyme loci at which dissimilar alleles occurred, and clustering was performed from a matrix of genetic distances by the average-linkage method [15].

Serogrouping and serotyping

Serogroups were determined by slide agglutination with antisera specific for the A, B, C, W135, X, Y, and Z polysaccharides. Serotyping was performed with monoclonal antibodies for antigens 1, 2a, 2b, 2c, 4, 8, 14, 15, P1.1, P1.2, P1.3, P1.15, and P1.16, using a dot-blot method as described by Wedege et al [16]. Protein-extracts for electrophoresis of enzymes were used as antigen preparations.

Enzyme-linked immunosorbent assays (ELISA)

Serum IgG antibody levels against outer membrane antigens from a B:15:P1.7,16 strain (44/76) were determined in ELISA as described by Harthug et al [17]. Briefly, outer membrane vesicles (OMV) extracted with 0·2 M lithium acetate, pH 5·8, at a concentration of 4 μg/ml were used to coat polystyrene microtitre plates overnight at 33 °C. Sera in at least a 1:200 dilution were incubated overnight and IgG reacting with the OMV antigens was detected by alkaline phosphatase-conjugated-swine-anti-human IgG (Orion Research, Finland). On all plates, twofold dilution series of a positive reference serum (starting dilution 1:100), in triplicate, and a 1:100 dilution of a negative reference serum, in duplicate, were added. Results were scaled relative to the positive standard (arbitrarily given the value 1000 U/ml) by a logit-log transformation of the OD values [18].

Bactericidal assay

Bactericidal antibodies against the strain isolated from the index case (strain 11/86) were studied essentially as described by Høiby et al [19]. Human serum from an individual without bactericidal antibodies was used as complement source. Sera were tested up to 80% final dilution by using a higher serum volume in the first wells (100 μl undiluted serum with 12·5 μl bacterial inoculum and 12·5 μl complement). Bactericidal titres are given as the lowest percentage of serum giving 50% or more killing of strain 11/86.

RESULTS

Index case

The meningococcal blood isolate (11/86) from the patient was serogroup B, serotype 15:P1.7,16 and ET-5, as the majority of the strains isolated from patients in Norway since the beginning of the present epidemic in the mid-1970s [10] has been.

Carriage rate

The day following the hospitalization of the patient, 23 of the 30 (77%) recruits belonging to the troop of the patient and one of their officers were found to be carrying *N. meningitidis* in their throats (Tables 1, 2). Two weeks later, the carriage rate had decreased to 60%, as five of the recruits (nos. 6, 12, 22, 27, and 29) were no longer carrying their strains. By 10 June, two additional individuals (nos. 8 and 20) had lost their strains while no. 12 had apparently reacquired meningococci. None of the nine non-carriers at the first sampling acquired the organisms during the rest of the sampling period. The carriage rate among the recruits in troop B, where no case of meningococcal disease occurred, was similar to that of troop A (Table 2).

Genotypic diversity

A total of 81 colonies of *N. meningitidis* from 76 positive throat cultures was analysed by multilocus enzyme electrophoresis. All but one (IP1) of the 14 enzyme loci assayed were polymorphic (Table 3). Twenty-seven distinctive multilocus enzyme genotypes were identified (Table 3), among which the mean genetic diversity per locus was 0·46. The pairs of morphologically different colonies isolated from five of the primary cultures proved identical in their multilocus enzyme genotypes.

The genetic relationships of the 27 ETs are shown by the dendrogram (Fig. 1). In Fig. 1 the number of individuals from whom meningococci of each of the 27 ETs were identified is also shown. Seventeen of the 27 ETs (63%) were represented in only one individual each, while the isolates of the remaining 10 ETs were recovered from 2–6 recruits. *N. meningitidis* of 4 of the 10 latter ETs (ETs 1, 15, 16, and 22) were found in recruits belonging to both troops. Strains of ET-7, isolated from six individuals in troop A, were not detected among recruits of troop B.

None of the recruits or officers of troop A harboured bacteria of the clone ET-5

Table 1. *Characteristics of* N. meningitidis *isolated from carriers in a troop of military recruits in Norway where a case occurred*

Individual no.	Sampling date: 31 Jan.	13 Feb.	10 June	Sleeping room
1	0	0	NS	IA
2	0	NS	NS	IA
3	NG:4:– ;ET-16	NG:4:– ;ET-16	NG:4:– ;ET-16	IA
4	0	NS	0	IB
5	W:NT:P1.3;ET-7	NT:NT:P1.3;ET-7	NG:NT:– ;ET-7	IB
6	NG:NT:P1.2:ET-8	0	0	IB
7	NG:2a:P1.2;ET-22	NG:4:– ;ET-16	NG:4:– ;ET-16	IB
8	NG:NT:P1.3;ET-7	NG:NT:P1.3;ET-7	0	IIA
9	0	0	0	IIA
10	NG:15:P1.15;ET-1	B:15:P1.15;ET-1	NG:15:P1.15;ET-1	IIA
11	NG:NT:P1.2;ET-24	NS	NS	IIA
12	B:NT:P1.16;ET-25	0	B:NT:P1.16;ET-25	IIB
13	0	0	0	IIB
14	0	0	0	IIB
15	B:NT:– ;ET-18	NG:NT:– ;ET-18	NS	IIB
16	NG:NT:P1.3;ET-7*	NG:NT:P1.3;ET-7	W:NT:P1.3;ET-7	IIIA
17	NG:NT:P1.3;ET-7*	NG:NT:P1.3;ET-7	B:8:– ;ET-10	IIIA
18	B:8:– ;ET-10†	B:NT:– ;ET-10*	B:8:– ;ET-10	IIIA
19	NG:NT:– ;ET-19	NG:14:– ;ET-19	B:NT:P1.3;ET-12	IIIA
20	NG:4:– ;ET-2	NG:4:– ;ET-2	0	IIIB
21	NG:NT:P1.3;ET-7‡	W:NT:P1.3;ET-7	W:NT:P1.3;ET-7	IIIB
22	W:NT:P1.3;ET-7	0	0	IIIB
23	NG:NT:– ;ET-26	NG:NT:– ;ET-26	NG:NT:– ;ET-26	IIIB
24	Y:NT:P1.3;ET-9	Y:NT:P1.3;ET-9	NG:4:– ;ET-4	IVA
25	B:1:– ;ET-14	B:1:– ;ET-14	B:1:– ;ET-14	IVA
26	W:NT:P1.1;ET-6	W:NT:P1.1;ET-6	W:NT:P1.1;ET-6	IVA
27	NG:4:P1.15;ET-15	0	0	IVB
28	B:15:P1.3;ET-3	B:1:– ;ET-14	B:1:P1.16;ET-24	IVB
29	NG:4:P1.15;ET-17	0	0	IVB
30	0	0	NS	IVB
Officers				
31	0	NS	NS	
32	NG:NT:– ;ET-21	NG:14:– ;ET-21	NS	
33	0	0	NS	

NS, not sampled; NG, non-serogroupable; NT, non-serotypable; –, non-subtypable.
* The two morphologically different colonies examined were identical.
† A morphologically different colony was B:NT:– ;ET-10.
‡ A morphologically different colony was W:NT:– ;ET-7.

responsible for the case of disease, but two recruits in troop B, sampled 2 weeks after the onset of the disease, were carrying ET-5 *N. meningitidis* (Fig. 1).

Temporal changes of clones colonizing healthy individuals

The serogroup, serotype, and ET of the isolates obtained from the three subsequent throat samples of the recruits and officers in troop A are given in Table 1. Of the 24 individuals with a positive throat culture on 31 January, 2 (nos. 7 and 28) were colonized with meningococci of a different clone by the next sample 2 weeks later, and 4 individuals (nos. 17, 19, 24, and 28) had acquired a strain of a

Table 2. *Number of carriers and number of meningococcal clones identified in individuals from two troops of military recruits in a single camp in Norway*

Troop/time no.	No. individuals sampled	No. carriers	No. ETs	Isolates/ ET
Troop 1				
31 January	33	24 (73%)	19	1·26
13 February	29	18 (62%)	12	1·50
10 June	25	15 (60%)	11	1·36
Troop 2				
13 February	29	19 (66%)	10	1·90
Total				
13 February	58	37 (64%)	28	1·32
Total				
All samples	63	44 (70%)	28	1·57

Table 3. *Allele profiles at 14 enzyme loci in ETs of* N. meningitidis *isolated from military recruits in Norway*

	Alleles at indicated enzyme loci*													
ET	ME	G6P	PEP	IDH	ACO	GD1	GD2	ADH	FUM	ALK	IP1	IP2	ADK	UDH
1	1	3	1	1	4	2	3	1	3	5	2	5	2	3
2	3	1	7	7	4	1	3	2	2	2	2	3	2	3
3	3	1	9	7	1	1	1	3	1	2	2	3	2	3
4	2	1	8	7	4	1	3	2	1	8	2	3	2	4
5	1	1	7	8	4	2	3	2	1	1	2	3	2	3
6	3	3	4	7	4	1	1	2	1	3	2	3	2	4
7	3	3	4	5	4	1	1	2	1	3	2	3	2	4
8	3	3	4	9	4	1	2	2	1	3	2	3	2	3
9	3	3	2	7	4	1	2	2	1	3	2	3	2	3
10	3	3	4	14	4	1	3	2	1	3	2	3	2	3
11	3	3	4	12	4	1	3	0	1	3	2	5	2	2
12	3	3	4	12	4	1	3	0	1	3	2	3	2	2
13	3	3	4	12	4	1	3	0	1	3	2	5	2	3
14	1	3	4	7	4	1	3	2	1	3	2	5	2	3
15	1	3	5	2	4	1	3	2	1	8	2	3	2	3
16	1	3	5	1	4	1	3	2	1	8	2	3	2	3
17	1	3	4	9	0	1	0	2	1	8	2	3	2	3
18	1	3	1	7	2	1	3	0	1	8	2	3	2	3
19	2	3	2	9	4	1	3	3	1	8	2	3	2	3
20	1	3	2	9	2	1	5	2	1	3	2	3	2	3
21	2	3	5	1	0	1	2	2	1	3	2	3	2	3
22	4	3	4	5	2	1	4	0	1	8	2	3	2	3
23	4	3	4	5	2	2	4	0	1	8	2	3	2	3
24	3	3	5	5	0	2	3	2	1	2	2	5	2	3
25	3	3	5	7	0	2	3	2	1	2	2	5	2	3
26	1	4	0·5	7	2	1	3	2	1	3	2	3	2	5
27	3	0·5	5	6	4	1	1	3	3	2	2	3	3	3

* ME, malic enzyme; G6P, glucose 6-phosphate dehydrogenase; PEP, peptidase; IDH, isocitrate dehydrogenase; ACO, aconitase; GD1 and GD2, two glutamate dehydrogenases; ADH, alcohol dehydrogenase; FUM, fumarase; ALK, alkaline phosphatase; IP1 and IP2, two indophenol dehydrogenases; ADK, adenylate kinase; UDH, unknown dehydrogenase.

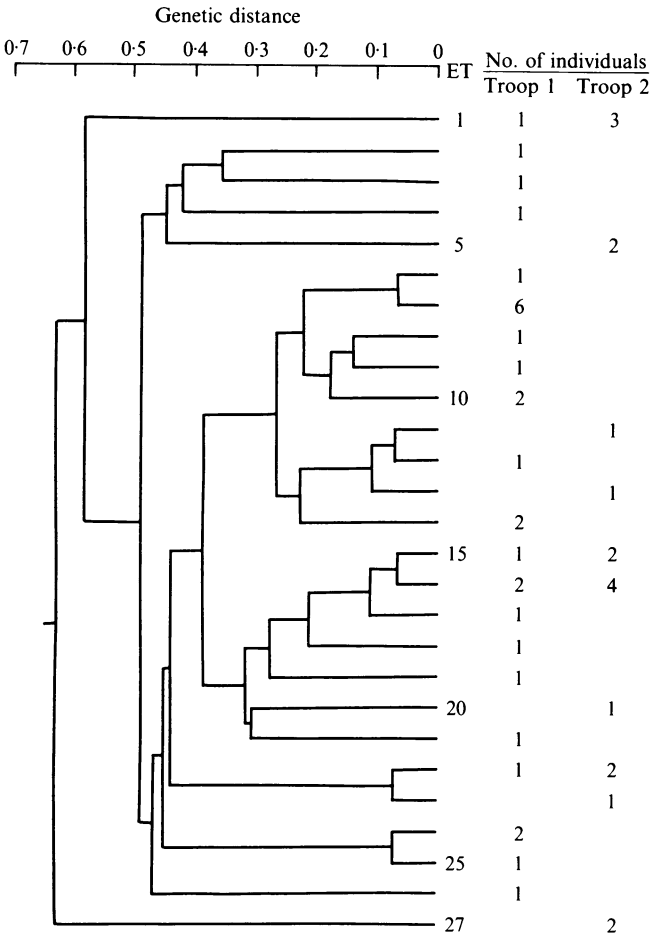

Fig. 1. Genetic relationships among 27 ETs of *N. meningitidis* isolates recovered from healthy carriers in two troops of military recruits in Norway. The dendrogram was generated by the average-linkage method of clustering from a matrix of coefficients of genetic distance based on 14 enzyme loci. ETs are numbered sequentially. The numbers of distinct carriers in each troop from whom individual ETs are identified are noted.

different clone between 13 February and 10 June. Nine of the 21 carriers (43%) at the first sample, who were subsequently examined twice, retained *N. meningitidis* of the same clone for the entire interval of time.

Four of the six changes of clones recorded among the carriers represented acquisition of bacteria of a clone previously identified in another carrier of the same troop: ET-10, which colonized individual no. 17, was previously found in individual no. 18 who shared the same sleeping-room; strains of ET-14 and ET-16, acquired by individuals nos. 28 and 7, respectively, had previously been identified in recruits nos. 25 and 3, living in the same apartment, but having different sleeping-rooms; and ET-24, representing a second strain acquisition by recruit no. 28, had been detected in recruit no. 11. Recruits nos. 11 and 28 did not share apartments. Meningococci of only one of the six acquired clones, ET-16, was also identified among carriers in troop B.

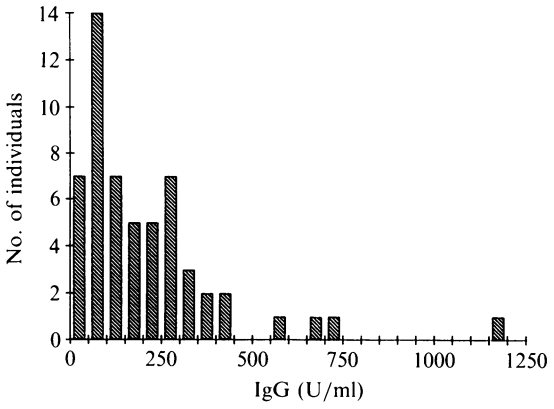

Fig. 2. Distribution of serum IgG levels (in U/ml) to outer membrane vesicle of *N. meningitidis* strain 44/76 measured in ELISA in 56 military recruits in Norway. Each bar represents a window of 50 IgG U/ml, except for the first which represents values less than 25 U/ml.

Phenotypic variation of the isolates of the same clone

Two of the five pairs of morphologically different colonies analysed from the same throat culture presented antigenic differences: one pair differed in both serogroup and subtype, and the other one in serotype (see footnotes, Table 1). Variation was also observed among consecutive isolates of the same ET in an individual, e.g. in recruits nos. 5 and 10. But there was no obvious temporal pattern in the antigenic variation.

Antibody studies

A total of 125 serum samples were tested for IgG antibodies against OMV from 44/76 and bactericidal activity against strain 11/86. The acute serum from the index case had very low IgG levels (4 U/ml) and serum at a concentration of more than 50% was necessary to obtain 50% killing of the infecting strain. With serum collected from the patient 3 months later, a serum concentration of only 0·4% was sufficient to a similar killing of the same strain. The IgG response, however, was moderate (52 U/ml) compared to that observed in other patients with systemic meningococcal disease caused by a B:15:P.16 strain [17].

In all but one recruit (no. 16), the multiple serum samples from individuals in troop A showed little variation over time in IgG and bactericidal antibody titres. The distribution of IgG and bactericidal antibody titres in sera from individuals from both troops (28 individuals from each troop) collected on 13 February are shown in Figs 2 and 3, respectively. No significant differences in mean antibody levels between individuals in troop A and B were observed. Most of the recruits (49 of 56, 87%) had more than 25 U/ml of IgG and at least a 50% killing of strain 11/86 in 50% dilutions of their sera (51 of 56, 91%). Of the 7 recruits with low IgG antibody level (less than 25 U/ml), 4 (nos. 1, 13, 14, and 30) were in troop A and 3 were in troop B. Another recruit in troop A (no. 4) was not sampled on 13 February but had low IgG titres in samples collected on 31 January and 10 June. Four of these eight individuals with low IgG titres needed serum concentration of 50% or higher to attain 50% killing of strain 11/86.

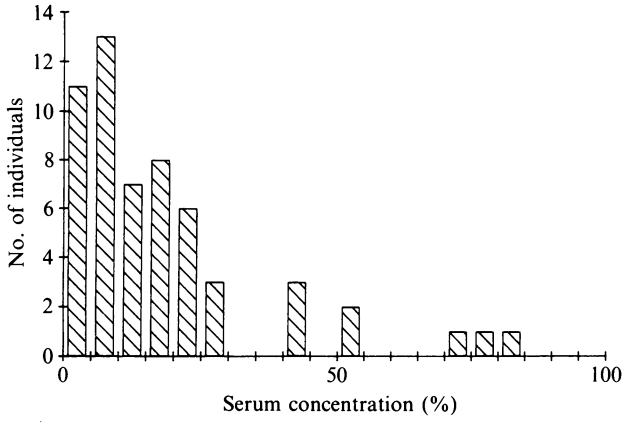

Fig. 3. Distribution of serum concentration (percent) giving 50% killing or more of *N. meningitidis* strain 11/86 in bactericidal assay in 56 military recruits in Norway. Each bar represents a window of 5% of serum dilution, except for the first which represents concentrations less than 2·5% serum. This first bar shows the individuals with sera with the highest bactericidal activity.

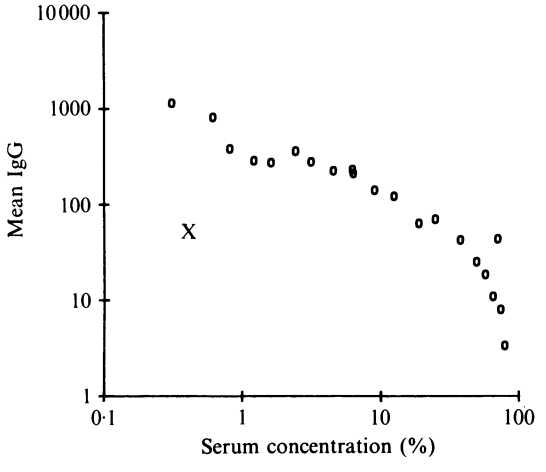

Fig. 4. Relationship between bactericidal titre of serum against *N. meningitidis* strain 11/86 (calculated as the percentage of serum giving 50% killing of the strain) and serum IgG level (in U/ml) against outer membrane vesicle of *N. meningitidis* strain 44/76. The comparison is based on 125 serum samples obtained from 62 individuals in a military camp in Norway. For each bactericidal titre the mean IgG level was calculated and plotted. X represents the values for serum from the index case taken 3 months after the onset of disease.

Generally, sera from the non-carriers had lower antibody levels than those from the carriers. The mean IgG level for the non-carriers in troop A was 87 U/ml compared to 243 U/ml for the carriers. Only 1 of the 8 recruits with IgG titres less than 25 U/ml had *N. meningitidis* in his throat.

No significant changes were observed in antibody levels in 4 of the 5 carriers who acquired a new strain. The remaining one (no. 17) had a fivefold increase in IgG titre and an increase in bactericidal activity from 19 to 6·3% serum to reach 50% killing of strain 11/86. Recruit no. 16 showed a threefold decrease in IgG and a decrease in bactericidal activity from 9 to 58% serum inducing 50% killing of

the patient strain between 31 January and 10 June, although he remained a carrier of the same strain.

The relationship between bactericidal activity against strain 11/86 and the mean IgG level against OMV of strain 44/76 of all tested serum samples is shown in Fig. 4. Sera with high bactericidal titres (low serum concentration needed for assuring 50% killing of the index strain) had high IgG levels measured in ELISA. One exception was the convalescent serum from the index case which was significantly more bactericidal than expected from its IgG level. From the data shown in Fig. 4, it appeared that about 25 U/ml of IgG was the lowest level giving 50% killing of strain 11/86 in 50% serum.

DISCUSSION

Close contacts of meningococcal cases are much more likely than control populations to harbour pathogenic strains in their throat and are at considerably increased risk of developing systemic meningococcal infection [20–22]. The carriage rate of *N. meningitidis* in a troop of military recruits, in which a case due to a B:15:P1.7, 16 strain of ET-5 had occurred, was higher than 70%, but none of the asymptomatic recruits carried *N. meningitidis* of the same clone as the patient. Subsequent throat samples did not reveal meningococci of the infecting strain among the close contacts of the case, showing that the patient had probably been infected from a source outside his troop, and that he had not transmitted the pathogenic strain to other members of his troop before becoming ill. Isolates identical in serogroup, serotype, and multilocus enzyme genotype to the patient strain were identified in two recruits of another troop in the same military camp. It was, however, unlikely that direct cross-infection had happened because little if any contact between these carriers and the patient had taken place. During the month preceding the episode of disease the recruits of troop A had had no leave of absence. In accidental laboratory infections, the onset of disease in a susceptible individual appears to occur only a few days after contact with the infecting strain. It is thus unlikely that the patient had been infected prior to arrival in the camp and the source of the infecting strain must remain unidentified. No further cases developed in the camp over the next 5 years.

Although there was a decrease in the carriage rate in troop A with time, nearly half of the carriers harboured the same strain over the entire 4-months period. The use of multilocus enzyme electrophoresis permitted the identification of the bacterial clones in a situation where the antigenic characteristics of the isolates showed considerable variation. Antibiotics to eradicate meningococcal carriage are only given exceptionally in Norway, and were not used in this situation.

The sensitivity of single swabbing in identifying meningococcal carriers has been questioned [21]. Together with a carriage rate of about 70%, the fact that no new carriers were detected by additional samples indicated that the tonsillopharyngeal swab procedure employed was adequate. Intermittent carriage of ET-25 by recruit no. 12 suggested, however, that the sample of 13 February may have been falsely negative. With the exception of the carriers who changed strains, the number of c.f.u. on primary plates was usually lower in the sample of 10 June than in the two first ones. Individuals who lost their strain had often a

relatively low number of c.f.u. in their last positive sample, suggesting a gradual elimination of the strain. We have observed among our laboratory workers one long-term carrier who usually grew 1–20 colonies, but sometimes was negative in between positive swabs.

While the ratio of isolates per ET was only slightly higher within each troop than that found earlier among non-associated carriers [23], several features indicated that transmission of meningococcal strains among the recruits of the same troop had taken place. While 18 of the 24 carries in troop A had unique ETs at the first sample, the remaining 6 carriers had strains of ET-7, which were not identified in troop B. The four ETs represented in both troops were clones relatively frequently met with in the *N. meningitidis* population in Norway, either from patients or carriers: ET-1 strains were isolated from several military recruits in another camp in 1987–8 (D. A. Caugant, unpublished data); ET-15 and ET-16 were closely related to strains assigned to a common clone-complex among Norwegian carriers, cluster D [23]; and, ET-22 was the second most frequent clone isolated from patients in Norway in 1986.

In a much larger survey of carriage among military personnel, Pether and colleagues concluded that acquisition of a new strain was a rare event [24]. In our study, we detected 6 acquisitions of which 4 involved a strain earlier detected in another recruit of the same troop. Only 1 of these 4 transmissions occurred between individuals sharing the same sleeping-room, suggesting that the sleeping conditions in the camp did not represent a dominating risk-factor for transmission of the meningococcal strains. The strains of ET-7 were recovered from individuals in 4 of the 8 recruits living in apartment III and in one recruit in each of apartments I and II. The recruits had been accommodated in those apartments for only a month before the samples were taken. However, the troop was constituted 3 months before, and the carriage pattern might still have reflected the living conditions from their previous training period, details of which we were unfortunately unable to reconstruct.

The multiple recovery of strains of ET-7 and the fact that 4 of the 6 acquisitions may have originated from individuals in the same troop suggest that transmission of somewhat low-virulent strains among military recruits is a frequent phenomenon. This study also illustrates the importance of carriage of low-virulent strains in the development of natural cross-reacting immunity in such populations [25]. Most recruits had bactericidal activity against the patient strain in 50% dilution of their sera, although only two carriers harboured a strain antigenically similar to that from the index case.

The levels of serum IgG measured by ELISA with OMV antigens generally corresponded well with the bactericidal titres obtained with human complement. Correlation between the two assays has also been observed in recent studies of sera from vaccinees in Norway [26, 27], and the correlation was significantly higher than that previously observed using baby rabbit complement [17]. Although it is now known how much functional antibody is detected in the ELISA test, our results indicate that IgG ELISA with the proper antigen may provide an indication about the bactericidal activity of the sera. However, exceptions are likely to be found, as illustrated by the convalescent serum specimens from the patient.

In the reference serum used in ELISA (1000 U/ml), the total amount of specific IgG reacting with OMV from strain 44/76 was 35 μg/ml, as detected with solid phase radioimmunoassay [17]. The index case had only 4 U/ml of IgG, corresponding to about 0·15 μg IgG/ml, at the onset of the disease, suggesting that such an IgG level is insufficient to assure protection. The small amount of antibody in the acute phase serum, however, may result from antigen excess and absorption of antibodies in the patient serum. A small number of other recruits also had very low antibody levels. One, a room-mate of the index case, also had only 4 U/ml of IgG and needed 80% serum concentration to reach 50% killing of the index strain. In spite of a low antibody level, this individual did not get meningococcal disease, providing additional evidence that the index case must have become ill rapidly after acquiring the infecting strain. More studies of pre-disease sera are needed to provide estimates of the protective levels of antibodies.

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