Geographical distribution of HIV-1 group O viruses in Africa

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Objective: To determine to what extent HIV-1 group O strains are present in different African countries.

Materials and methods: A total of 14 682 samples of sera from a range of patients from 12 different African countries were tested. All the sera were tested with an enzyme-linked immunosorbent assay (ELISA) using a combination of V3 peptides from ANT-70 and MVP-5180. Samples reactive in ELISA were retested in a line immunoassay (LIA-O). Samples reactive in ELISA were also retested with an in-house Western blot to determine the presence of antibodies to gp120 of HIV-1 ANT-70. Polymerase chain reaction was performed on HIV-1 group O and group O indeterminate sera.

Results: Of all the sera samples tested, only 19 sera had antibodies to group O V3 peptides exclusively and 46 were indeterminate for group O infection in LIA-O. The highest prevalence of HIV-1 group O infection among HIV-positive sera was observed in Cameroon (2.1%) and neighbouring countries, 1.1% in Nigeria and 0.9% in Gabon. The lowest rates were seen in west Africa: 0.07% in Senegal, 0.14% in Togo, 0.16% in Chad and 0.3% in Niger. Group O sera were observed in almost all the population categories tested. The ANT-70 V3 peptide in LIA-O was reactive with all of the sera considered to be HIV-1 group O antibody positive by LIA, versus 78.9% for the MVP-5180 peptide. Thirteen out of 19 group O samples of sera were tested in PCR. Eight samples were identified as group O by specific group O *pol* and/or V3 primers; in the remaining five samples no HIV RNA could be detected. Of the indeterminate sera samples, two were identified as group O.

Conclusion: In eight of the 12 countries tested, antibodies to group O viruses were identified. Numbers of HIV-1 group O viruses are low. Their presence is not restricted to Cameroon and neighbouring countries but can also be found in west and south-east Africa.

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Introduction

HIV-1 displays an unusually high degree of genetic variability *in vivo*. Analysis of HIV-1 genes of virus strains from different geographical locales has revealed that HIV-1 can be divided into two distinctive groups, M (major) and O (outlier) [1]. HIV-1 group M isolates can be further subdivided into at least 10 distinct genetic subtypes (A–J) [2–5].

The first HIV-1 group O viruses to be described were the ANT-70 and MVP-5180 strains, both isolated from Cameroonians. These isolates had only 50% homology with the other HIV-1 isolates in the *env* gene [6,7]. Additional HIV-1 group O variants have been described and the majority of the strains have been obtained from Cameroon or from Cameroonians living in Europe [8,9]. Group O virus was identified in Spain in an individual who often travelled to Equatorial Guinea and Cameroon [10]. Recently, group O infection was diagnosed in the US [11]. Sporadic cases of group O infection have been documented in other African countries like Gabon, Nigeria, Equatorial Guinea and Kenya, and in Benin a dual HIV-1 group M and O infection has been described. [12–16].

Analysis of group O viruses shows that these viruses are genetically very diverse [8]. These HIV viruses present a public health challenge because several of them escape detection by certain conventional screening tests and can give indeterminate Western blot patterns [17–19]. To diagnose HIV-1 group O infection different serological approaches have been used. Some researchers use a competitive immunoblot or a negative result in HIV-1 competitive assays [20–21]. Studies among HIV-infected individuals from different African countries, using an enzyme-linked immunosorbent assay (ELISA) based on the V3 peptide from ANT-70 with confirmation by a specific ANT-70 Western blot, indicated that HIV-1 group O infection is present in Cameroon and Gabon [12]. Sequence data on a limited number of samples confirmed that this strategy can lead to the identification of HIV-1 viruses from group O [22].

Up until now little has been known about the spread of HIV-1 group O viruses in Africa. It is important to monitor these viruses in order to adapt HIV-testing strategies for blood screening and serodiagnosis if required.

Materials and methods

Populations studied

Sera from different population categories of 12 African countries were studied: Burkina Faso, Burundi, Cameroon, Congo, Gabon, Mali, Niger, Nigeria, Senegal, Chad, Togo and Zambia.

Samples were collected from AIDS patients, tuberculosis patients, sexually transmitted disease (STD) patients, prostitutes, blood donors and pregnant women. Table 1 summarizes the population categories tested and the HIV status of the samples in the different countries. Data for Nigeria, Senegal and Togo have been partially published earlier [23]. In total we tested 14 682 samples of sera: 1427 from blood donors (260 HIV-positive, 150 HIV-indeterminate, 1017 negatives); 2489 from pregnant women (522 HIV-positive, 87 HIV-indeterminate, 1880 negatives); 7406 from infectious diseases and tuberculosis patients (5007 HIV-positive, 272 HIV-indeterminate, 2127 negatives); 961 from STD patients (187 HIV-positive, 30 HIV-indeterminate, 744 negatives); and 2399 from prostitutes (1102 HIV-positive, 49 HIV-indeterminate, 1248 negatives).

Among the 14 682 sera samples, 7016 were HIV-negative and 550 were HIV-indeterminate, i.e. those samples in which there was the presence of antibodies to Gag and/or Pol proteins only, without reaction to Env proteins on HIV-1 Western blot or line immunoassay (LIA) HIV-1/2. A total of 7078 were HIV-positive: 2781 HIV-1, 380 HIV-2, 376 HIV-1 and -2 and 3541 were defined as HIV-positive by a positive reaction in two different HIV-1 and -2 screening assays.

Serology

All the sera were tested for group O antibodies by an ELISA using a combination of V3 peptides from ANT-70 and MVP-5180 (Innogenetics, Antwerp, Belgium). Sera reactive by ELISA were retested in an LIA, in which biotinylated V3 peptides from different group O and M HIV-1 viruses (consensus HIV-1 group M; M-Mal; O-ANT-70; O-VI686, which is a Gabonese HIV-1 group O isolate [22]; and O-MVP-5180) were applied as a streptavidin complex in parallel lines on nylon strips (Innogenetics). Samples reactive in ELISA were also retested on an in-house Western blot for the presence of antibodies to gp120 of HIV-1 ANT-70 as previously described [24]. Sera were considered positive for HIV-1 group O antibodies if they reacted on LIA exclusively with group O peptides whether or not there was reaction on the ANT-70 Western blot. Samples reactive with group O and M peptides with or without reaction to gp120 on ANT-70 Western blot were considered as indeterminate for HIV group O antibodies and were further analysed by polymerase chain reaction (PCR). The testing algorithm is summarized in Fig. 1.

PCR

Viral RNA was extracted from 50 µl of plasma by the extraction method of Chomczynski and Sacchi [25].

Table 1. Different population groups and their HIV status in the 12 African countries studied.

	HIV-1	HIV-2	HIV-1 and -2	HIV pos	HIV ind	HIV neg	Total
Burkina Faso (n=568)							
AIDS patients, TB pregnant women	206		49	143	36	134	568
Burundi (n=686)							
Blood donors				13	1	329	343
TB patients				84	10	249	343
Cameroon (n=1143)							
Blood donors				118	18	308	444
Pregnant women	21	1		18	23	134	197
Infectious diseases				174	3	325	502
Chad (n=619)							
Infectious diseases,TB				619			619
Congo (n=702)							
Blood donors	4				3	164	171
Pregnant women	9				4	106	119
Infectious diseases	275				26	111	412
Gabon (n=701)							
Pregnant women	90	5	1		40	375	511
Infectious diseases	36	2	1		2	26	67
TB patients	76	1	1		21	24	123
Mali (n=1016)							
Infectious disease, TB	244	2	44	445		106	841
Prostitutes	62	7	12			94	175
Niger (n=2619)							
Blood donors	48		15	62	128	216	469
Pregnant women	4			36		36	76
Infectious diseases	598		165	233	111	27	1134
STD patients	29		11	22	12	402	476
Prostitutes	187	2	46	1	22	206	464
Nigeria (n=2083)							
Pregnant women				11	20	1011	1042
Infectious diseases				14	11	120	145
TB patients				8	12	220	240
STD patients				30	18	222	270
Prostitutes				120	26	240	386
Senegal (n=2432)							
Pregnant women	9	15	2			218	244
Infectious diseases	531		22		40	182	775
TB patients	22						22
STD patients	16	1					17
Prostitutes	314	344	7		1	708	1374
Togo (n=1113)							
Infectious diseases,TB				670		443	1113
Zambia (n=1000)							
Pregnant women				300			300
Infectious diseases				242		160	402
TB patients				100			100
STD patients				78		120	198

pos, Positive; ind, indeterminate; neg, negative; TB, tuberculosis; STD, sexually transmitted disease.

Briefly, virus is lysed by guanidium thiocyanate, followed by a phenol/chloroform-isoamyl alcohol extraction and RNA is precipitated by isopropanol. Extracted RNA is resuspended in 5 µl sterile water.

Reverse transcription was performed on 5 µl of extracted RNA in a final volume of 20 µl containing 10 pmol of each reverse primer, 0.4 mmol of each dNTP and 5 units of avian myeloblastosis virus reverse transcriptase (RT) (Promega, France). The cDNA product was amplified by nested PCR using primers in the *pol*

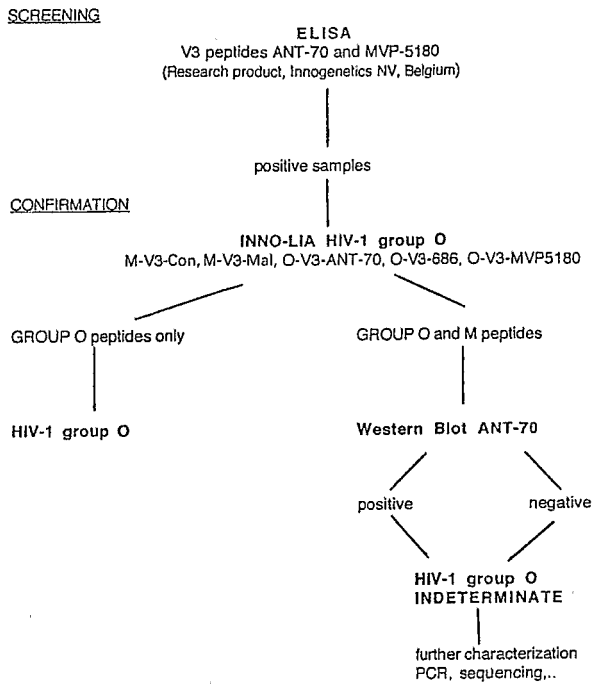

Fig.1. Serological testing algorithm for the detection of HIV-1 group O infection. ELISA, enzyme-linked immunosorbent assay; LIA, line immunoassay.

region as previously described. The outer primers were common for group O and M viruses and one of the inner primers was replaced by a specific group O primer to identify an HIV-1 group O infection with a 100% sensitivity and specificity [26,27]. For some serum samples PCR was carried out with primers specific for the V3 region of group O viruses. We used V70-1 (5′ TTGTACACATGGCATTAGGCCAACA GTAAGT 3′) and V70-4 (5′ TGGATCCTACAAT AAAAGAATTCTCCAGACA 3′) as outer primers and V70-2 (5′ TGAATTCTAATATTGAAT GGGA CACTCTCT 3′) and V70-5 (5′ GTTCTCCATAT ATCTTTCATATCTCCCTA 3′) as inner primers. PCR was performed in a final volume of 50 µl containing 0.2 mmol of each dNTP, 20 pmol primer, 2.5 U Taq DNA polymerase (Promega) and 5 µl of the cDNA.

PCR conditions were as follows: 35 cycles were performed, 20 sec at 94°C, 30 sec at 50 °C and 1 min at 72°C with an initial denaturation of 2 min at 94°C and a final extension of 5 min at 72°C. The second PCR round was performed on 2 µl of the amplified product from the first round and the cycling conditions were identical, with the exception of the group O *pol* primers where the optimal annealing temperature was 56°C.

Samples were considered as positive for HIV-1 group O if they reacted with the specific group O *pol* and/or *env* primers.

Results

Prevalence of HIV-1 group O antibodies

Table 2 shows for the different countries the number and percentage of sera positive for HIV-1 group O antibodies. All these sera were positive in the ANT-70/MVP-5180 mixed ELISA and reacted specifically with V3 peptides from group O on the LIA. Fifteen of these 19 group O sera also reacted with the gp120 of ANT-70 on Western blot. Based on these strict serological criteria, group O sera were present in seven of the 12 countries tested: Cameroon, Gabon, Niger, Nigeria, Senegal, Chad and Togo. Of the 19 HIV group O sera, all were initially characterized as HIV-positive, 18 were HIV-1 and one was HIV-1 and -2-positive. None of the HIV-2-positive sera were reactive with group O peptides. The highest numbers of HIV-1 group O sera were observed in Cameroon (2.1%) and the neighbouring countries Nigeria (1.1%) and Gabon (0.9%) whereas the lowest values were observed in the West African countries Senegal (0.07%), Togo (0.14%), Chad (0.16%) and Niger (0.3%) . Group O sera were identified in pregnant women, AIDS and tuberculosis patients, blood donors and prostitutes.

Table 2. Number of HIV-1 group O and group O indeterminate sera among HIV-positive sera in the different countries.

	HIV group O		HIV group O indeterminate	
Country	n*/N‡	%	n†/N‡	%
Burkina Faso	0/398	0.0	6/398	1.5
Burundi	0/97	0.0	0/97	0.0§
Cameroon	7/332	2.1	2/332	0.6
Chad	1/619	0.16	17/619	2.7
Congo	0/288	0.0	2/288	0.7¶
Gabon	2/213	0.9	0/213	0.0
Mali	0/816	0.0	4/816	0.5
Niger	5/1459	0.3	1/1459	0.07
Nigeria	2/183	1.1	0/183	0.0
Senegal	1/1283	0.07	1/1283	0.07
Togo	1/670	0.14	3/670	0.44
Zambia	0/720	0.0	7/720	0.97

*No. of sera reactive with group O only or †group O- and M-V3 peptides in LIA-O. ‡Total number of HIV antibody-positive sera tested. §Two HIV-O indeterminate sera were observed among HIV-negative sera in Burundi. ¶One HIV-O indeterminate serum sample was observed in HIV-indeterminate serum in Congo.

In total, 46 samples of sera were indeterminate for the presence of group O antibodies, i.e. the sera reacted simultaneously with group O V3 peptides and the V3 consensus of group M- or V3-Mal. The proportion of group O-indeterminate sera was relatively high compared with the number of sera that were confirmed group O. The majority of these 46 sera were HIV-positive, but one was indeterminate for HIV antibodies and two were negative. Group O-indeterminate sera were observed in nine of the 12 countries tested. The overall prevalence of HIV-O-indeterminate samples was 0.69% of the HIV-positive sera and ranged from 0% to 2.6% in the different countries.

Antibody reactions to the different group O and M peptides and proteins

Table 3 summarizes the antibody reactions to the different group O peptides. On LIA-O, all of the 19 (100%) group O sera reacted with the ANT-70 peptide, 18 out of 19 (94.7%) with the VI686 peptide and 15 out of 19 (78.9%) with the MVP-5180 peptide. For the HIV-O indeterminate sera, the majority (82.9%) reacted with the ANT-70 peptide but only 36.2 % and 48.9% reacted with the VI686 and MVP-5180 peptide respectively. Some of the indeterminate sera reacted only with the MVP-5180 peptide in contrast to the sera that were confirmed group O. In Mali and Congo, we observed samples of sera reacting strongly with the MVP-5180 peptide and the M-V3 consensus without antibodies to gp120 on ANT-70 Western blot.

Table 3. Reactivity of HIV-1 group O and group O-indeterminate sera with the different group M and O peptides on LIA and with ANT-70 gp120 on Western blot.

	HIV-1 group O		HIV-1 group O indeterminate	
Peptides	n*/N†	%	n*/N†	%
M-V3-Con	0/19	0	40/46	86.9
M-V3-Mal	0/19	0	37/46	80.4
O-V3-ANT-70	19/19	100	38/46	82.6
O-V3-686	18/19	94.7	16/46	34.7
O-V3-MVP-5180	15/19	78.9	22/46	47.8
O-Western blot ANT-70	15/19	78.9	14/46	30.4

*No. of sera reactive. †No. of sera tested.

PCR

Only minute volumes of serum were available for the majority of samples serologically considered to be HIV-1 group O or group O-indeterminate, and therefore not all of them were tested by PCR. In addition, storage conditions (several freeze-thaw cycles and storage at -20°C, which causes deterioration of RNA) were not optimal to perform RT-PCR. Among the 19 group O sera, 13 were tested by PCR with the universal group O/M primers to confirm the presence of RNA in the sample and with the specific group O *pol* primers for confirmation of group O. Five were reactive with the universal *pol* primers and five were identified as group O with the specific group O *pol* primers, whereas in eight no viral RNA could be detected by PCR with the universal *pol* primers. In addition, PCR was carried out with the specific group O V3 primers on 10 samples from which nine had been tested also with the *pol* primers. Only three samples were reactive with the V3 group O primers, two of these three samples were initially negative for the universal O/M *pol* primers and one was not tested with the *pol* primers.

Of 38 of the 46 sera identified as group O-indeterminate by serology, RT-PCR was performed with the

universal *pol* primers to confirm the presence of RNA and with specific group O *pol* primers to confirm group O infection. Twenty of the 38 samples were reactive with the universal *pol* primers and only two of them were identified as group O with the specific group O *pol* primers. The 18 remaining samples were negative by *pol* PCR. Of the two samples reactive with group O *pol* primers one was from Zambia and one was from Cameroon. All of the nine group O-indeterminate samples (five were positive and four were negative with universal *pol* primers) tested with the specific V3 primers were negative with this primers (Table 4). Overall, in the group O and group O-indeterminate sera together, RNA could be detected in 28 (54.9%) out of 51 samples tested.

Table 4. Reactivity of group O and group O indeterminate sera by PCR with different primers sets.

	Group O No. reactive / no. tested	Group O indeterminate No. reactive / no. tested
Universal *pol* primers	5/13*	20/38†
Group O *pol* primers	5/5	2/20
Group O V3 primers	3‡/10	0/9

*† Sera were first tested with the universal *pol* primers and those reactive were retested with the specific group O *pol* primer. ‡One out of three sera was negative with the universal and specific *pol* primers; one out of three was reactive with the universal *pol* but not with group O *pol* primer; one out of three was not tested with *pol* primers.

Antibody reaction to gp120 of ANT-70 on Western blot

Ten samples were identified as group O by PCR, of which two were indeterminate by group O serology because they reacted simultaneously with group O and group M V3 peptides. Nine of these 10 samples reacted with the gp120 of ANT-70 on Western blot and one was negative. Eighteen samples were identified as group M by PCR and among them five were clearly reactive on the ANT-70 gp120, three showed a weak reaction and 10 were negative. These data indicate that the majority of HIV-1 group O sera are reactive with gp120 of ANT-70 although some sera remain negative. About 50% (eight out of 18) of the group M sera that cross-reacted with group O V3 peptides on LIA were also reactive with gp120 of ANT-70 on Western blot. These data show that HIV-1 group O Western blot is not sensitive enough and is also not specific enough.

Discussion

This study on more than 14 600 sera from different population groups and in 12 African countries showed that the seroprevalence of HIV-1 group O viruses is rather low, ranging from absent to 2.1% of all the HIV-positive sera tested. HIV-1 group O infection was observed in eight of the 12 countries included in the study, however. The highest percentage of group O sera among HIV-positive samples was observed in Cameroon (2.1%), followed by Nigeria (1.1%) and Gabon (0.9%), whilst the lowest percentages were seen in west Africa. Our data show that group O infection is not limited to Cameroon and its neighbouring countries but is also present in west African countries such as Senegal, Togo and Niger and even in the south-east African country Zambia. All HIV-1 group O sera were detected among samples which were initially identified as being HIV-positive. The ANT-70/MVP-5180 V3 ELISA had good specificity because only three out of 7016 HIV antibody-negative sera were reactive with group O peptides on LIA-O and all remained group O indeterminate in group O LIA.

All group O sera reacted with the ANT-70 peptide with or without simultaneous reaction to the other group O peptides VI686 or MVP-5180, none reacted only to the MVP-5180 or VI686 peptide. Compared with HIV-1 group O sera, a relatively high number of group O-indeterminate sera was observed and this was the case in almost all the countries studied. The majority of the group O-indeterminate sera were HIV-positive with the exception of two sera which were HIV-negative and one which was HIV-indeterminate.

Our data indicate that the ANT-70 V3 peptide detects more group O sera than the MVP-5180 and the VI686 peptides. The V3 sequence of the ANT-70 virus strain is close to the consensus sequence of HIV-1 group O viruses described [6–8] and sequenced in our laboratory (unpublished results). Further examination is needed, however, to discover whether the peptides that we used for screening and confirmation of HIV-1 group O infection are the most sensitive for detecting and diagnosing the diverse range of group O viruses. Preliminary results on the performance of the ANT-70 V3 peptide on group O sera confirmed by sequence data in our laboratory indicate that this peptide detects all group O infections. The significance of a strong serological reaction simultaneously with the MVP-5180 peptide and the M-V3 consensus as observed in one serum from Congo and one from Mali remains to be elucidated.

Eight out of 19 sera, reacting exclusively with group O V3 peptides and in which RNA could be detected by RT-PCR, were all confirmed by PCR as group O by either specific group O pol or V3 primers. Two out of 20 (10%) of the serologically group O-indeterminate sera were identified as group O by PCR. These data indicate that the majority of the individuals whose sera react simultaneously with group O- and M-V3 peptides are infected with an HIV-1 group M virus. Another possibility is the development of dual infection with an HIV-1 group M and group O virus whereby HIV group O expression may be suppressed at certain

stages of the infection, although this can only explain a minority of the group O indeterminate sera on LIA.

The value of a reactivity with the gp120 of ANT-70 on Western blot seems very limited and remains to be determined. The Western blot was not sufficiently sensitive as one out of the 10 PCR-confirmed group O sera remained negative on gp120. Almost half (eight out of 18) of group O indeterminate sera, identified as group M by PCR, were also reactive with gp120 indicating that ANT-70 Western blot was also not specific enough. Our strategy, based on the use of V3 peptides from ANT-70 and MVP-5180, detects the majority of the group O sera. It could be that the criteria that we used were too strict since two sera were classified as group O-indeterminate. If less strict criteria are used, however, a large number of sera would be classified as group O and would overestimate the prevalence of group O infection. Further studies on 'gold standard' sera, i.e. sera from individuals proven to be confirmed with group O viruses based on sequence and phylogenetic analysis, are necessary to evaluate the intrinsic sensitivity of this and other strategies to diagnose group O infection.

Unfortunately, it was not possible to obtain demographic data for the majority of group O sera from West Africa, such as a history of travelling to Cameroon or neighbouring countries, or sexual contacts with people from these regions. Only from the group O sample from Senegal was it possible to obtain more epidemiological information. The serum was from a Senegalese woman who never left the country, but her husband had worked for several years in Cameroon and died from AIDS a few years ago.

In conclusion, group O viruses are actually present in very low numbers in Africa but further prospective studies are necessary to monitor the true prevalence of these viruses in Cameroon, its neighbouring countries and West Africa, especially to determine if the prevalence of these viruses increases. Future studies should also analyse the risk factors associated with group O infection.

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