Yellow Fever Vaccine. IV. Reactogenicity and Antibody Response in Volunteers Inoculated With a Vaccine Free From Contaminating Avian Leukosis Viruses (36161)

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The development of avian leukosis virus (ALV)-free primary and secondary yellow fever (YF) vaccine seed lots has been described (8). Subsequent publications dealt with (i) an evaluation of ALV-free seed lots with respect to neurovirulence characteristics, development of viremia and antibody production in monkeys inoculated by the intracerebral route (9); and (ii) a study of the antibody response in rhesus monkeys inoculated with ALV-contaminated and -free YF vaccine by the conventional subcutaneous route (10). This report deals with our evaluation of the reactogenicity and antibody response in volunteers inoculated with these vaccines.

Materials and Methods. Volunteers. Two separate volunteer studies were performed. The first study employed 180 inmates of the U.S. Federal Penitentiary at Lewisburg, PA. The second study was conducted with 1502 U.S. Army recruits at Fort Ord, CA. All volunteers were inoculated subcutaneously with 0.5 ml of either conventional ALV-contaminated or -free YF vaccine by needle and syringe (Lewisburg) or jetgun (Ft. Ord). Subsequently, the volunteers were monitored for subjectives and objective reactions to vaccination.

Vaccines. All YF vaccine lots were manufactured by the National Drug Company to meet all prescribed standards of a licensed product. Five different lots of ALV-contaminated and 4 lots of ALV-free vaccine were used. Table I shows the vaccine lots employed, total number of volunteers inoculated, and the potency of the vaccines. Freeze-dried vaccine was reconstituted with sterile 0.85% saline and used within a 2 hr period.

Viruses. The method of preparation of the infectious stock YF 17D strain virus employed in the plaque neutralization (PN) tests has been described (5).

Antigens. The hemagglutination (HA) antigens were prepared by sucrose–acetone extraction (1) of suckling mouse brains harvested when animals sickened.

Animals. Newborn mice (NBM; 1–3 days old) and weanling mice (WM; 16–20 g, 24–28 days old; Swiss albino, NIH General Purpose strain) used for titrating vaccine were obtained from the Rodent and Rabbit Production Section, Laboratory Aids Branch, NIH. In our studies complete litters (containing 6–16 NBM) and 10 WM were used at each dilution point, 0.03 ml was inoculated by the intracerebral route, and LD_{50} end points were determined at 21 days by the Kärber method [cited in Ref. (3)] and expressed as $\log_{10}$ values.

Cell culture. The MA-104 embryonic rhesus monkey kidney cell cultures (originally obtained from Microbiological Associates, Inc., Bethesda, MD) were prepared in a routine manner established by the Cell Biolo-

TABLE I. Numbers of Volunteers and Potency of Yellow Fever Vaccines Employed.[a]

Study (group no.)	ALV status	Vaccine lot no.	No. of volunteers inoculated	Vaccine potency[b]	
				Mfr	DBS
Lewisburg	Contaminated	6838	89	6.5[c]	6.7
	Free	6677	91	5.9	ND
Ft. Ord					
1	Contaminated	6883M9	166	6.7	6.0
2		7069	246	6.2	6.4
3		6547D9	249	6.3	6.5
4		7068	166	6.4	6.3
5	Free	6677	219	6.0	ND
6		6553D9	151	5.9	6.4
7		6554D9	146	5.6	6.1
8		6555D9	153	6.0	6.1

[a] Abbreviations: ALV = avian leukosis virus; Mfr = manufacturer; DBS = Division of Biologics Standards; ND = not done.

[b] Vaccine potency was determined by both the manufacturer and the DBS employing the standard weanling mouse intracerebral test.

[c] Mouse intracerebral LD_{50} (in $\log_{10}$ values) per 0.5 ml of inoculum used to immunize volunteers.

gy Section of our Laboratory. The method of preparation has been described (5).

Serological studies. Sera were obtained from all volunteers prior to (pre-) and 28 days after (post-) inoculation with YF vaccine. In the Lewisburg study, sera were also obtained 1 year after vaccination; when the serological tests were performed with these sera obtained 1 year after vaccination, aliquots of the serum samples obtained from the same volunteers at 28 days were included in the same tests. Sera were kept at 4° from the time of blood collection and during processing until time of final storage at —20°.

Plaque neutralization (PN) test utilizing the constant serum-varying virus dilutions technique has previously been described (6). The difference in titer between pre- and post-immunization serum samples represents the neutralizing capacity of the serum and is expressed as neutralization index (NI) below.

Hemagglutination-inhibition (HI) test employing the basic HI procedure of Clarke and Casals (1), modified for use in microtiter equipment (4), has been described (7). The highest dilution of serum which completely inhibited HA was recorded as the serum antibody titer.

Complement-fixation (CF) test. The CF procedure used in our laboratory has been described (7). Five 50% hemolytic units of complement and 4 to 8 units of antigen were added to serial 2-fold dilutions of heat-inactivated (56° for 30 min) sera; the hemolytic system was added after incubation at 4° for 18 hr and the mixture was incubated at 37° for 30 min with shaking of the plates at 10 and 20 min.

Results. Immunization of volunteers. Table I shows the numbers of volunteers and the potency of the yellow fever vaccine lots employed. In the first study (Lewisburg), conventional ALV-contaminated YF vaccine was given to 89 and ALV-free vaccine administered to 91 inmates. In the second study (Ft. Ord), four different lots of conventional vaccine were given to 827 and four lots of ALV-free vaccine to 675 army recruits. The potency of all lots employed was satisfactory.

Vaccine reactogenicity. The total number of subjective and objective reactions was minimal, and similar in all vaccine groups, indicating a comparably low degree of reactogenicity of the 9 vaccine lots employed.

Antigenicity of YF vaccines. The results of the serological tests revealed that the ALV-free and -contaminated vaccine lots were indistinguishable (Table II). When the most sensitive PN test was employed, the antibody

TABLE II. Results of Serological Tests Performed on Volunteers Inoculated with ALV-Contaminated and -Free Yellow Fever (YF) Vaccine.[a]

Group	ALV-status of vaccines: Contaminated — No. in group	HI (%)[b]	PNT LNI	PNT %[c]	CF No. tested	CF %[d]	Free — No. in group	HI %[b]	PNT LNI	PNT %[c]	CF No. tested	CF %[d]
Lewisburg study												
initial survey												
(28 days)	89(65)[e]	62(71)[f]	2.2[g]	99	ND		91(68)[e]	69(78)[f]	2.2[g]	98	ND	
1 year survey[h]												
(28 days)	22(18)	86(77)	2.9	100	ND		17(10)	77(100)	2.7	100	ND	
(1 yr)		45(45)	3.5	100	ND			47(70)	2.9	100	ND	
Ft. Ord study												
Group 1[i] 5[j]	25(22)	92(91)	3.1	100	24	12(3)[k]	23(17)	96(100)	3.1	100	25	12(3)[k]
2 6	23(19)	91(95)	2.7	100	24	0	23(22)	100(100)	2.8	100	25	12(3)
3 7	25(23)	100(100)	2.8	100	25	4(1)	25(21)	92(91)	2.4	100	24	4(1)
4 8	25(14)	92(100)	3.0	100	25	16(4)	25(16)	96(100)	2.9	100	25	12(3)
Ft. Ord total	98(78)	94(96)	2.9	100	98	8(8)	96(76)	96(97)	2.8	100	99	10(10)

[a] Abbreviations: ALV = avian leukosis virus; HI = hemagglutination-inhibition; PNT = plaque neutralization test; CF = complement fixation; LNI = $\log_{10}$ neutralization index; ND = not done.

[b] Percentage of individuals developing a 4-fold or greater rise in HI antibody titer.

[c] Percentage of individuals having an LNI of ≤ 0.7.

[d] Percentage of individuals developing a 4-fold or greater rise in CF antibody titer.

[e] Number with no YF HI antibody in preinoculation serum.

[f] Percent of individuals not having YF HI antibody in preinoculation serum who developed 4-fold or greater rise in HI antibody.

[g] Average value

[h] At the 1 year survey, serological tests were performed with the serum samples obtained at the time of the 28 day and 1 year postinoculation surveys (see Materials and Methods).

[i] First column contains the group numbers of those receiving the conventional ALV-contaminated vaccines.

[j] Second column contains the group numbers of those receiving ALV-free vaccines.

[k] Numbers (in parentheses) actually developing 4-fold or greater rise.

TABLE III. Number and Percentage of Volunteers with Hemagglutination-Inhibition Antibodies to Several Arboviruses.[a]

Group	ALV status[b]:	No. inoculated +	No. inoculated −		Arbovirus antigens: YF(17D) +	YF(17D) −	YF(FN) +	YF(FN) −	WN +	WN −	Langat +	Langat −	SF +	SF −	Chik +	Chik −	Bunyam +	Bunyam −
Lewisburg study		89[c]	91[d]	No.	24	23	31	25	62	61	7	9	3	3	0	0	0	1
				%	27	25	35	28	70	67	7.9	9.9	3.4	3.3	0	0	0	1.1
Ft. Ord study																		
I		25	23	No.	3	6	6	7	8	7	6	3	0	1	0	0	0	0
				%	12	26	24	30	32	30	24	13	0	4.3	0	0	0	0
II		23	23	No.	4	1	5	3	8	4	3	1	0	0	0	0	0	0
				%	17	4.3	22	13	35	17	13	4.3	0	0	0	0	0	0
III		25	25	No.	2	4	7	6	5	10	4	3	0	0	0	0	0	0
				%	8.0	16	28	24	21	40	17	12	0	0	0	0	0	0
IV		25	25	No.	11	9	13	13	11	13	1	3	0	3	0	0	0	0
				%	44	36	52	52	44	52	4.0	12	0	12	0	0	0	0
Total		187	187	No.	44	43	62	54	94	95	21	19	3	7	0	0	0	1
				%	24	23	33	29	50	51	11	10	1.6	3.7	0	0	0	0.5

[a] Abbreviations: YF = yellow fever; WN = West Nile; SF = Semliki Forest; Chik = Chikungunya; Bunyam = Bunyamwera.

[b] ALV status of vaccines administered to vaccinees: + = ALV-contaminated; − = ALV-free.

[c] Number of volunteers inoculated with ALV-contaminated vaccines.

[d] Number of volunteers inoculated with ALV-free vaccines.

TABLE IV. The Presence of Yellow Fever Hemagglutination-Inhibition Antibodies Related to Prior Vaccination in the Lewisburg Volunteers.[a]

History of yellow fever vaccination	HI antibody status of pre-serum	ALV status of study vaccine groups		Total
		Contaminated	Free	
With		49[b]	32	81
	Pos	17	13	30
	Neg	32	19	51
Without		40	59	99
	Pos	17	10	27
	Neg	23	49	72
Total		89	91	180
	Pos	34	23	57
	Neg	55	68	123

[a] Abbreviations: HI = hemagglutination-inhibition; ALV = avian leukosis virus.
[b] Number of volunteers.

conversion rate was 100% in all of the 8 Ft. Ord study groups and 98 to 99% in the 2 Lewisburg groups. This indicated the high degree of antigenicity of the 17D yellow fever vaccine.

The results of the less sensitive HI test essentially reflected the similarity of the ALV-free and -contaminated vaccines and the high degree of antigenicity of the 17D yellow fever vaccine. It should be noted that the higher HI response of the Ft. Ord groups was due, in our opinion, to our improved technical competency in performing all aspects of the HI test.

The poor CF response to vaccination with 17D strain was not unexpected and generally reflected previous experience (2).

Survey of arbovirus HI antibodies in sera of volunteers prior to inoculation with YF vaccine. The sera of the volunteers were examined for the presence of preexisting HI antibodies to some arboviruses, including members of Group A: Semliki Forest (SF) and Chikungunya (Chik) viruses; Group B mosquito-borne: the 17D and French neurotropic (FN) strains of YF and West Nile (WN) viruses; tick-borne: Langat virus; and the Bunyamwera group, the prototype Bunyamwera virus. The presence of HI antibody most likely reflected the variable history of arboviral disease and vaccination of the volunteers (Table III). Comparing the Lewisburg and Ft. Ord volunteers, a higher percentage of the Lewisburg group exhibited WN and YF antibodies. This was probably due to the fact that because of their older age the Lewisburg volunteers may have had a greater chance of exposure to disease caused by viruses closely related to WN antigens, such as St. Louis encephalitis and dengue. In addition, many of the Lewisburg volunteers had already been in the U.S. Military service where they routinely received YF vaccine. Table IV shows the numbers of Lewisburg volunteers related to a history of prior YF vaccination.

The history of prior YF vaccination in the Ft. Ord volunteers was not recorded. It would be expected that very few of these individuals would have been inoculated with YF vaccine prior to their induction into the U.S. Army, for YF vaccine is ordinarily administered only to individuals anticipating travel into a YF endemic area.

HI antibody response to several arbovirus antigens after inoculation with YF vaccine. Following administration of YF vaccine serological tests were performed with several arbovirus antigens to determine: (i) the effect of preexisting antibody upon the antibody response to YF vaccine; and (ii) the effect of YF vaccination upon eliciting an antibody

TABLE V. Percentage of Volunteers Developing Four-fold or Greater Rise in Hemagglutination-Inhibition (HI) Antibody After Inoculation with Yellow Fever Vaccine.[a]

Group	Yellow fever antibody status of pre-serum	ALV status[b]:	Arbovirus antigens													
			YF(17D)		YF(FN)		WN		Langat		SF		Chik		Bunyam	
			+	−	+	−	+	−	+	−	+	−	+	−	+	−
Lewisburg study	Pos		42[c]	43	65	44	15	10	14	11	0	0	0	0	0	0
	Neg		71[d]	78	88	92	22	23	1.2	3.7	0	0	—	—	—	0
Ft. Ord study																
I	Pos		100	83	83	100	13	14	0	33	—	0	—	—	—	—
	Neg		91	100	89	94	5.9	13	5.3	0	0	0	0	0	0	0
II	Pos		75	100	40	100	0	0	0	0	—	—	—	—	—	—
	Neg		95	100	94	100	0	5.3	0	0	0	0	0	0	0	0
III	Pos		100	100	57	83	0	20	0	33	—	—	—	—	—	—
	Neg		100	91	94	84	0	6.7	0	9.1	0	0	0	4	0	4
IV	Pos		82	89	92	85	46	31	0	33	—	—	—	—	—	—
	Neg		100	100	100	100	14	8.3	17	9.1	0	0	0	0	0	0
Total	Pos		61[e]	65[f]	69[g]	69[h]	16	14	4.8	21	0	—	—	—	—	0
	Neg		91[e]	88[f]	91[g]	93[h]	16	13	5.0	5.6	0	—	0	0.5	0	0.5

[a] Abbreviations: same as Table IV.

[b] ALV status of vaccines administered to vaccinees: + = ALV-contaminated; − = ALV-free.

[c] Percentage of volunteers with preexisting antibody to an antigen developing a four-fold or greater rise in HI antibody titer to the same antigen; multiplication of these percentages times the number inoculated in the corresponding ALV status group (shown in Table IV) gives the numbers of volunteers developing a four-fold or greater antibody response.

[d] Percentage of volunteers with no preexisting antibody to an antigen developing a four-fold or greater rise in HI antibody titer to the same antigen.

[e–h] Chi-square analysis of the raw data showed a level of significance of 1%.

response broadly reacting to other arboviruses. The results, shown in Table V, essentially demonstrate that preexisting YF antibody had a suppressive effect upon the HI antibody response to YF vaccine; chi-square analysis indicated that these results were significant (level of significance 1%). Preexisting WN antibody had no effect. Too few volunteers had preexisting antibodies to the other arboviruses to determine the effect of this parameter upon the response to YF vaccine.

Analysis of geometric mean HI antibody titers generally reflected what was shown from analysis of the fourfold and greater antibody responses.

Administration of YF vaccine did elicit antibodies capable of reacting with WN, and less so with Langat, HA antigens.

Discussion. The results of these studies suggest that the removal of ALV, which heretofore existed as an adventitious contaminant in the yellow fever 17D vaccine since its development in the middle 1940's, exercised no demonstrable deleterious effect upon the reactogenicity and antigenicity of resulting vaccines in man. The results of the more sensitive and reproducible PN test demonstrate the high degree of antigenicity of the 17D vaccine—a fact generally accepted by the scientific community on the basis of over 25 years of experience with this vaccine in the "field." From the small amount of data obtained thus far from these volunteer studies, it appears that the presence of preexisting yellow fever antibodies may interfere with the antibody response to yellow fever vaccine, if we can extrapolate from our HI data alone. This should not be a source of concern because individuals with preexisting YF antibody would be expected to be immune to yellow fever, the aim of vaccination.

On the contrary, the presence of preexisting YF antibody appeared not to interfere with the antibody response to yellow fever vaccine in rhesus monkeys (10). This might be due to the fact that YF antibody in these rhesus monkeys was not specifically induced by previous exposure to yellow fever virus, but rather represented cross-reactions from antibody induced by previous exposure to other Group B arboviruses indigenous to that part of India where the rhesus monkeys habitated.

The development of antibodies capable of cross-reacting with WN, and less so with Langat, arbovirus antigens may have been the result of either an additive effect of administering yellow fever virus upon a background of preexisting Group B arbovirus antibodies or an anamnestic response induced by antigens shared by YF and other Group B arboviruses. It is difficult to assess the significance of the development of HI antibodies capable of cross-reacting with WN, and less so with Langat, antigens. Whether these cross-reacting antibodies reflect immunity to these other arbovirus diseases remains to be determined.

In this present study, no attempt was made either to standardize or to determine the dose of vaccine virus for an optimal antibody response. Antigenicity studies presently underway may resolve these questions.

Summary. Removal of avian leukosis viruses (ALV) which have contaminated the yellow fever (YF) 17D vaccine since its development in the middle 1940's, had no effect upon the reactogenicity and antigenicity of this vaccine in man. From results of plaque neutralization (PN) tests, the high degree of antigenicity of the 17D vaccine was confirmed.

Preexisting yellow fever antibody appeared to interfere with the antibody response to YF vaccine. Administration of YF vaccine did elicit antibodies capable of cross-reacting with West Nile, and less so with Langat, arbovirus antigens.

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