The total influenza vaccine failure of 1947 revisited: Major intrasubtypic antigenic change can explain failure of vaccine in a post-World War II epidemic

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Although vaccine-induced immunity to influenza A virus is continually challenged by progressively selected mutations in the virus's major antigens (antigenic drift), virus strains within a subtype (e.g., H1N1) are antigenically cross-reactive. Although cross-immunity diminishes as further mutations accumulate, necessitating frequent changes in vaccine strains, older vaccines are usually partially protective. The post-World War II epidemic of 1947 is notable for the total failure of a vaccine previously effective in the 1943–44 and 1944–45 seasons. We have combined extensive antigenic characterization of the hemagglutinin and neuraminidase antigens of the 1943 and 1947 viruses with analysis of their nucleotide and amino acid sequences and have found marked antigenic and amino acid differences in viruses of the two years. Furthermore, in a mouse model, vaccination with the 1943 vaccine had no effect on infection with the 1947 strain. These findings are important, because complete lack of cross-immunogenicity has been found previously only with antigenic shift, in which antigenically novel antigens have been captured by reassortment of human and animal strains, sometimes leading to pandemics. Although the 1947 epidemic lacked the usual hallmarks of pandemic disease, including an extensive increase in mortality, it warns of the possibility that extreme intrasubtypic antigenic variation (if coupled with an increase in disease severity) could produce pandemic disease without the introduction of animal virus antigens.

It is generally accepted that epidemics of influenza A virus infection are of two distinct types: the first, which occur annually or biannually, are regional epidemics in partially immune populations, and the second, which occur at irregular and unpredictable intervals ranging from 11 to 30 years or more, confront essentially nonimmune populations and are global (pandemic) in distribution. Regional, or interpandemic, influenza is associated with progressive evolution of the virus by its acquisition of cumulative point mutations affecting antigenic sites of the hemagglutinin (HA) and neuraminidase (NA) antigens of the virus (antigenic drift), whereas pandemics follow the introduction of wholly new antigens, novel to human experience, probably through genetic reassortment of human and animal viruses (antigenic shift).

The worldwide influenza epidemic of influenza in 1947 after the close of World War II has never fit neatly into either epidemic category. So different were the strains of 1946–47 that initial classification of human influenza A viruses designated them as "A prime" (1) and somewhat later as "H1" in contrast to the "H0" designation of all earlier strains (2). Currently, based principally on antigenic cross-reactivity and more recently and definitively on sequence homology, the H0 and H1 strains are grouped together in the H1 category, and thus this significant bit of history (and its categorical and epidemiological implications) has been forgotten (3). On the basis of subsequent antigenic analysis and genomic sequence data, the 1947 virus is clearly of the H1N1 subtype, but the rapid post-World War II dissemination of the virus, the low levels of serum antibody to the virus in

humans at that time (4), and the almost total failure of a vaccine containing earlier H1N1 strains (5) have remained puzzling.

In short, the categorical analysis of the virus has not been in accord with its epidemiologic manifestations. In the present paper we demonstrate unidirectional antigenic changes in the prototype 1947 H1N1 strain, A/Fort Monmouth/1/47, from the antecedent A/Weiss/43 virus (and another 1943 strain), that may explain both the failure of vaccine containing the Weiss strain and the extent of the 1947 epidemic.

Materials and Methods

Viruses and Their Genealogy. Strains of A/Fort Monmouth/1/47 (H1N1) (FM-1) from three laboratories were compared initially and found to be similar in antigenicity and HA sequence. Thereafter, our studies focused on the strain present in the laboratory of one of us (E.D.K.) since 1951 and used previously in immunologic studies. This strain was known to be avirulent in mice and presumably was closest in genealogy to the strain initially isolated in eggs in 1947. A/Weiss/43 (H1N1) was a component [together with A/PR/8/34 (H1N1) and B/Lee/40] of the 1947 vaccine. A second 1943 strain, A/Marton/43, was generously provided as lyophilized virus by H. F. Maassab (University of Michigan, Ann Arbor), and two others, A/Huston/43 and A/DSP/43, were from the files of the Centers for Disease Control. It is important to note that although both the Weiss and Marton strains had been isolated initially in ferrets (the principal experimental host used for influenza studies in 1943), in 1971 the Marton strain was reisolated from clinical material in eggs to create an egg passage line (E9), which has been used in this study. The principal strains and their passage histories before their use in the experiments described herein are listed in Table 1.

Strains of the later period of H1N1 prevalence (1977–1986) include A/USSR/90/77, A/India/6263/80, A/Chile/1/83, and A/Taiwan/1/86. All of these strains had been isolated and passaged only in eggs. These latter strains had been examined extensively and compared previously with respect to their antigenic relationships (6).

Antisera. Antisera used in hemagglutination-inhibition (HI) and NA-inhibition (NI) tests were prepared in rabbits as secondary response sera (6).

Serologic Testing. Serologic testing was done as described (6). Antigenic relatedness was determined by the method of Archetti

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Table 1. Passage history and source of viruses studied

Strain	Passage	Source	GenBank accession nos. HA	GenBank accession nos. NA
Weiss/43	F3M3E67	CDC	AF494247	AF494252
Marton/43	E9	Ann Arbor	AF494248	AY122326
DSP/43/43	E8	CDC	AF494246	N/A
Huston/43	E11	CDC	AF494251	N/A
Fort Monmouth/47	EX	RI	AF494250	AF494253
Rhodes/47	F7M7E9	CDC	AF494249	AY122327

F, ferret; E, egg (chick embryo); EX, passage number unknown; M, mouse; CDC, Centers for Disease Control; N/A, not applicable; RI, Rockefeller Institute.

and Horsfall (7) by calculation of homologous and heterologous titer ratios. The formula used compensates for differing homologous titers of the antisera used in the analyses in order for antigenic relationships to be defined by a single value (see also ref. 6). Plaque-inhibition tests were carried out in Madin–Darby canine kidney (MDCK) cell monolayer cultures as described (8).

Vaccination and Protection of Mice. BALB/c female mice averaging 26 g in weight were injected i.p. with formalin-inactivated A/Weiss/43 virus as described in *Results*, bled for antibody studies 14 days after a second boosting infection (day 35), and then challenged by intranasal injection under light ether anesthesia with the indicated dose of virus. Lungs were removed 3 days after challenge and titrated for the presence of plaque-forming virus.

Nucleotide Sequencing. Viral RNA was purified directly from viral material with the Qiagen (Chatsworth, CA) RNeasy kit. The HA1 domain of the HA gene was amplified by reverse-transcription followed by PCR. PCR-derived double-stranded DNA was used as a template for dye terminator sequencing by

Table 2. Antigenic relatedness of influenza A (H1N1) viruses from 1943 and 1947

Virus	HI*		% rel†	NI‡		% rel†
	Weiss/43	Marton/43		Weiss/43	Marton/43	
Weiss/43	5120	5120	71	1835	795	32
Marton/43	1280	2560		132	545	
	Weiss/43	FM-1/47		Weiss/43	FM-1/47	
Weiss/43	2560	240	6	2023	923	22
FM-1/47	40	4280		222	2239	
	Weiss/43	Rhodes/47		Weiss/43	Rhodes/47	
Weiss/43	1280	20	3	1731	105	14
Rhodes/47	10	160		256	800	
	Marton/43	FM-1/47		Marton/43	FM-1/47	
Marton/43	7680	80	<1	403	195	34
FM-1/47	10	2560		552	2239	
	Marton/43	Rhodes/47		Marton/43	Rhodes/47	
Marton/43	2560	10	3	1353	774	65
Rhodes/47	20	640		1221	358	

*Reciprocal dilution endpoint in HI titration.
†Calculated by the method of Archetti and Horsfall (7).
‡Reciprocal dilution endpoint in NI titration.

Table 3. Markedly diminished inhibition of post-1943 viruses with antisera to A/Weiss/43, A/Marton/43, and Huston/43 viruses

Virus	Antisera A/Weiss/43	Antisera A/Marton/43	Antisera Huston/43
Weiss/43	1,920	2,560	2,500
Marton/43	320	1,760	1,280
Huston/43	160	2,560	1,280
DSP/43	320	1,280	—
FM-1/47	28	25	40
Rhodes/47	40	10	40

Reciprocal of HI (H1) endpoint titers of polyclonal rabbit antisera titrated in duplicate. Results are expressed as initial serum dilutions prior to the addition of virus and red blood cells.

using a Model 373A or 3100 DNA sequencer (Amplicon Biosystems, Foster City, CA). Four primers complementary to the plus-sense RNA strand were used to sequence the HA1 domain. Five primers complementary to the plus-sense RNA strand were used to sequence the NA. The primers' sequencing is available on request.

The sequences generated in this study have been deposited in the GenBank database under the accession codes shown in Table 1. The remaining sequences were obtained from the GenBank database.

Results

Antigenic Relatedness of HA and NA Antigens of the 1943 Strains, Weiss and Marton, and the 1947 Viruses FM-1 and Rhodes. The antigenic relatedness of the HA and NA antigens of the 1943 and 1947 prototype viruses was defined first by reciprocal HI and NI titrations with broadly reactive hyperimmune polyclonal rabbit antisera. The results are presented in Table 2. First, it is apparent that although the HA antigens of the 1943 strains were closely related (71%), the viruses were antigenically distinguishable from one another, particularly with respect to their NA antigens. Nonetheless, both 1943 strains stand apart from both 1947 strains. The 1947 strains resembled each other closely in that neither was inhibited significantly in HI tests by antisera to either

Table 4. Weiss/43 vaccine is ineffective in reducing pulmonary virus replication of FM-1/47 virus in mice

Group	Vaccine	Antibody response HI*	Antibody response NI†	Challenge Virus	Challenge PFU‡	Lung virus Mean, PFU§
A	Weiss/43	56	1/5	FM-1/47	10^6	9×10^4
B	Weiss/43	28	1/5	FM-1/47	10^4	8×10^4
C	—	<16	0/5	FM-1/47	10^6	20×10^4
D	—	<16	0/5	FM-1/47	10^4	2×10^4
E	Weiss/43	64	2/5	Weiss/43	10^6	6×10^2
F	Weiss/43	64	2/5	Weiss/43	10^4	<10
G	—	<16	0/5	Weiss/43	10^6	3×10^4
H	—	<16	0/5	Weiss/43	10^4	4×10^4
I	—	<16	0/5	—	—	<10

*Geometric mean HI antibody titer against Weiss virus. HI titers of individual mice in groups A and B ranged from <16 to 512 and in groups E and F from <16 to 512, but even in those mice in groups A and B with the highest titers, suppression of FM-1 virus replication was not demonstrated.
†Number of mice showing significant increase in serum antibody to Weiss virus measured in the NI test (no detectable increase in HI or NI antibodies was found against the FM-1 virus).
‡Plaque-forming units (PFU) of virus inoculated.
§PFU of challenge virus per 0.1 g of lung 3 days after intranasal challenge.

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Table 5. Effect of antisera to immediately antecedent strains on next-emerging strain of epidemiological significance in the 1977–1986 period of H1N1 prevalence

Virus	Antisera		
	USSR/77	India/80	Chile/83
India/80	1,280*		
	1,024†		
Chile/83		12,800*	
		512†	
Taiwan/86			6,400*
			256†

*Plaque-inhibition titer endpoint.
†Inhibition endpoint of HI titration.

Table 6. Effect of USSR/77 antiserum on subsequently evolving H1N1 variants

Virus	USSR/77 Antiserum	
	Plaque reduction*	HI
USSR/77	12,800	2,560
India/80	12,800	1,280
Chile/83	12,800	320
Taiwan/86	3,200	80

In this later period, spanning 9 years, no abrupt antigenic change of the same magnitude is seen comparable to that which occurred between 1943 and 1947 with the Weiss/43 and FM-1/47 viruses.
*Reciprocal of serum dilution at the endpoint.

1943 virus, and antisera to the vaccine strain, Weiss, did not cause significant (i.e., >25%) inhibition of the 1947 viruses in NI tests. With respect to the HA antigens, relatedness was unidirectional in the sense that antibodies to Weiss antigens were virtually noninhibitory to either 1947 virus, although antibodies to the antigens of FM-1/47 virus were inhibitory to, i.e., recognized epitopes in, the antecedent Weiss and Marton strains. The markedly diminished inhibition of post-1943 viruses by antisera to both Weiss and Marton viruses is summarized in Table 3.

Similarly, tests of *in vitro* neutralization of virus infection in Madin–Darby canine kidney cell cultures (data not shown) demonstrated not only virus dissimilarity but a lack of measurable effect of Weiss antiserum on suppression of FM-1 virus replication. The fact that Weiss antiserum had no significant effect on FM-1 virus in any test suggests that both HA and NA antigens had mutated significantly, an inference later supported by the studies of HA and NA sequences described below. A similar lack of effect on FM-1 virus replication was found with antiserum to the still earlier A/PR/8/34 H1N1 virus strain also used in the 1947 vaccine (data not shown).

Differences among the NA antigens of 1943 and 1947 strains are less extensive than with the HA antigens, and Weiss and Marton are discordant with respect to their inhibition of the Rhodes/47 NA.

Failure of a Weiss/43 Virus Vaccine to Protect Mice Against FM-1 Virus Infection: Vaccination and Protection of Mice. BALB/c female mice in groups of five were injected i.p. with two successive doses of formalin-treated A/Weiss/43 virus vaccine or a PBS control, bled for studies of serologic response at 35 days, and challenged by intranasal infection under light ether anesthesia with varying doses of either homologous (Weiss) or heterovariant (FM-1)

virus. The results, as summarized in Table 4, demonstrate the induction at 35 days of homologous H1 and N1 antibody and a 100-fold or greater reduction in pulmonary virus in the case of homologous challenge but no protection against challenge with FM-1 virus.

Antigenic Variation and Cross-Immunity Among H1N1 Strains in the 1977–1986 Period. Because no event comparable to the 1947 epidemic has occurred since the reintroduction of H1N1 subtype in 1977, we examined the antigenic relationships among epidemiologically significant strains in the 9-year period from 1977 to 1986 (Tables 5 and 6). Antigenic cross-reactivity was seen among the four strains studied, and although progressive diminution of inhibition was observed, in no case was antibody to earlier strains nonreactive with subsequently emerging viruses, even after 9 years. The results shown in plaque-reduction tests are particularly significant, because these tests in cell cultures measure effects on actual *in vitro* infection and reflect the presence of both neutralizing or plaque size-reducing antibodies (8).

Studies of HA and NA Nucleotide Sequences. Although differences in antigenic phenotype were the critical point of departure for this study, such differences (or similarities) are not definitive criteria for the establishment of genealogical relationships among viruses. Therefore, complete HA1 and NA nucleotide sequences were obtained and analyzed. Amino acid sequences were deduced and then analyzed with reference to the number of differences overall between viruses and with respect to their known antigenic sites.

Amino Acid Sequences of HA Antigens of 1943 and 1947 Prototype Strains. The fundamental similarities of the 1943 prototypes and their marked dissimilarities from those of 1947 are epitomized by comparison of their amino acid sequences at the Sa, Sb, and Ca1

Table 7. Antigenic site changes in the HA gene

	Antigenic site												
	Sa				Sb			Ca1		Ca2		Cb	
	163	165	166	129	192	196	197	173	273	145	225	78	81
1943–1947													
Weiss/43	Y	N	N		Q	Q		A	S	R	D	L	E
Marton/43	N	N	N		Q	Q		G	S	S	G	Q	E
FM/1/47	K	S	K		K	R		E	P	S	G	L	K
Rhodes/47	K	K	K		R	R		E	S	S	G	L	K
1977–1986													
USSR/90/77				K	K	R	T				G		
India/6263/80				K	K	R	T				D		
Chile/1/83				K	K	R	T				N		
Taiwan/1/86				N	R	H	A				G		

Table 8. Comparative no. of amino acid changes in antigenic sites of the HAs of H1 viruses in the post-Weiss/43 and post-USSR/77 periods

Antiserum	Virus	Interval, years	No. of amino acids	Changes per year	Changes in antigenic sites	No. antigenic sites involved
Weiss/43 vs.	FM-1/47	4	34	8.5	10	5
	Rhodes/47	4	36	9.0	9	5
USSR/77 vs.	India/80	3	12	4.0	1	1
	Chile/83	6	15	2.5	1	1
	Taiwan/86	9	21	2.3	4	2

sites of the HA (ref. 9; Table 7, 1943–1947). The differences in sequence at Sa, Ca1, and Cb of Weiss and Marton strains may comprise the basis of their minor antigenic differences.

It is reassuring that despite their different passage histories, the 1947 strains are identical at 7 of 11 antigenic sites' amino acids. FM-1 differs from Weiss and Marton in all of the 11 amino acids that comprise the five H1 antigenic sites, whereas Rhodes has only one Ca1 amino acid in common with the 1943 viruses at position 273 in site Ca1.

HA Antigenic Site Changes During the 1977–1986 Period. Compared with the earlier period, remarkably few changes at antigenic sites characterize viruses of the 1977–1986 period until the appearance of the Taiwan strain in 1986, when different amino acid substitutions appeared at Sa, Sb, and Ca2 sites (Table 7, 1977–1986). Robertson noted five amino acid substitutions between residues 189 and 197 comprising part of the Sb site at the antigenically significant tip of the HA molecule (10). It is worth noting that in a crowded boys' school population, vaccine containing the preceding Chile strain had little effect on two successive epidemics from which Taiwan-like virus was recovered (11). No changes from the USSR/77 strain occurred in any of the strains at the Ca1 and Cb sites (data not shown in Table 7).

Comparative Number of HA Amino Acid Changes During the Early and Late Periods of H1N1 Prevalence. Table 8 contrasts the changes in a 4-year interval in the case of the early strains with 3–9-year intervals in evolution of the later viruses. The general point to be made is the ≈2-fold greater number of total changes in HA1 comparing the Weiss-to-FM-1 4-year period with the USSR-to-Chile interval of 6 years. Furthermore, the earlier changes involved five antigenic sites rather than only one in the later period. Even after 9 years, in the case of Taiwan virus, changes involved only two sites. Previous studies of sequential variants of epidemiological importance have revealed that, in general, each significant drift variant has at least four amino acid substitutions located in two or more defined antigenic sites (12).

Changes in NA Antigenic Sites (See Tables 9 and 10). In contrast to the situation with the HAs of the 1943 and 1947 viruses in which sharp differences are evident in both antigenic and nucleotide sequence analyses, analyses of the NAs are less easy to interpret and present some dichotomies. To begin with, all four NAs are cross-reactive antigenically to some degree. By sequence analysis, Marton/43 and Rhodes/47 are almost identical, although they are less related on antigenic analysis. Their similarity cannot be ascribed to host-passage histories, which are very different (see Table 1).

On the other hand, the NAs of the two 1947 strains have in common only 39 of 49 amino acids (78%) in antigenic sites yet are ≈100% related antigenically. A more extreme dichotomy has been observed with two HA variants of A/Shanghai/11/87 (H3N2), which, although only 40% antigenically related by analysis with polyclonal rabbit antibody, differed by only a single amino acid at HA1 site 186 (13). Such occasional nonconcordances are to be expected, depending on the location of the specific sites involved in virus-antibody reactions, because they may or may not affect functionally important sites on viral surface proteins. Furthermore, the determination of antigenic relationships can be influenced by the species of antibody and the method of immunization used (13, 14).

There are precedents for the cocirculation of intrasubtypic NA variants (15, 16) and also for the slower evolutionary rate of NA proteins (6). In the present case, perhaps genetically distinguishable but antigenically similar NAs have arrived at the same place by convergent evolution under the suppressive effects of population antibody evident even before 1943.

Antigenic site changes in the NAs of the 1977–1986 period were minimal in the first 6 years and until the introduction of the Taiwan/86 strain at the end of the 9-year period.

Discussion

A cardinal concern in this revisitation of the past of viruses over 50 years old is how representative these viruses may be of those circulating at that time, recognizing that with repeated laboratory passage such viruses are subject to "laboratory drift" and

Table 9. Antigenic site changes in the NA gene from 1943 to 1947

	VII	II		III	(VIII)*						V
	153	342	347	368	385	386	392	393	397	400	434
Weiss/43	T	Y	N	D	E	T	F	V	I	M	K
Marton/43	S	Y	N	N	E	T	F	E	V	V	K
FM-1/47	S	N	D	N	D	P	L	V	I	M	N
Rhodes/47	S	Y	N	N	E	T	F	T	V	V	K

Shown are only the antigenic sites in which amino acid changes have occurred, and then only the amino acid positions that have changed are shown; there are actually 46 amino acids in 8 antigenic sites.
*This antigenic site is not defined as an antigenic site for N2 but it is included here because there are significant changes at this site in the N1 model.

Table 10. Antigenic site changes in the NA gene from 1977 to 1986

	Amino acid at antigenic site										
	VI	I		II		(VIII)*					V
	199	332	334	339	342	386	390	392	397	399	434
USSR/77	D	K	R	D	N	P	N	L	I	A	K
India/80	D	K	S	D	T	T	N	L	V	A	K
Chile/83	D	K	S	D	T	T	N	L	V	A	K
Taiwan/86	N	E	S	N	T	T	D	S	V	P	N

Shown are only the antigenic sites in which amino acid changes have occurred, and then only the amino acid positions that have changed are shown; there are actually 46 amino acids in 8 antigenic sites.
*This antigenic site is not defined as an antigenic site for N2 but it is included here because there are significant changes at this site in the N1 model.

changes associated with isolation or passage in different host systems (17, 18). Virus changes associated with host adaptation have been associated often with single site mutations of the HA, which pleiotropically can have minor effects on antigenicity (13, 19). We have taken pains to employ two of the few available representatives of both the 1943 and 1947 periods in our present analysis and have shown that they differ within each year in amino sequences involving their antigenic sites despite the fact that one virus in each year (Weiss/43 and Rhodes/47) had been recovered initially in ferrets. In comparisons of 1943 with 1947 strains, regardless of origin, marked and similar differences were discerned. This result was not unexpected in view of the failure of the 1943 vaccine in healthy young soldiers in 1947 and our present demonstration of 3–5% antigenic relatedness of the HAs of the viruses of the two periods. It should be stressed that differences of this degree are more characteristic of intersubtype HA differences caused by the importation of novel antigens from animal sources (17) than variations within a subtype.

The vaccine failure of 1947 clearly was not the result of administration of a nonimmunogenic vaccine: (*i*) homologous response to the Weiss component was good (4), (*ii*) the vaccine was protective in the 1943–44 and 1944–45 epidemics (1, 20), and (*iii*) in our animal model a freshly made Weiss-strain vaccine failed to reduce replication of FM-1 virus in mice but was protective against Weiss-virus challenge. Similarly, tests of standard (National Institutes of Health) vaccine in 1947 failed to raise neutralizing antibodies in mice against either the FM-1 or LF1 1947 strains, but even when highly diluted it did induce neutralizing antibodies to its component Weiss and PR8 viruses (4).

Why did a virus as different as FM-1/47 not cause pandemic infection? In the sense of global distribution of the virus, it did, but other signs of true pandemic disease were lacking, including notably a significant effect on mortality, although disease in young military patients was as severe as in earlier epidemics (21). It is possible (even probable) that in a population experienced with H1N1 viruses, no matter how apparently different with respect to HA and NA antigens, a degree of immunity to FM-1-like strains existed sufficient to moderate the epidemic but insufficient to prime for an adequate heterovariant vaccine response. Also, the 34–65% antigenic relationship of the NA antigens of our two 1947 strains to the Marton 1943 strain hints at the possibility of some cross protection through the NA antigens such as that seen in the pandemic of 1968 (22).

The question arises, "whence came the viruses of 1947?" Probably not as mainstream extensions of the Weiss-like viruses of 1943. More likely, as Nakajima *et al.* (23) have suggested in the case of the Taiwan-like viruses of 1986, they emerged as a side branch from other H1N1 viruses of the earlier period that had not produced significant epidemics at that time. Indeed, an exception to the good protection afforded to most troops vaccinated with Weiss-containing vaccine in 1943–44 was encountered at one installation in California in which the incidence of influenza was less moderated than in other outbreaks studied at the same time (20), and the virus isolated from the outbreak differed slightly from the Weiss vaccine antigen. Unfortunately, this "Olsen strain" is not available for study.

The present study is important in demonstrating an extreme of intrasubtypic antigenic variation that, under other circumstances with a virus of greater intrinsic virulence, might present a pandemic threat even without the recruitment of novel antigens from animal sources.

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