Mucosal and Systemic Humoral Immunity Differences between Sexes during Influenza Vaccination and Viral Challenge National Institute of

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	BACK	GROUND							RESULTS:
 Sex-linked differences in influenza disease, with more severe presentations in females, 					Table 2a: Prechallenge systemi	c antibody titers (vac	cinated subgroup))	
are not well understood b	ut have been ob	served in anima	I and human studies	1,2,3,4		Male (n=17)	Female (n=20)	Difference (95% Cl)	p-value
					Age, y, mean, (std)	31.5 (6.2)	36 (8.2)	-4.5 (-9.3 to 0.4)	0.0682
 In our previous challenge 	-	_			Serum log ₂ HAI titer, mean, (std)	6.79 (2.52)	6.97 (2.04)	-0.18 (-1.15 to 0.79)	0.7085
baseline hemagglutination symptoms and to have me		7 ·	•		Serum log ₂ NAI titer, mean, (std)	6.49 (1.24)	7.07 (1.25)	-0.57 (-1.41 to 0.26)	0.1711
inhibition (NAI) titers in re		_			Serum HA IgG, mean, (std)	422989 (82860)	430263 (80093)	-7274 (-62001 to 47453)	0.7886
					Serum HA Stalk IgG, mean, (std)	216841 (50528)	199005 (58052)	17836 (-18406 to 54078)	0.3246
 We performed another hu 	ıman influenza c	hallenge study,	which also included	data on	Serum NA IgG, median, (25, 75%tile)	5967 (4482-9996)	4732 (3178-5789)		0.2087
mucosal immunity and wh	•	•••	eplicate our previous	analysis	Serum Total IgG, mean, (std)	1084 (235)	1055 (193)	29 (-117 to 175)	0.6874
in a more immunologically	y varied population	on.			Serum Total IgA, mean, (std)	227 (75)	201 (88)	26 (-29 to 81)	0.3438
	OB IF	CTIVES			Table 2c: Postchallenge day 7 s	systemic antibody tite	ers		
				<u> </u>		Male (n=34)	Female (n=40)	Difference (95% Cl)	p-value
 To determine the different influence obellence 	ices in clinical ou	tcomes betwee	n men and women a	fter	Serum log ₂ HAI titer, median, (25, 75%tile)	7.32 (4.57-9.32)	7.82 (6.07-9.32)		0.2981
influenza challenge.					Serum log ₂ NAI titer, mean, (std)	5.82 (1.58)	6.02 (1.44)	-0.2 (-0.91 to 0.51)	0.5736
 To determine immunologi 	ical differences i	n systemic and	mucosal immunitv be	etween	Serum HA IgG, mean, (std)	537946 (159069)	550595 (127723)	-12649 (-80475 to 55176)	0.7106
men and women after inf		•	•		Serum HA Stalk IgG, mean, (std)	174205 (69929)	167766 (69902)	6439 (-26087 to 38966)	0.6942
			0		Serum NA IgG, median, (25, 75%tile)	4879 (3669-7575)	5019 (3575-8557)		0.8504
 To verify the findings of o 	our previous sex	differences anal	ysis.		Serum Total IgG, mean, (std)	1002 (207)	996 (189)	5 (-89 to 99)	0.9083
					Serum Total IgA, mean, (std)	220 (98)	189 (80)	30 (-12 to 73)	0.1553
	MEI	HODS			Table 2e: Postchallenge day 56	systemic antibody ti	ters		
 Seventy-four healthy adult 	Its completed the	e study. Forty-or	ne were given seaso	nal		Male (n=34)	Female (n=40)	Difference (95% Cl)	p-value
quadrivalent vaccine (tho		_	-		Serum log ₂ HAI titer, median, (25, 75%tile)	8.32 (6.32-9.32)	7.82 (6.57-9.32)		0.7473
unvaccinated. All participa		nfluenza challer	nge with		Serum log ₂ NAI titer, median, (25, 75%tile)	7.32 (7.32-9.32)	8.82 (7.32-9.32)		0.1143
A/Bethesda/MM2/H1N1 c	challenge virus.				Serum HA IgG, median, (25, 75%tile)	368904 (244699- 453201)	353219 (273796- 431504)		0.8526
Necel and blood complex	ware collected r	riar to vocionat	ion 20 dave after va	adination	Serum HA Stalk IgG, mean, (std)	136716 (63842)	123303 (65468)	13412 (-17152 to 43978)	0.3842
 Nasal and blood samples one day prior to challenge 			-		Serum NA IgG, median, (25, 75%tile)	4926 (3290-8203)	5214 (3223-9157)		0.7913
after challenge.	e, r days aller of	ialienge, zo uay	s aller challenge, an	u Ju uays	Serum Total IgG, mean, (std)	1123 (240)	1093 (212)	30 (-78 to 139)	0.5776
and on anongo					Serum Total IgA, mean, (std)	238 (92)	199 (84)	39 (-2.75 to 82)	0.0664
 IgA titers against HA/NA/I 	HA stalk from na	sal samples and	I IgG titers against H	A/NA/HA	Comparisons for means performed using t-	-tests, for medians using Wi	ilcoxon rank-sum tests,	and for proportions using tests	of proportions. Abbrevia
stalk, serum HAI, and ser	rum NAI assays f	rom serum sam	ples were performed	1.	20 T	*	<u>*</u> ۲ <mark>*</mark>		*
	1 4						iter		
Clinical and immunologica		-	•		<u>15</u> 15 -			• • •	•
visually. Means were calc normally distributed varia		•			$\begin{array}{c c} \mathbf{F} & \vdots & \vdots \\ \mathbf{F} & \vdots $: • •• : :
normally distributed varial	•	•	• • •						
Wilcoxon rank-sum tests.	•		•	•			S A III		
	-		· · ·				<u> </u>		
F	RESULTS: V	ACCINAT	ION				And And		
Table 1a: Prevaccine syste	emic antibody tite	rs			₀ <mark>↓┙╃┦┛╶╹┩┦┛╶╹┩╿┛╶╹┩</mark>	┍┛╴┛┩┛╴┛┩┛		,,,,,,,,,,,,,,,,,,,,, ,,,,,,,,,,,,,,,	
	Male (n=18)	Female (n=23)	Difference (95% Cl)	p-value	ion ion ted ted of		i noi	on ted ted de de	N ^{OE}
Age, y, mean, (std)	31.3 (6.1)	34.6 (8.7)	-3.3 (-8.0 to 1.4)	0.159	ccination ination ccinated inated lenge	nallenge nge	evaccination ination	Vaccinated inated lienge of the second secon	allett
Serum log ₂ HAI titer, mean, (std)	6.16 (1.95)	6.41 (1.50)	-0.25 (-1.39 to 0.88)	0.6515	Prevace postulace Nac Unvac Postch Postch	of stoll	evace that h	Vac unvac oster oster oster	
Serum log ₂ NAI titer, mean, (std)	6.77 (1.72)	6.76 (1.62)	0.01 (-1.06 to -1.08)	0.9855	Pre Pos Day NUM Po 28Po	50 C	Pre Pos ani	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	
Serum HA IgG, mean, (std)	215395 (103823)	215165 (84099)	229 (-61068 to 61527)	0.994	Day Day Day Day		\sim	s Ds Day Day	
Serum HA Stalk IgG, mean, (std)	111268 (52693)	99382 (56198)	11887 (-22690 to 46464)	0.4906	15 א ד *	*		· •	
Serum NA IgG, median, (25, 75%tile)	5286 (3372-7584)	4806 (2762-7285)		0.5761			Figure 1: Nasal	IgA antibody titers by ELI	SA against HA,
Table 1b: Postvaccine syst	temic antibody tit	ers			:		•	A obtained prevaccination	•
, ,	Male (n=18)	Female (n=23)	Difference (95% CI)	p-value		····		, day -1 prechallenge (sep	•
Serum log ₂ HAI titer, mean, (std)	7.21 (1.60)	7.19 (1.42)	0.03 (-0.96 to 1.01)	0.9588				us), 7 days postchallenge, and 56 days postchallenge	•
Serum log ₂ NAI titer, mean, (std)	6.77 (1.29)	7.41 (1.27)	-0.65 (-1.47 to 0.18)	0.1215				titers and error bars denot	• •
Serum HA IgG, mean, (std)	381788 (59549)	388913 (77718)	-7125 (-51091 to 36840)	0.7446				e bars represent males an	
ocham na 150, mean, (sta)	331,00 (33343)	555515 (77716)	, 123 (31031 (0 30040)	0.7 ++0			•	es. Dots represent individu	
Serum HA Stalk IgG, mean, (std)	243375 (42664)	225299 (64462)	18075 (-16444 to 52595)	0.2955			02	med. * denote significant	
Serum NA IgG, median, (25, 75%tile)	5798 (4832-10595)	6778 (3597-8620)		0.8825		۱ ۱ ۱ کړ کړ ک	than 0.05.	sum tests with unadjusted	p-values less
Comparisons for means performed	using t-tests and for n	nediane using Wilcow	on rank-sum tests		ination ination inater inater lieng	nallenge allenge			
Abbreviations: HA, hemagglutinin; H	HAI, HA inhibition; NA,	-		b		hai stchai			
deviation; %tile, percentile; CI, conf	idence interval				Pret post and in TPOS 8005	56P03			
Acknowledgement: This research	arch was supported	d by the Intramura	I Research program of	the NIH	Day Day Day Day Day	•			
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	BACKO	ROUND							RESULTS:
 Sex-linked differences in i 	influenza disease	e. with more sev	vere presentations in	females.	Table 2a: Prechallenge systemic ar	ntibody titers (vac	cinated subaroup)	
are not well understood by			-			Male (n=17)	Female (n=20)	Difference (95% CI)	p-value
				-	Age, y, mean, (std)	31.5 (6.2)	36 (8.2)	-4.5 (-9.3 to 0.4)	0.0682
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symptoms and to have mo			-		Serum HA IgG, mean, (std)	422989 (82860)	430263 (80093)	-7274 (-62001 to 47453)	0.7886
inhibition (NAI) titers in res		2							
	•	0			Serum HA Stalk IgG, mean, (std)	216841 (50528)	199005 (58052)	17836 (-18406 to 54078)	0.3246
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mucosal immunity and wh		• •			Serum Total IgG, mean, (std)	1084 (235)	1055 (193)	29 (-117 to 175)	0.6874
in a more immunologically	-	•••		j	Serum Total IgA, mean, (std)	227 (75)	201 (88)	26 (-29 to 81)	0.3438
		CTIVES			Table 2c: Postchallenge day 7 system	emic antibody tite	ers		
	ODJE	CIIVES				Male (n=34)	Female (n=40)	Difference (95% CI)	p-value
 To determine the difference 	ces in clinical out	comes betweer	n men and women af	fter	Serum log ₂ HAI titer, median, (25, 75%tile)	7.32 (4.57-9.32)	7.82 (6.07-9.32)		0.2981
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men and women after infl	luenza vaccinatic	on and after influ	uenza challenge.		Serum HA Stalk IgG, mean, (std)	174205 (69929)	167766 (69902)	6439 (-26087 to 38966)	0.6942
					Serum NA IgG, median, (25, 75%tile)	4879 (3669-7575)	5019 (3575-8557)		0.8504
 To verify the findings of or 	ur previous sex c	lifferences analy	ysis.		Serum Total IgG, mean, (std)	1002 (207)	996 (189)	5 (-89 to 99)	0.9083
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	MET	HODS			Table 2e: Postchallenge day 56 sys	temic antibody fi	ters		
 Seventy-four healthy adul 	Its completed the	study Forty-on	e were given seasor	nal		Male (n=34)	Female (n=40)	Difference (95% CI)	p-value
quadrivalent vaccine (thou	-	• •	•		Serum log ₂ HAI titer, median, (25, 75%tile)	8.32 (6.32-9.32)	7.82 (6.57-9.32)		0.7473
unvaccinated. All participa	• •	•	-		Serum log ₂ NAI titer, median, (25, 75%tile)	7.32 (7.32-9.32)	8.82 (7.32-9.32)		0.1143
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						453201)	431504)		
 Nasal and blood samples 	were collected p	rior to vaccinati	on, 28 days after vac	ccination.	Serum HA Stalk IgG, mean, (std)	136716 (63842)	123303 (65468)	13412 (-17152 to 43978)	0.3842
one day prior to challenge	-		•		Serum NA IgG, median, (25, 75%tile)	4926 (3290-8203)	5214 (3223-9157)		0.7913
after challenge.	,		e enter enter 9e, ent	- - -	Serum Total IgG, mean, (std)	1123 (240)	1093 (212)	30 (-78 to 139)	0.5776
U					Serum Total IgA, mean, (std) Comparisons for means performed using t-tests	238 (92)	199 (84)	39 (-2.75 to 82)	0.0664
 IgA titers against HA/NA/H stalk, serum HAI, and seru Clinical and immunological visually. Means were calcondrived variated normally distributed variated Normally distributed variated Wilcoxon rank-sum tests. 	um NAI assays fr al outcomes were ulated for normal bles. Proportions bles using t-tests	rom serum sam e compared. No ly distributed va s were compare , and non-norm	ples were performed rmality was determin ariables and medians d using tests of prop ally distributed variat	ned s for non- ortions, ples using	Hard Titler		A Stalk IgA Titer		
R	RESULTS: V		ION		₩ 5-111 III III III		Anti-4		
Table 1a: Prevaccine syste	mic antihody tite	rs			₀→ <mark>┛╃┛╶╹┥┛╶╹┥┛╶╹┥╹╴╹┥</mark> ╹				II_II II
TANTO TALI TOVOCONIC SYSLE	Male (n=18)	Female (n=23)	Difference (95% CI)	p-value	ϕ^{0} ϕ^{0} ϕ^{0} ϕ^{0} ϕ^{0}	\$° \$°	· 70.		. A
Age, y, mean, (std)	31.3 (6.1)	34.6 (8.7)	-3.3 (-8.0 to 1.4)	0.159	Naccination Naccinated inated allenge aller	de d	ination ination	Asceinated instead lenge allenge	allenz
Serum log ₂ HAI titer, mean, (std)	6.16 (1.95)	6.41 (1.50)	-0.25 (-1.39 to 0.88)	0.6515	Prevacci vacci vacci nacci postcha postcha	tche	3CC1 3CC1	ac vacu tchia tchia tch	le.
Serum \log_2 NAI titer, mean, (std)	6.77 (1.72)	6.76 (1.62)	0.01 (-1.06 to -1.08)	0.9855	ρ_{10}^{0} ρ_{21}^{0} γ_{11}^{0} γ_{11}^{0} γ_{20}^{0} ρ_{20}^{0} ρ_{20}^{0}	<u>,</u>	pre post in	UII 1 P03 2 P03 2 P05	
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Serum NA IgG, median, (25, 75%tile)	5286 (3372-7584)	4806 (2762-7285)		0.5761	15 * * .	*			
				0.5701			•	IgA antibody titers by ELIS A obtained prevaccination	•
Table 1b: Postvaccine system						· ·		day -1 prechallenge (sep	
	Male (n=18)	Female (n=23)	Difference (95% Cl)	p-value		• ••	•	us), 7 days postchallenge,	•
Serum log ₂ HAI titer, mean, (std)	7.21 (1.60)	7.19 (1.42)	0.03 (-0.96 to 1.01)	0.9588				and 56 days postchallenge	•
Serum log ₂ NAI titer, mean, (std)	6.77 (1.29)	7.41 (1.27)	-0.65 (-1.47 to 0.18)	0.1215				iters and error bars denot	- -
Serum HA IgG, mean, (std)	381788 (59549)	388913 (77718)	-7125 (-51091 to 36840)	0.7446	And		•	e bars represent males an es. Dots represent individu	•
Serum HA Stalk IgG, mean, (std)	243375 (42664)	225299 (64462)	18075 (-16444 to 52595)	0.2955			are log ₂ transfor	med. * denote significant of sum tests with unadjusted	comparisons by
Serum NA IgG, median, (25, 75%tile)	5798 (4832-10595)	6778 (3597-8620)		0.8825		\$° \$°	than 0.05.		
Comparisons for means performed Abbreviations: HA, hemagglutinin; H deviation; %tile, percentile; CI, confi	HAI, HA inhibition; NA,	•			Prevaccination on the steed instead in	oe noe noe stchallenge			

RESULTS:		ENCE
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viations: HA, hemagglutinin; HAI, HA inhibition; NA, neuraminidase; NAI, NA inhibition; std, standard deviation; %tile, percentile; CI, confidence interval

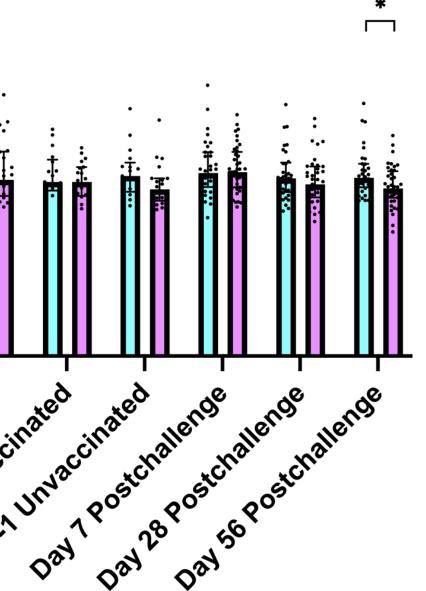


Table 2b: Prechallenge systemic antibody titers (unvaccinated subgroup)								
	Male (n=17)	Female (n=20)	Difference (95% Cl)	p-value				
Age, y, mean, (std)	33.1 (10.9)	34.5 (7.0)	-1.3 (-7.6 to 5.0)	0.6671				
Serum log ₂ HAI titer, mean, (std)	3.83 (2.47)	4.34 (1.78)	-0.51 (-1.98 to 0.97)	0.4883				
Serum log ₂ NAI titer, mean, (std)	6.85 (1.74)	7.02 (1.34)	-0.17 (-1.23 to 0.89)	0.7438				
Serum HA IgG, mean, (std)	267070 (111602)	274241 (91640)	-7171 (-76409 to 62067)	0.8341				
Serum HA Stalk IgG, mean, (std)	115135 (54674)	112691 (50012)	2444 (-32854 to 37741)	0.8888				
Serum NA IgG, median, (25, 75%tile)	3726 (3353-4227)	3525 (3031-6218)		0.7749				
Serum Total IgG, mean, (std)	1098 (210)	1074 (225)	23 (-122 to 168)	0.7464				
Serum Total IgA, mean, (std)	224 (108)	192 (84)	32 (-34 to 97)	0.347				

Table 20. Postchallenge day zo systemic antibody liters							
	Male (n=34)	Female (n=40)	Difference (95% Cl)	p-value			
Serum log ₂ HAI titer, median, (25, 75%tile)	6.32 (5.32-8.32)	7.32 (5.32-7.32)		0.5809			
Serum log ₂ NAI titer, mean, (std)	7.05 (1.42)	7.53 (1.14)	-0.48 (-1.10 to 0.13)	0.123			
Serum HA IgG, mean, (std)	408238 (148513)	381906 (121438)	26332 (-38339 to 91002)	0.4188			
Serum HA Stalk IgG, mean, (std)	186115 (57915)	180701 (55829)	5414 (-21480 to 32308)	0.6891			
Serum NA IgG, median, (25, 75%tile)	5548 (4079-9733)	5968 (3807-10109)		0.6942			
Serum Total IgG, median, (25, 75%tile)	1026 (924-1122)	1065 (936-1226)		0.5223			
Serum Total IgA, median, (25, 75%tile)	223.5 (165-276)	187 (152-253)		0.1888			
Table 2f: Postchallenge clinical outco	omes						
	Male (n=34)		Female (n=40)	p-value			
Presence of symptoms, proportion	0.82		0.85	0.7582			
Presence of shedding, proportion	0.71		0.73	0.8557			
Days of symptoms, median, (25, 75%tile)	3.5 (1.25-6)		4.5 (3-6.25)	0.3288			
Days of shedding, median, (25, 75%tile)	2 (0-3)		1 (0-2)	0.3449			
No. of symptoms, median, (25, 75%tile)	3 (1-4)		3.5 (1.75-5.25)	0.3559			

• In contrast to our previous findings, no differences in clinical outcomes or systemic NAI titers were observed between men and women, which may be due to differences in selection criteria (not restricting participation to volunteers with low HAI titers), or the lower sample size in this study.

- observed in the unvaccinated subgroup prior to challenge.
- literature in women with influenza disease.

- influenza A H1N1 virus of swine origin in Japan. PLoS One 2011;6:e19409. JAMA 2009:302:1872-9.
- Infect Dis. 2022 Feb 15;225(4):715-722.



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Table 2b: Drachallance exclamic antibody titors (unvaccinated subgroup)

Table 2d. Postchallenge day 28 systemic antibody titers

CONCLUSIONS

 However, significant differences in mucosal antibody titers were observed, with women having lower prevaccine mucosal titers than men against HA stalk and NA. Lower mucosal NA titers in women were also

• The differences in mucosal titers diminished after vaccination and after challenge, but reappeared 8 weeks after challenge with significant differences observed between men and women in anti-HA IgA, anti-HA stalk IgA, and anti-NA IgA. Women were observed to have lower titers than men for all 3 measurements.

• Further studies are necessary to replicate the observation of lower baseline and convalescent mucosal titers in women and to determine whether this plays a role in the more severe outcomes reported in the

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