

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

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BACKGROUND

Sex-linked differences in influenza disease, with more severe presentations in females, are not well understood but have been observed in animal and human studies.^{1,2,3,4}

In our previous challenge studies (which primarily recruited participants with low baseline hemagglutination inhibition [HAI] titers), women were more likely to have symptoms and to have more symptoms than men. They also had lower neuraminidase inhibition (NAI) titers in response to challenge.⁵

We performed another human influenza challenge study, which also included data on mucosal immunity and which provided an opportunity to replicate our previous analysis in a more immunologically varied population.

OBJECTIVES

To determine the differences in clinical outcomes between men and women after influenza challenge.

To determine immunological differences in systemic and mucosal immunity between men and women after influenza vaccination and after influenza challenge.

To verify the findings of our previous sex differences analysis.

METHODS

Seventy-four healthy adults completed the study. Forty-one were given seasonal quadrivalent vaccine (though only 37 underwent challenge) and 37 were kept unvaccinated. All participants underwent influenza challenge with A/Bethesda/MM2/H1N1 challenge virus.

Nasal and blood samples were collected prior to vaccination, 28 days after vaccination, one day prior to challenge, 7 days after challenge, 28 days after challenge, and 56 days after challenge.

IgA titers against HA/NA/HA stalk from nasal samples and IgG titers against HA/NA/HA stalk, serum HAI, and serum NAI assays from serum samples were performed.

Clinical and immunological outcomes were compared. Normality was determined visually. Means were calculated for normally distributed variables and medians for non-normally distributed variables. Proportions were compared using tests of proportions, normally distributed variables using t-tests, and non-normally distributed variables using Wilcoxon rank-sum tests. Adjustment was not performed for multiple comparisons.

RESULTS: VACCINATION

Table 1a: Prevacine systemic antibody titers

	Male (n=18)	Female (n=23)	Difference (95% CI)	p-value
Age, y, mean, (std)	31.3 (6.1)	34.6 (8.7)	-3.3 (-8.0 to 1.4)	0.159
Serum log ₂ HAI titer, mean, (std)	6.16 (1.95)	6.41 (1.50)	-0.25 (-1.39 to 0.88)	0.6515
Serum log ₂ NAI titer, mean, (std)	6.77 (1.72)	6.76 (1.62)	0.01 (-1.06 to -1.08)	0.9855
Serum HA IgG, mean, (std)	215395 (103823)	215165 (84099)	229 (-61068 to 61527)	0.994
Serum HA Stalk IgG, mean, (std)	111268 (52693)	99382 (56198)	11887 (-22690 to 46464)	0.4906
Serum NA IgG, median, (25, 75%tile)	5286 (3372-7584)	4806 (2762-7285)		0.5761

Table 1b: Postvaccine systemic antibody titers

	Male (n=18)	Female (n=23)	Difference (95% CI)	p-value
Serum log ₂ HAI titer, mean, (std)	7.21 (1.60)	7.19 (1.42)	0.03 (-0.96 to 1.01)	0.9588
Serum log ₂ NAI titer, mean, (std)	6.77 (1.29)	7.41 (1.27)	-0.65 (-1.47 to 0.18)	0.1215
Serum HA IgG, mean, (std)	381788 (59549)	388913 (77718)	-7125 (-51091 to 36840)	0.7446
Serum HA Stalk IgG, mean, (std)	243375 (42664)	225299 (64462)	18075 (-16444 to 52595)	0.2955
Serum NA IgG, median, (25, 75%tile)	5798 (4832-10595)	6778 (3597-8620)		0.8825

Comparisons for means performed using t-tests and for medians using Wilcoxon rank-sum tests. Abbreviations: HA, hemagglutinin; HAI, HA inhibition; NA, neuraminidase; NAI, NA inhibition; std, standard deviation; %tile, percentile; CI, confidence interval

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RESULTS: CHALLENGE

Table 2a: Prechallenge systemic antibody titers (vaccinated subgroup)

	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
Serum log ₂ HAI titer, mean, (std)	6.79 (2.52)	6.97 (2.04)	-0.18 (-1.15 to 0.79)	0.7085
Serum log ₂ NAI titer, mean, (std)	6.49 (1.24)	7.07 (1.25)	-0.57 (-1.41 to 0.26)	0.1711
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
Serum NA IgG, median, (25, 75%tile)	5967 (4482-9996)	4732 (3178-5789)		0.2087
Serum Total IgG, mean, (std)	1084 (235)	1055 (193)	29 (-117 to 175)	0.6874
Serum Total IgA, mean, (std)	227 (75)	201 (88)	26 (-29 to 81)	0.3438

Table 2c: Postchallenge day 7 systemic antibody titers

	Male (n=34)	Female (n=40)	Difference (95% CI)	p-value
Serum log ₂ HAI titer, median, (25, 75%tile)	7.32 (4.57-9.32)	7.82 (6.07-9.32)		0.2981
Serum log ₂ NAI titer, mean, (std)	5.82 (1.58)	6.02 (1.44)	-0.2 (-0.91 to 0.51)	0.5736
Serum HA IgG, mean, (std)	537946 (159069)	550595 (127723)	-12649 (-80475 to 55176)	0.7106
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
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

Table 2e: Postchallenge day 56 systemic antibody titers

	Male (n=34)	Female (n=40)	Difference (95% CI)	p-value
Serum log ₂ HAI titer, median, (25, 75%tile)	8.32 (6.32-9.32)	7.82 (6.57-9.32)		0.7473
Serum log ₂ NAI titer, median, (25, 75%tile)	7.32 (7.32-9.32)	8.82 (7.32-9.32)		0.1143
Serum HA IgG, median, (25, 75%tile)	368904 (244699-453201)	353219 (273796-431504)		0.8526
Serum HA Stalk IgG, mean, (std)	136716 (63842)	123303 (65468)	13412 (-17152 to 43978)	0.3842
Serum NA IgG, median, (25, 75%tile)	4926 (3290-8203)	5214 (3223-9157)		0.7913
Serum Total IgG, mean, (std)	1123 (240)	1093 (212)	30 (-78 to 139)	0.5776
Serum Total IgA, mean, (std)	238 (92)	199 (84)	39 (-2.75 to 82)	0.0664

Comparisons for means performed using t-tests, for medians using Wilcoxon rank-sum tests, and for proportions using tests of proportions. Abbreviations: HA, hemagglutinin; HAI, HA inhibition; NA, neuraminidase; NAI, NA inhibition; std, standard deviation; %tile, percentile; CI, confidence interval

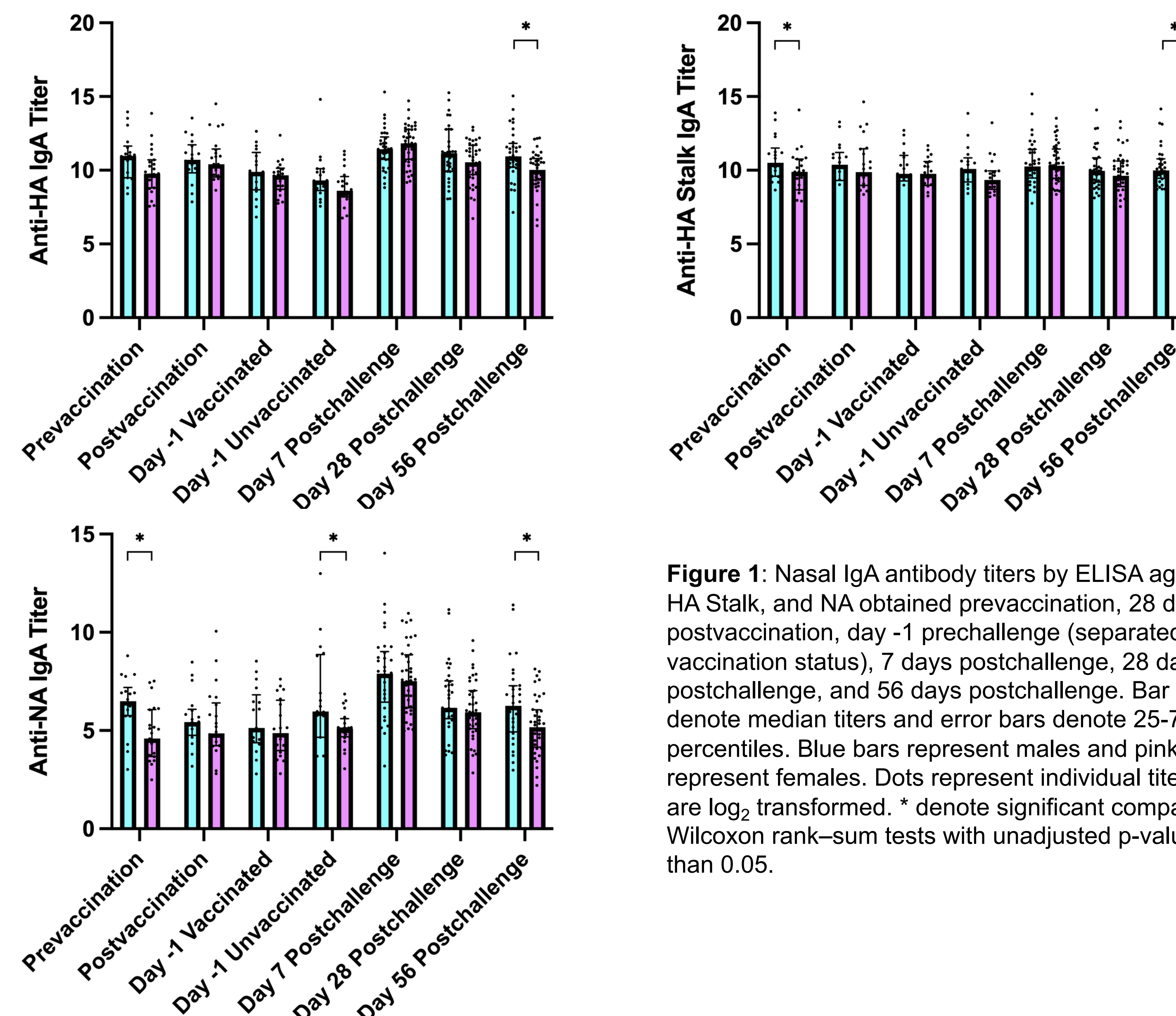


Figure 1: Nasal IgA antibody titers by ELISA against HA, HA Stalk, and NA obtained prevaccination, 28 days postvaccination, day -1 prechallenge (separated by vaccination status), 7 days postchallenge, 28 days postchallenge, and 56 days postchallenge. Bar graphs denote median titers and error bars denote 25-75th percentiles. Blue bars represent males and pink bars represent females. Dots represent individual titers. Titters are log₂ transformed. * denote significant comparisons by Wilcoxon rank-sum tests with unadjusted p-values less than 0.05.

Table 2b: Prechallenge systemic antibody titers (unvaccinated subgroup)

	Male (n=17)	Female (n=20)	Difference (95% CI)	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 2d: Postchallenge day 28 systemic antibody titers

	Male (n=34)	Female (n=40)	Difference (95% CI)	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 outcomes

	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

CONCLUSIONS

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

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