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Immunogenetics of Seasonal Influenza Vaccine Response*

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Abstract

Seasonal influenza causes significant morbidity, mortality, and economic costs. Vaccines against influenza, though both safe and effective, are imperfect. Notably, these vaccines result in significant immune response variability across the population. The mechanism for this variability, in part, appears to lie in the polymorphisms of key immune response genes. Despite the importance of this variability, little in the way of genetic polymorphisms and its association with vaccine immune response to viral vaccines has been performed. Herein, we review and synthesize what is known about the immune response pathway and influenza viral immunity and then present original data from our laboratory on the immunogenetic relationships between HLA, cytokine and cytokine receptor gene polymorphisms and the variations in humoral immune response to inactivated seasonal influenza vaccine. Finally, we propose that a better understanding of vaccine immunogenetics offers insight towards the development of better influenza vaccines.

Keywords

Influenza Vaccines; Active Immunity; Genetic Predisposition to Disease; Polymorphism; Genetic; HLA Antigens; Cytokines; Receptors; Cytokines and Polymorphism; Single Nucleotide

Introduction

Even where annual influenza immunization programs are in place, annual seasonal epidemics and periodic pandemic influenza pandemics continue to be a major cause of high morbidity and mortality worldwide which results in approximately 250,000 to 500,000 deaths per year [1,2]. Still, the best opportunity for reducing the impact of influenza virus infection and subsequent morbidity and mortality in the human population remains influenza immunization [3]. The presence of circulating neutralizing antibody to the major surface glycoproteins hemagglutinin and neuraminidase is sufficient to protect against influenza infection and disease

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[4]. While the importance of cytotoxic T cell responses is less clear, they appear to decrease the severity of infection and significantly lower morbidity and mortality rates [5–7]. Two types of vaccines are currently licensed for prevention of yearly epidemic influenza: trivalent inactivated influenza vaccine (TIV) and live attenuated influenza vaccine (LAIV) [8].

Genetic polymorphisms appear to be important in explaining variations in immune response to influenza. For example, a recent population-based study in Utah identified persons who died as a result of influenza virus infection in the past 100 years; investigators determined their genetic relationships, and suggested evidence for a heritable predisposition to death due to influenza viral infection and genetic susceptibility to severe seasonal and pandemic influenza [9,10]. While a fair amount of information is known about the generation of the immune response to influenza vaccines [11,12], few studies have focused on the immunogenetics of seasonal influenza vaccine immune response. Despite the use of a constant formulation, including the quality and quantity of vaccine antigens, the number of doses administered and the route of administration, the protective immune response to influenza immunization among healthy humans is quite heterogeneous (within a given season) [11,13]. The spectrum of this heterogeneity includes immunologic failure to mount protective neutralizing antibody responses against influenza virus hemagglutinin protein [14]. A better understanding of the optimal host immune response and the influence of host gene polymorphisms is essential for both the development of improved influenza vaccines and for understanding the mechanism for population immune response variability [15].

Influenza Virus and the Immune Response Pathway

The pathway by which protective immune responses to influenza develops is a multi-step process: virus must be “recognized” by receptors in order to cause infection as well as activating toll-like receptors (TLRs) and other sensors, triggering innate immune responses and stimulating antigen presenting, cytokine, chemokine, and cytokine/chemokine receptor genes, in turn leading to immune responses via secretion of cytokines as intercellular messengers to stimulate Th1 and Th2 adaptive immune responses and other immune effector molecules (Figure 1). Of the 10 human TLRs that are central to antiviral innate immunity, TLR3 (which recognizes double-stranded RNA), TLR7 and TLR8 (which both recognize single-stranded RNA), and TLR9 (which recognizes single-stranded RNA) are endosomal and specialize in viral recognition [16]. The replicative intermediate of influenza virus, the double-stranded RNA, is recognized by TLR3 and signals through both the PI3K/Akt signaling pathway and TRIF-dependent pathway, resulting in the activation of INF- β and IFN-inducible genes (IL6, IL8 and RANTES) from macrophages and dendritic cells [16–18]. Recent studies have uncovered a parallel innate signaling pathway mediated by recognition of single-stranded RNA and double-stranded RNA by cytoplasmic RNA helicases, such as retinoic acid inducible gene I (RIG-I) and melanoma differentiation-associated gene 5 (mda5), which contribute to the antiviral state of infected cells and trigger antiviral responses [16,19]. Therefore, single-nucleotide polymorphisms (SNPs) in TLR (specifically TLR3), RIG-1 and other adapter family genes in persons vaccinated against influenza could be important to study given their central role.

In addition to their own direct anti-viral properties, cytokines (and chemokines) and their receptors play a key role in mediating both innate and adaptive immune responses to influenza. For example, the influenza hemagglutinin suppresses transcription of the IL12p35 subunit which leads to downregulation of neutrophil function and Th1-type responses [20]. The influenza nonstructural protein 1 (NS1) has multiple direct effects on host immune responses, suppressing inflammatory cytokine secretion and antigen presentation [21,22]. Additionally, cytokines, such as IL-12, IL-7, IL-23, GM-CSF and others have been used as adjuvants along with inactivated influenza vaccines to enhance resulting immune responses [23,24]. Thus,

polymorphisms in cytokine and cytokine receptor genes affect the functional activity of these molecules, thereby altering the resultant immune responses to influenza infection or vaccination.

The human leukocyte antigen (HLA) gene complex is the most genetically polymorphic region in the human genome and the most important genetic regulator of adaptive immunity. HLA class I and class II molecules are responsible for antigen presentation to CD8+ and CD4+ T helper cells, respectively. Several studies of immune responses to measles, mumps, rubella, vaccinia, and other viral vaccines have demonstrated the influence of HLA allelic variation on immune response [25–29]. The production of influenza neutralizing antibody is under the control of antigen-specific CD4+ helper T cells, which recognize exogenously derived influenza virus antigens in association with HLA class II molecules. However, no correlation between influenza-specific CD4+ T cells and humoral responses has been found [30]. There are exceptions in that HLA class I molecules may present exogenous antigens and class II molecules may present endogenous peptides that have not come from endosomes (i.e. cross-presentation) [31]. Hence, it is important to understand to what degree HLA allelic variations modulate humoral immune responses following seasonal influenza A (H1N1 and H3N2 subtypes) and B vaccines in healthy individuals.

The Importance of HLA Genes on Influenza Vaccine Immunity

Early studies suggested that HLA-Bw35 may influence the early phase of the immune response to influenza A antigens and that HLA-Bw16 was associated with decreased antibody response after influenza A vaccination [32,33]. A study examining the role of HLA antigens and antibody response after live attenuated intranasal influenza A vaccination has linked the HLA-Bw16 allele to a lower influenza-specific antibody response [34]. However, the association between HLA-Bw16 and resistance to infection with live attenuated influenza A virus vaccine could not be confirmed in other studies [33,35].

An increased frequency of HLA-DRB1*0701 and a decreased frequency of HLA-DQB1*0603-9/14 was found in nonresponders to the trivalent influenza virus subunit vaccine when compared with matched responders to the same vaccine [36]. The same study described an increased frequency of the HLA-DQB1*0303 allele, which is part of an HLA-DRB1*07-containing haplotype, among influenza virus vaccine nonresponders [36]. This is significant, because it potentially identifies individuals who may not be protected by existing vaccination strategies [37]. Further, following subunit vaccination, individuals with the HLA-DRB1*0701 allele were found to recognize identical CD4+ T-cell epitopes from influenza A virus hemagglutinin [38]. On the other hand, HLA serogroups, DR3 and DR4, were found to be associated with decreased immune responsiveness to influenza vaccine in patients with type 1 diabetes [39].

Other studies have also identified the importance of class II HLA-DR alleles in seasonal influenza A immune responses [40]. Further investigation of the role of HLA polymorphisms and immune responses to seasonal influenza vaccination is clearly necessary given these early data, and the importance of HLA genes as the primary means by which viral antigens are presented to immune cells.

Associations Between HLA Alleles and Influenza A Vaccine Virus H1-Specific Hemagglutination Inhibition Titer

To assess the spectrum of humoral immune responses among healthy subjects after seasonal influenza A vaccination, we collected serum samples from healthy 18–40 year old subjects (n=184; Caucasian males) who were enrolled from December 2005 to March 2006 at the Naval

Health Research Center (NHRC) in San Diego, CA. Medical records for 156 of the 184 subjects confirmed receipt of seasonal influenza vaccine containing A/H1N1 New Caledonia/20/99, A/H3N2 California/7/2004 and B/Shanghai/361/2002 influenza virus antigens. We were given assurances from the NHRC that the other 28 individuals, although undocumented, were immunized as well, but as a precaution we ran a series of sensitivity analyses excluding these 28 subjects. Results for 156 subjects were similar to those based on all 184 individuals (data not shown). Sera were tested for antibody titers against both hemagglutinin proteins from H1 (A/H1N1 New Caledonia/20/99) and H3 (A/H3N2 Panama/2007/99) serotypes of influenza A viruses by a hemagglutination inhibition (HAI) assay using a starting dilution of 1:10 and 0.65% guinea pig erythrocytes [41]. Consistent with the literature, we considered an HAI antibody titer of 1:40 or more sufficient to inhibit influenza virus infection and indicative of immunity [42,43]. HAI assays against whole influenza A/H1N1 New Caledonia/20/99 virus detected protective post vaccination serum antibody titers of >1:40 (range 1:40 to 1:640) in 167 (90.8%) of 184 volunteers following the 2005–2006 trivalent influenza vaccine, containing H1N1 antigens from this virus. HAI titers to the H3N2 strain of >1:40 (range 1:40 to 1:640) elicited by influenza A/H3N2 Panama/2007/99 virus antigens were detected in 156 (84.8%) of 184 subjects. Importantly, HAI testing revealed that 9.2% (n=17) of subjects were seronegative (HAI titer of 1:10 to 1:20) for influenza (H1N1) virus and 15.2% (n=28) of subjects were seronegative for influenza (H3N2) virus. We found that 2.7% (n=5) of these subjects were seronegative for both H1 and H3 antigens [44].

Given the critical role of HLA molecules in antigen presentation and T cell recognition, host genetic factors logically modulate the serologic response to influenza virus vaccination. For this reason, we examined associations between HLA gene polymorphisms and HAI in these subjects. The median value for H1-antibody titer for the 2005–2006 season was 1:160. The HLA-A locus was significantly associated with variations in influenza H1 post vaccination antibody titer (global p-value 0.007). Specifically, A*1101 (median antibody titer of 1:640; p=0.0001) and A*6801 (median antibody titer of 1:320, p=0.09) alleles were associated with higher median levels of influenza H1 vaccine-induced antibodies. Specific B (global p-value 0.269) and C (global p-value 0.613) alleles suggestive of being positively associated with influenza H1-specific antibodies included HLA-B*3503 (median antibody titer of 1:40; p=0.02), HLA-B*1401 (median antibody titer of 1:60; p=0.06), and HLA-C*0802 (median antibody titer of 1:80; p=0.05); however, these results must be viewed with caution given the global p-values. Global associations between class II loci and influenza H1-specific HAI did not reach statistical significance; however individually, the DRB1*1104 (median antibody titer of 1:320; p=0.04), DRB1*1601 (median antibody titer of 1:320; p=0.02), DQB1*0502 (median antibody titer of 1:320; p=0.03), and DPA*0202 (median antibody titer of 1:320; p=0.06) alleles were marginally associated with higher HAI, whereas DRB1*1303 (median antibody titer of 1:30; p=0.04) was associated with lower HAI antibodies. These data provide further preliminary evidence that HLA class I A genes have important immunogenetic associations with circulating H1-specific antibody titers following seasonal influenza A vaccine.

Associations Between HLA Alleles and Influenza A Vaccine Virus H3-Specific Hemagglutination Inhibition Titer

The HAI antibody responses to trivalent influenza vaccine, containing H3N2 California/7/2004 strain antigens, were also investigated. The median value for the HAI H3-antibody titer was 1:80. We found no associations with any of the HLA-A, B or C alleles and HAI antibody titer. Global statistical tests for association also failed to show statistically significant associations between H3-specific circulating antibody levels and HLA class II alleles, likely due to small sample size and insufficient statistical power to detect associations given the overall lower mean antibody response. However, when examining DRB1 alleles individually, DRB1*0901 (median antibody titer of 1:40; p=0.07) approached statistical significance with lower HAI

antibody levels. Thus, HLA alleles may have limited associations with H3-specific antibody responses to influenza A vaccination, and it is not clear to what extent HLA gene polymorphisms contribute to H3-specific response to influenza vaccine.

Associations Between Cytokine and Cytokine Receptor SNPs and Influenza A Vaccine Virus H1-Specific Hemagglutination Inhibition Titer

We also analyzed the relationship between cytokine and cytokine receptor SNPs and humoral responses in recipients of seasonal influenza vaccine. We genotyped healthy subjects (n=184; 18–40 year old Caucasian males) for a panel of 586 cytokine and cytokine receptor SNPs (monomorphic SNPs or those with call rates less than 80% were excluded) using the Illumina platform. Associations between SNPs and influenza H1-specific antibody titer were examined using analysis of covariance (ANCOVA). Study subjects received trivalent influenza vaccine containing the H1N1 New Caledonia/20/99 strain. Thirty-nine significant associations (range of p-values 0.002 to 0.046) were found between SNPs belonging to cytokine (IL6, IL18, IL12A, IL12B, IFNG) and cytokine receptor (IL1R, IL2RG, IL4R, IL10RB, IL12RB, IFNAR2, TNFRSF1A) genes and variations in HAI antibody levels to influenza H1 antigens. Table 1 shows associations between SNPs in coding and regulatory regions of cytokine and cytokine receptor genes and influenza A virus H1-specific HAI antibody levels. Interestingly, SNPs within three major cytokine and cytokine receptor genes, IL6 (rs1800796; p=0.005), IL12B (rs3212227; p=0.042) and IL1R1 (rs3732131; p=0.046) demonstrated associations with influenza H1-induced antibody responses in an allele dose-related manner. For example, the presence of SNP allele C in the IL12B gene and SNP allele G in the IL1R1 gene resulted in a dose-related decrease of H1-specific HAI titer from 1:160 to 1:80 and from 1:160 to 1:60, respectively. In addition, very high HAI titers (median antibody titer of 1:640; p=0.005) were observed in the presence of minor SNP allele G in the IL6 gene. Importantly, IL-6, IL1b, and TNF have been implicated in immune-mediated lung pathology during severe influenza [45, 46] and death caused by influenza virus A/H5N1 infection [45,47]. The Th1-like TNF- is an important immunomodulator and proinflammatory cytokine that has also been implicated in the pathogenesis and development of many infectious diseases, including influenza [48]. IL-1 is a proinflammatory cytokine known to cause fever in humans in subnanomolar concentrations [49,50]. It has also been demonstrated that human blood plasmacytoid dendritic cells, when stimulated with influenza virus and CD40L *in vitro*, undergo a maturation process characterized by up-regulation of HLA proteins, adhesion and costimulatory molecules and drive a potent Th1-type polarization, which is mediated by the synergistic effect of IL-12 and type I interferon (IFN- α/β) [51]. In contrast, the Th2-like cytokine IL-6, is a potent cofactor of IL-1 in IgM synthesis and of IL-5 in IgA synthesis and play a role in Ig production [52]. We also found associations between several intronic SNPs in both cytokine and cytokine receptor genes and HAI levels; however, lower frequencies for minor alleles were observed for these SNPs (data not shown). Further, a linkage disequilibrium (LD) pattern was observed among seven intronic SNPs located in two LD blocks belonging to the IL18 (rs5744256 and rs1834481) and IL12B (rs2421047, rs3213093, rs3181218, rs3181217, and rs3212219) genes (data not shown) and two SNPs (rs3171425 and rs1058867) in the 3'UTR region of the IL10RB gene. These preliminary data suggest that SNPs present in cytokine and cytokine receptor genes are associated with H1-induced antibody titers, some in an allele-dose related manner, and may influence H1-specific antibody response(s) following influenza vaccination.

Associations Between Cytokine and Cytokine Receptor SNPs and Influenza A Vaccine Virus H3-Specific Hemagglutination Inhibition Titer

Associations between SNPs and influenza A virus H3-specific HAI titers were also assessed in these subjects who received trivalent influenza vaccine containing the H3N2 California/

7/2004 strain. Forty-seven significant associations (range of p-values 0.002 to 0.049) were found between SNPs belonging to cytokine (IL1B, IL6, IL18, IL12B, IFNG, IFNB1) and cytokine receptor (IL1R2, IL1RN, IL2RA, IL10RA, IL10RB, IL12RB2, TNFRSF1B) genes and variations in HAI antibody levels to seasonal influenza H3 antigens. Of these, 13 SNPs in coding and regulatory regions of cytokine and cytokine receptor genes demonstrated associations with influenza A virus H3-specific antibody levels; however, 3 SNPs (rs7873167, rs1364612, and rs1364613) located in the 3' region of the IFNB1 gene showed LD. A heterozygous variant GA for non-synonymous SNPs within the interleukin 12 receptor gene (rs2307153; Asp465Gly; p=0.03) and TNF receptor 2 gene (rs5746026; Lys232Glu; p=0.04) demonstrated associations with a lower H3-specific HAI titer (median antibody titer of 1:40). In contrast, a minor allele T variant of rs12722605 (p=0.03) located in the 3' region of the IL2 receptor gene was associated with a higher H3-specific antibody titer (median antibody titer of 1:240) (Table 2). In addition, we found significant associations between two SNPs in the IL10 receptor gene (rs4252243; p=0.02 and rs4252249; p=0.02) and influenza H3 specific humoral immune responses. Importantly, a SNP in the IL10 promoter region has been shown to confer a significantly decreased risk (p=0.04) of an adverse response to inactivated influenza vaccine [48]. Finally, the presence of minor SNP allele G in the IL6 gene (rs1800796) resulted in an increase of both H1- and H3-specific HAI titers from 1:160 to 1:640 (p=0.005) and from 1:80 to 1:160 (p=0.01), respectively. Thus, we identified significant associations between H3-specific humoral immune responses and polymorphisms of cytokine and cytokine receptor immune response genes.

Discussion

The published investigations regarding the immune pathway and influenza viral infection support the importance of understanding the influence of immune response gene polymorphisms on the heterogeneity of influenza vaccine responses. Our results demonstrate multiple associations between variations in humoral immunity and HLA, cytokine and cytokine receptor gene polymorphisms. These data suggest that these genes are important transcriptional targets during the immune response to influenza virus. The detection of genetic variation in a given population is important for understanding its role in influenza vaccine immune response variation. Jin and Wang reviewed the rationale and value of studying SNP polymorphisms and associations with immune responses – so-called “immunogenetic profiling” [53]. By combining information about the immunogenetic impact of multiple gene family pathways critical to developing immune responses, such immunogenetic studies may help to develop an enhanced mechanistic understanding of the immune response to seasonal influenza vaccines [54].

Identifying associations between variations in vaccine immune responses and polymorphisms of key immune response genes is also essential to developing newer vaccines. This may become particularly important in the development of universal influenza vaccines whereby one might receive only a few doses of influenza vaccine during a lifetime to generate protective immune responses to highly conserved influenza proteins. Knowledge of key immunogenetic associations could allow design of a vaccine that circumvents immunogenetic restrictions. As stated by one investigator, “Just as pharmacogenetics has suggested ways of designing drugs to minimize population variability, understanding mechanisms of immunogenetic variation may lead to new vaccines designed specifically to minimize immunogenetically based vaccine failure” [55]. Thus, understanding and defining associations between key immune response gene polymorphisms and subsequent immune response can aid in designing new vaccines. This understanding may provide general principles for viral vaccines that may guide our understanding of their immunogenicity leading to the rational development of vaccines against other novel influenza viruses by determining the genetic basis for immune response variation.

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References

1. World Health Organization. Influenza. [Accessed March 5, 2008]. www.who.int/mediacentre/factsheet/fs211/en/. Revised March 2003. April 2006
2. Monto AS. Influenza: quantifying morbidity and mortality. *Am J Med* 1987;82(6A):20–5. [PubMed: 3591814]
3. Palese P, Garcia-Sastre A. Influenza vaccines: present and future. *J Clin Invest* 2002;110(1):9–13. [PubMed: 12093881]
4. Brydak LB, Roszkowska-Blaim M, Machala M, Leszczynska B, Sieniawska M. Antibody response to influenza immunization in two consecutive epidemic seasons in patients with renal diseases. *Vaccine* 2000;18:3280–6. [PubMed: 10869773]
5. Belshe RB, Gruber WC, Mendelman PM, Mehta HB, Mahmood K, Reisinger K, et al. Correlates of immune protection induced by live, attenuated, cold-adapted, trivalent, intranasal influenza virus vaccine. *J Infect Dis* 2000;181(3):1133–7. [PubMed: 10720541]
6. Hikono H, Kohlmeier JE, Ely KH, Scott I, Roberts AD, Blackman MA, et al. T-cell memory and recall responses to respiratory virus infections. *Immunol Rev* 2006;211:119–32. [PubMed: 16824122]
7. Flynn KJ, Belz GT, Altman JD, Ahmed R, Woodland DL, Doherty PC. Virus-specific CD8+ T cells in primary and secondary influenza pneumonia. *Immunity* 1998;8(6):683–91. [PubMed: 9655482]
8. Smith NM, Bresee JS, Shay DK, Uyeki TM, Cox NJ, Strikas RA. Prevention and Control of Influenza: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 2006;55(RR10):1–42. [PubMed: 16874296]
9. Mubareka S, Palese P. Human genes and influenza. *J Infect Dis* 2008;197(1):1–3. [PubMed: 18171276]
10. Albright FS, Orlando P, Pavia AT, Jackson GG, Cannon Albright LA. Evidence for a heritable predisposition to death due to influenza. *J Infect Dis* 2008;197(1):18–24. [PubMed: 18171280]
11. Cox RJ, Brokstad KA, Ogra P. Influenza virus: immunity and vaccination strategies. Comparison of the immune response to inactivated and live, attenuated influenza vaccines. *Scand J Immunol* 2004;59(1):1–15. [PubMed: 14723616]
12. McElhaney JE, Xie D, Hager WD, Barry MB, Wang Y, Kleppinger A, et al. T cell responses are better correlates of vaccine protection in the elderly. *J Immunol* 2006;176(10):6333–9. [PubMed: 16670345]
13. Gardner EM, Gonzalez EW, Nogusa S, Murasko DM. Age-related changes in the immune response to influenza vaccination in a racially diverse, healthy elderly population. *Vaccine* 2006;24(10):1609–14. [PubMed: 16260072]
14. Gelder CM, Lambkin R, Hart KW, Fleming D, Williams OM, Bunce M, et al. Associations between human leukocyte antigens and nonresponsiveness to influenza vaccine. *J Infect Dis* 2002;185(1):114–7. [PubMed: 11756990]
15. Poland, GA.; Ovsyannikova, IG.; Jacobson, RM.; Kaslow, RA., editors. Genetic influences on the immune response to vaccines. Oxford: Oxford University Press; 2008. Genetic Susceptibility to Infectious Diseases; p. 27p. 414-29.

16. Meylan E, Tschopp J. Toll-like receptors and RNA helicases: two parallel ways to trigger antiviral responses. *Mol Cell* 2006;22(5):561–9. [PubMed: 16762830]
17. Koyama S, Ishii KJ, Kumar H, et al. Differential role of TLR- and RLR-signaling in the immune responses to influenza A virus infection and vaccination. *J Immunol* 2007;179(7):4711–20. [PubMed: 17878370]
18. Le Goffic R, Pothlichet J, Vitour D, et al. Cutting Edge: Influenza A virus activates TLR3-dependent inflammatory and RIG-I-dependent antiviral responses in human lung epithelial cells. *J Immunol* 2007;178(6):3368–72. [PubMed: 17339430]
19. Berghall H, Siren J, Sarkar D, Julkunen I, Fisher PB, Vainionpaa R, et al. The interferon-inducible RNA helicase, mda-5, is involved in measles virus-induced expression of antiviral cytokines. *Microbes Infect* 2006;8(8):2138–44. [PubMed: 16782388]
20. Noone CM, Lewis EA, Frawely AB, Newman RW, Mahon BP, Mills KH, et al. Novel mechanism of immunosuppression by influenza virus haemagglutinin: selective suppression of interleukin 12p35 transcription in murine bone marrow-derived dendritic cells. *J Gen Virol* 2005;86(Pt 7):1885–90. [PubMed: 15958666]
21. Newby CM, Sabin L, Pekosz A. The RNA binding domain of influenza A virus NS1 protein affects secretion of tumor necrosis factor alpha, interleukin-6, and interferon in primary murine tracheal epithelial cells. *J Virol* 2007;81(17):9469–80. [PubMed: 17596305]
22. Fernandez-Sesma A, Marukian S, Ebersole BJ, Kaminski D, Park MS, Yuen T, et al. Influenza virus evades innate and adaptive immunity via the NS1 protein. *J Virol* 2006;80(13):6295–304. [PubMed: 16775317]
23. Aspinall R, Pido-Lopez J, Imami N, Henson SM, Ngom PT, Morre M, et al. Old rhesus macaques treated with interleukin-7 show increased TREC levels and respond well to influenza vaccination. *Rejuvenation Res* 2007;10(1):5–17. [PubMed: 17378748]
24. Williman J, Lockhart E, Slobbe L, Buchan G, Baird M. The use of Th1 cytokines, IL-12 and IL-23, to modulate the immune response raised to a DNA vaccine delivered by gene gun. *Vaccine* 2006;24(21):4471–4. [PubMed: 16140432]
25. Poland GA, Ovsyannikova IG, Jacobson RM, Vierkant RA, Jacobsen SJ, Pankratz VS, et al. Identification of an association between HLA class II alleles and low antibody levels after measles immunization. *Vaccine* 2001;20(3–4):430–8. [PubMed: 11672906]
26. Jacobson RM, Poland GA, Vierkant RA, Pankratz VS, Schaid DJ, Jacobsen SJ, et al. The association of class I HLA alleles and antibody levels following a single dose of measles vaccine. *Hum Immunol* 2003;64(1):103–9. [PubMed: 12507820]
27. Ovsyannikova IG, Jacobson RM, Dhiman N, Vierkant RA, Pankratz VS, Poland GA. HLA and cytokine receptor gene polymorphisms associated with heterogeneous immune responses to mumps viral vaccine. *Pediatrics* 2008;121(5):e1091–9. [PubMed: 18450852]
28. Ovsyannikova IG, Jacobson RM, Vierkant RA, Jacobsen SJ, Pankratz VS, Poland GA. Human leukocyte antigen class II alleles and rubella-specific humoral and cell-mediated immunity following measles-mumps-rubella-II vaccination. *J Infect Dis* 2005;191(4):515–9. [PubMed: 15655774]
29. Oseroff C, Kos F, Bui HH, Peters B, Pasquetto V, Glenn J, et al. HLA class I-restricted responses to vaccinia recognize a broad array of proteins mainly involved in virulence and viral gene regulation. *Proc Natl Acad Sci USA* 2005;102(39):13980–5. [PubMed: 16172378]
30. Gioia C, Castilletti C, Tempestilli M, Piacentini P, Bordi L, Chiappini R, et al. Cross-subtype immunity against avian influenza in persons recently vaccinated for influenza. *Emerging Infectious Diseases* 2008;14(1):121–8. [PubMed: 18258091]www.cdc.eid
31. German RN, Castellino F, Han R, Ries e Sousa, Romagnoli P, Sadegh-Nasseri S, et al. Processing and presentation of endocytically acquired protein antigens by MHC class II and class I molecules. *Immunol Rev* 1996;151:5–30. [PubMed: 8872483]
32. Cunningham-Rundles S, Brown A, Gross D, Braun D, Hansen JA, Good RA, et al. Association of HLA in immune response to influenza-A immunization. *Transplant Proc* 1979;11:1849–52. [PubMed: 394432]
33. Mackenzie JS, Wetherall JD, Fimmel PJ, Hawkins BR, Dawkins RL. Host factors and susceptibility to influenza A infection: the effect of ABO blood groups and HL-A antigens. *Dev Biol Stand* 1977;39:355–62. [PubMed: 604120]

34. Spencer MJ, Cherry JD, Terasaki PI. HL-A antigens and antibody response after influenza A vaccination. Decreased response associated with HL-A type W16. *N Engl J Med* 1976;294(1):13–6. [PubMed: 1105178]
35. Mackenzie JS, Wetherall JD, Flower RL, Fimmel PJ, Dawkins RL. HLA antigens and the response to influenza A virus. *Vox Sang* 1979;37(4):201–8. [PubMed: 494590]
36. Gelder CM, Lambkin R, Hart KW, Fleming D, Williams OM, Bunce M, et al. Associations between human leukocyte antigens and nonresponsiveness to influenza vaccine. *J Infect Dis* 2002;185:114–7. [PubMed: 11756990]
37. Lambkin R, Novelli P, Oxford J, Gelder C. Human genetics and responses to influenza vaccination: clinical implications. *Am J Pharmacogenomics* 2004;4(5):293–8. [PubMed: 15462607]
38. Gelder C, Davenport M, Barnardo M, Bourne T, Lamb J, Askonas B, et al. Six unrelated HLA-DR-matched adults recognize identical CD4⁺ T cell epitopes from influenza A haemagglutinin that are not simply peptides with high HLA-DR binding affinities. *Int Immunol* 1998;10(2):211–22. [PubMed: 9533449]
39. Ruben FL, Fireman P, LaPorte RE, Drash AL, Uhrin M, Vergona R. Immune responses to killed influenza vaccine in patients with type 1 diabetes: Altered responses associated with HLA-DR3 and DR4. *J Lab Clin Med* 1988;112:595–602. [PubMed: 3263455]
40. Danke NA, Kwok WW. HLA class II-restricted CD4⁺ T cell responses directed against influenza viral antigens postinfluenza vaccination. *J Immunol* 2003;171(6):3163–9. [PubMed: 12960344]
41. World Health Organization Global Influenza Programme. WHO Manual on Animal Influenza Diagnosis and Surveillance. 2008. p. 28. <http://www.who.int/csr/resources/publications/influenza/whocdscsrncs20025.pdf>
42. Hehme N, Engelmann H, Kuenzel W, Neumeier E, Saenger R. Immunogenicity of a monovalent, aluminum-adsorbed influenza whole virus vaccine for pandemic use. *Virus Res* 2004;103(1–2):163–71. [PubMed: 15163505]
43. Michiels B, Philips H, Coenen S, Denekens J, Van Royen P. Serum antibodies against circulating influenza strains among vaccinated and unvaccinated general practitioners during two consecutive years (2002–2003). *Vaccine* 2006;24(16):3145–52. [PubMed: 16490288]
44. Ovsyannikova, IG.; Jacobson, RM.; Haralambieva, IH.; Dhiman, N.; Watson, DA.; Vierkant, RA., et al. Distinct HLA, cytokine, and cytokine-receptor genotypes influence the human antiviral H1- and H3- specific antibody responses to seasonal influenza vaccination. 11th Annual Conference on Vaccine Research; Baltimore, MD. May 5–7, 2008; Abstract S17
45. Kaiser L, Fritz RS, Straus SE, Gubareva L, Hayden FG. Symptom pathogenesis during acute influenza: interleukin-6 and other cytokine responses. *J Med Virol* 2001;64(3):262–8. [PubMed: 11424113]
46. Fritz RS, Hayden FG, Calfee DP, Cass LM, Peng AW, Alford WG, et al. Nasal cytokine and chemokine responses in experimental influenza A virus infection: results of a placebo-controlled trial of intravenous zanamivir treatment. *J Infect Dis* 1999;180(3):586–93. [PubMed: 10438343]
47. La Gruta NL, Kedzierska K, Stambas J, Doherty PC. A question of self-preservation: immunopathology in influenza virus infection. *Immunol Cell Biol* 2007;85(2):85–92. [PubMed: 17213831]
48. Tang YW, Li H, Wu H, Shyr Y, Edwards KM. Host single-nucleotide polymorphisms and altered responses to inactivated influenza vaccine. *J Infect Dis* 2007;196(7):1021–5. [PubMed: 17763324]
49. Dinarello CA. The IL-1 family and inflammatory diseases. *Clin Exp Rheumatol* 2002;5 (Suppl 27):S1–S13. [PubMed: 14989423]
50. Dinarello CA. Infection, fever, and exogenous and endogenous pyrogens: some concepts have changed. *J Endotoxin Res* 2004;10(4):201–22. [PubMed: 15373964]
51. Cella M, Facchetti F, Lanzavecchia A, Colonna M. Plasmacytoid dendritic cells activated by influenza virus and CD40L drive a potent TH1 polarization. *Nat Immunol* 2000;1(4):305–10. [PubMed: 11017101]
52. Kunimoto DY, Nordan RP, Strober W. IL-6 is a potent cofactor of IL-1 in IgM synthesis and of IL-5 in IgA synthesis. *J Immunol* 1989;143(7):2230–5. [PubMed: 2789259]
53. Jin P, Wang E. Polymorphism in clinical immunology - From HLA typing to immunogenetic profiling. *J Transl Med* 2003;1(1):8. [PubMed: 14624696]

54. Poland GA, Ovsyannikova IG, Jacobson RM, Smith DI. Heterogeneity in vaccine immune response: The role of immunogenetics and the emerging field of vaccinomics. *Clin Pharmacol Ther* 2007;82(6):653–64. [PubMed: 17971814]
55. Spielberg SP. Therapeutics and toxicology. *Curr Opin Pediatr* 1998;10:201–2. [PubMed: 9608900]

Figure.

Immune response pathway representing a set of candidate gene classes encompassing both innate and adaptive immune response genes to influenza virus. These genes include: 1) viral binding surface receptor and innate receptor genes (natural killer receptors, Toll-like receptors, retinoic acid-inducible gene I-like receptors, and protein kinase receptors) for influenza virus, which regulate viral recognition and detection of infection, 2) HLA (class I and class II) genes that bind and present naturally processed viral peptides to T cells, 3) cytokines and their receptor genes that serve to shape and control both humoral and cellular immune responses responsible for viral neutralization and clearance, 4) adhesion molecule and chemokines and their receptor genes that serve to regulate the chemotaxis and infiltration of leucocytes into infected tissues, and 5) immune effector molecules (mannose binding lectins, perforin, granzymes) that serve to neutralize virus and mediate cell cytotoxicity.

Associations between SNPs in coding and regulatory regions of cytokine and cytokine receptor genes and influenza virus H1-specific hemagglutination inhibition

SNP ID	Gene (Function)	Geno-type	N	Median HAI antibody titer (IQR) ¹	P-value ²
rs1800796	IL6 (5'near gene)	CC	156	160 (80, 320)	0.005
		CG	27	320 (80, 640)	
		GG	1	640 (640, 640)	
rs3212227	IL12B	AA	123	160 (80, 320)	0.042
	(3'UTR)	AC	50	120 (40, 320)	
		CC	11	80 (40, 320)	
rs4149621	TNFRSF1A	AA	179	160 (80, 320)	0.043
	(5'near gene)	AG	5	80 (40, 80)	
rs3732131	IL1R1	AA	158	160 (80, 320)	0.046
	(3'UTR)	AG	20	80 (80, 160)	
		GG	6	60 (20, 80)	
rs2069861	IL6	GG	160	160 (80, 320)	0.042
	(3'near gene)	GA	23	160 (80, 320)	
		AA	1	640 (640, 640)	
rs3171425	IL10RB	GG	57	160 (80, 320)	0.045
	(3'UTR)	GA	96	160 (40, 320)	
		AA	31	160 (40, 320)	

A-Adenine, G-Guanine, C-Cytosine, T-Thymine, 3'UTR-3' untranslated region.

¹ Genotype-specific median HAI antibody titer and corresponding interquartile range (IQR).

² SNP-specific p-value testing if HAI antibody titers differ across SNP genotypes. SNPs in bold demonstrated an allele dose-related immune response.

Associations between SNPs in coding and regulatory regions of cytokine and cytokine receptor genes and influenza virus H3-specific hemagglutination inhibition

SNP ID	Gene (Function)	type	N	Median HAI antibody titer (IQR)	p-value ²
rs2228150	IL2RA (syn)	GG	175	80 (40, 160)	0.003
		GA	8	160 (120, 320)	
rs4252249	IL10RA (syn)	GG	139	80 (40, 160)	0.023
		GA	41	80 (80, 160)	
rs2307153	IL12RB2 (nonsyn)	AA	3	80 (80, 160)	0.031
		GG	176	80 (40, 160)	
rs315952	IL1RN (syn)	GA	8	40 (20, 60)	0.035
		AA	87	80 (40, 160)	
		AG	78	80 (40, 160)	
		GG	19	160 (40, 160)	
rs5746026	TNFRSF1B (nonsyn)	GG	175	80 (40, 160)	0.047
		GA	9	40 (40, 80)	
rs1800796	IL6 (5' near gene)	CC	156	80 (40, 160)	0.012
		CG	27	80 (80, 160)	
		GG	1	160 (160, 160)	
rs4252243	IL10RA (5' near gene)	GG	139	80 (40, 160)	0.016
		GA	42	80 (80, 160)	
		AA	3	80 (80, 160)	
rs12722605	IL2RA (3' near gene)	AA	127	80 (40, 160)	0.029
		AT	51	80 (40, 160)	
		TT	6	240 (40, 320)	
rs315951	IL1RN (3'UTR)	CC	88	80 (40, 120)	0.037
		CG	77	80 (40, 160)	
		GG	19	160 (40, 160)	
rs1364613	IFNB1 (3' near gene)	AA	142	80 (40, 80)	0.041
		AC	37	80 (40, 160)	
		CC	5	160 (40, 160)	

A-Adenine, G-Guanine, C-Cytosine, T-Thymine, Syn-synonymous, Nonsyn-nonsynonymous, 3'UTR-3' untranslated region.

¹ Genotype-specific median HAI antibody titer and corresponding interquartile range (IQR).

² SNP-specific p-value testing if HAI antibody tiers differ across SNP genotypes.