

Mucosal Immunization and Adjuvants

Hideki Hasegawa, Elly van Reit and Hiroshi Kida

Abstract The goal of the influenza vaccine is to prevent influenza virus infection and control the yearly seasonal epidemic and pandemic. However, the presently available parenteral influenza vaccine induces only systemic humoral immunity, which does not prevent influenza virus infection on the mucosal surface. Secretory IgA antibodies play an important role in preventing natural infection. Moreover, the IgA antibody response mediates cross-protection against variant viruses in animal models. Thus, a mucosal influenza vaccine that induces mucosal immunity would be a powerful tool to protect individuals from the influenza virus. Although the function of the mucosal immune system, especially in the respiratory tract, is not completely understood, there are several studies underway to develop mucosal influenza vaccines. Here, we will review current knowledge concerning the induction of IgA, the role of B-cell production of influenza virus specific IgA antibodies in anti-influenza immunity, and the role of humoral memory responses induced upon vaccination.

Contents

1	Introduction.....	372
2	The Use of Secretory IgA Antibodies for the Prevention of Influenza Virus Infection.....	372
3	The Characteristics of IgA Antibodies.....	373
4	IgA Antibody Production in Mucosal Tissues.....	374
5	Innate Sensing and Mucosal Adjuvants.....	375
6	Mucosal Vaccine Design.....	376
	References.....	378

H. Hasegawa (✉) · E. van Reit
Department of Pathology, National Institute of Infectious Diseases, Toyama 1-23-1,
Shinjuku, Tokyo 162-8640, Japan
e-mail: hasegawa@nih.go.jp

H. Kida
Research Center for Zoonosis Control, Hokkaido University, Sapporo, Japan

1 Introduction

Influenza viruses belong to the *Orthomyxoviridae* family, which is categorized into three genera, namely *influenzavirus A*, *B*, and *C*. *Influenzavirus A* comprise several subtypes based on the unique combination of two surface proteins, hemagglutinin (HA) and neuraminidase (NA). The seasonal influenza A virus infects millions of individuals each year, with the highest risk of complications occurring in young, elderly, and immunocompromised patients. For example, *influenzavirus A* can lead to fatal encephalopathy in infants and pneumonia in the elderly.

In addition, the avian and/or swine *influenzavirus A* has caused a pandemic every few decades by emerging each time as a genetically novel virus. The most recent pandemic in 2009 was caused by the *influenzavirus A* (H1N1) pdm09 of swine origin. Cases of highly pathogenic avian *influenzavirus A* H5N1 and H7N9 infection and fatal pneumonia have been also reported, with many individuals developing acute respiratory distress syndrome (ARDS) (La Gruta et al. 2007).

Vaccination is the most effective method to control both seasonal and newly evolved pandemic strains of the influenza virus. However, currently used parenteral influenza vaccines are only effective against strains that are closely antigenic to the vaccine strains. Thus, the yearly seasonal vaccine contains multiple influenza virus strains, including influenza virus A strains H1N1 and H3N2, and influenza virus B strain. However, there is an urgent need for improved cross-protection because antigenic mismatches between seasonal vaccines and circulating virus strains. It is also difficult to predict the newly evolved strains such as A/H5N1 and A/H7N9. Ideally, a universal influenza vaccine that induces a strong and long-lasting memory response and cross-protects against drifted variants, as well as against several subtypes of the influenza virus, which induce hetero-subtypic immunity, should be developed. While mucosal secretory IgA (S-IgA) antibodies show cross-protection against variant influenza viruses in mouse models, rational design of IgA antibody-inducing vaccines has so far been hampered by a lack of knowledge about local and tissue-specific immune responses and IgA antibody function (Matzinger and Kamala 2011). Consequently, the importance of IgA antibodies in immunity and the mechanisms by which IgA antibody responses are induced and maintained are just beginning to be established (Brandtzaeg 2007). In this review, we discuss the different mechanisms involved in the induction of S-IgA antibodies during influenza virus infection and vaccination and provide insight into how this information could be used to improve vaccine design.

2 The Use of Secretory IgA Antibodies for the Prevention of Influenza Virus Infection

The respiratory mucosal surface is the first line of defense against influenza virus infection. For example, pre-existing S-IgA antibodies on the surface of mucosal epithelial cells can eliminate a pathogen before it infects respiratory epithelial

cells, thereby providing immediate immunity (Renegar et al. 2004) in a process defined as immune exclusion (Stokes et al. 1975). S-IgA antibodies can also disarm viruses within infected secretory epithelial cells and redirect antigens to the lumen after they have entered the lamina propria (Brandtzaeg 2007). All of these responses are non-inflammatory in nature because, unlike IgG antibodies, IgA antibodies do not fix complement and do not activate the inflammatory complement pathway (Yel 2010). Therefore, a strong S-IgA response is critical for prevention of influenza virus infection especially in case of pathogenic strains for their severe clinical outcomes. Although it is difficult to study the functions of S-IgA and serum antibodies independently, mucosal vaccination and influenza virus infection in knockout mice, which lack poly Ig receptor expression and fail to secrete IgA antibodies from the mucosal surface, show that S-IgA antibodies protect against both homologous and heterologous influenza virus strains (Asahi et al. 2002, 2004). Moreover, transfer of S-IgA antibodies from respiratory tract washings from immunized to naïve mice has been shown to protect against challenge with a homologous or drifted strain (Tamura et al. 1991). Several studies in mice also have shown induction of strong homosubtypic, as well as modest heterosubtypic, cross-protective IgA antibodies.

Since the influenza vaccine is generally administered intramuscularly or subcutaneously, S-IgA antibodies are generally not produced in large quantities; however, intranasal and intradermal influenza vaccinations can produce an effective IgA antibody response (Amorij et al. 2010). The most common route for the influenza virus to enter the host is via the respiratory tract. Therefore, intranasal immunization is the most widely explored route of mucosal vaccination against influenza. FluMist[®] (MedImmune, LLC), a live attenuated influenza virus vaccine, is the only nasal vaccine on the market. However, the rational design of S-IgA vaccines has been hampered by a lack of knowledge on the mechanisms by which IgA antibodies are induced (Brandtzaeg 2007).

3 The Characteristics of IgA Antibodies

After IgG, IgA is the second most abundant isotype in the serum; however, approximately 70 % of all antibodies in mucosae are IgA (Macpherson et al. 2008). In the human serum, IgA antibodies are present mostly as monomeric IgA₁ (Yel 2010), while S-IgA antibodies are found as dimeric IgA₂, although tri- and tetrameric molecules also exist. These polymeric IgA antibodies consist of monomeric IgA molecules connected by one or more J (Joining) chains. After binding to the secretory component (SC), the ectodomain of the polymeric Ig receptor (pIgR), the polymeric IgA is secreted as S-IgA antibodies. Since the cross-protective characteristics of nasal IgA antibodies depend on the polymeric nature of IgA, understanding the molecular structure, development, and function of these higher order polymeric IgA antibodies may be important for the rational design of cross-protective vaccines (Taylor and Dimmock 1985; Song et al. 1995; Renegar et al. 1998).

IgA₂ antibodies develop mostly at sites colonized by a wide range of microbiota, including urogenital and distal intestinal tracts. For example, intestinal bacteria instruct dendritic cells (DC) to produce IgA antibodies (Massacand et al. 2008). IgA antibodies are also present in the respiratory tract, which is not populated with many commensals; however, the predominant isotype is IgA₁.

And IgA₁- specific proteases can cleave bonds within human IgA1 molecules, but these specific bonds are only present in IgA molecules in higher primates and not in the mouse (Weiser et al. 2003).

4 IgA Antibody Production in Mucosal Tissues

The inductive site of the mucosal immune system can be divided in two different sites, namely inductive and effector sites. The inductive site includes mucosa-associated lymphatic tissue (MALT), and local and regional draining lymph nodes. Antigens are directly taken from the mucosal surface with an important role of microfold (M) cells and antigen-presenting cells (APC). Antigen-specific antibody producing B-cells can be developed at two different inductive sites, namely extra-follicular and germinal centres (GC), and their induction involves T-cell-dependent or -independent mechanisms (Cerutti 2008). The GC is a specialized environment that supports affinity maturation, which is mediated by activation-induced deaminase (AID) induced somatic hypermutation (Honjo et al. 2004). In addition, AID participates in the production of the preferred antibody class by influencing class switch recombination (CSR) of the heavy chain (Honjo et al. 2004; Zaheen and Martin 2010). Most IgA memory B-cells (BMem) and long-lived IgA plasma cells develop in the GC of peripheral lymphoid organs and that step requires T-cell help via CD40L (CD154) and TGFβ1. T-cell-independent B-cell class switching in the GC might be mediated by interactions with (DC) and stromal cells, including follicular DC (Puga et al. 2010).

At extrafollicular mucosal sites, antibodies can develop both with and without the help of T-cells, with the latter process involving B-cell activating factor (BAFF) and a proliferation-inducing ligand (APRIL) (Chen and Cerutti 2010; Rothaeusler and Baumgarth 2010). Although hypermutation, which is necessary for affinity maturation, is minimal at these sites (MacLennan et al. 2003), antigen-specific antibody producing B-cells at this site can prevent reinfection (Lee et al. 2005) generate an IgG- and IgA-producing BMem subset (Berkowska et al. 2011).

IgA CSR mechanisms have been studied mostly in the gut where they are influenced by specific environmental factors that are mainly created by commensal bacteria and their products (Massacand et al. 2008). While the respiratory tract is not populated with as many commensals as the intestinal tract, it would appear to be protected from the influenza virus by commensals in the gut because CD4 and CD8 T-cell number and the IgA antibody response were reduced in mice treated with an antibiotic. Immunity against the influenza virus was restored by nasal administration of lipopolysaccharides (LPS) but also by rectal administration of

Toll-like receptor (TLR) agonists (e.g., LPS, CpG, polyI:C) (Ichinohe et al. 2011). These findings indicate that signals from distal mucosal regions can support immune priming in the mucosal effector in the respiratory tract. Additional studies are needed to determine whether distal regions contribute to immunity in the respiratory tract.

5 Innate Sensing and Mucosal Adjuvants

Influenza viruses activate pattern recognition receptors belonging to several different families, namely the TLR family, the RIG-I like receptor (RLR) family, the Nod-like receptor (NLR) family (Pang and Iwasaki 2011), and the C-type lectin family (Londrigan et al. 2011). To improve vaccine efficacy, members of the pattern recognition receptor family, which are not activated by influenza virus infection, can be employed. For example, flagellin, which activates TLR5, promotes IgA production and heterosubtypic protection when incorporated into the membrane of influenza virus-like particles (Wang et al. 2010). Similarly, PolyI:PolyC₁₂U, activating TLR3, has been shown to induce heterosubtypic protection through IgA antibodies after administration of an intranasal vaccine (Ichinohe et al. 2007). Moreover, the TLR3 ligand acts synergistically with the TLR-2 ligand zymosan (Ainai et al. 2010).

Recently, several models have demonstrated the importance of TLR signaling in CSR. Early studies have shown only two signals to induce CSR in naïve B-cells, namely, the presentation of antigenic peptides on MHC class II molecules after antigen binding to the B-cell receptor, and the activation of these B-cells via cytokines and the interaction of CD40-CD40L with antigen-specific T-cells. Presently, TLR signaling is thought to involve an important third signal (Bekeredjian-Ding and Jegu 2009). A recent study has shown that MyD88 can induce a protective immune response during primary, but not secondary, influenza virus infection. The IgA level in MyD88^{-/-}-TRIF^{-/-} mice is reduced in the saliva during secondary infection; however, in serum and nasal wash, the level, which was induced in a TLR-independent manner, is similar to those in wild type mice (Seo et al. 2010). Furthermore, TLRs play a role in both T-cell-dependent and -independent IgA responses in mucosal and systemic antibody levels (Bessa et al. 2009).

Some APCs such as plasmacytoid DCs (pDC), Tip-DCs (TNF and Inducible nitric oxygen species (iNOS) Producing DC) and LAPCs have been reported to with the IgA response. In addition, pDCs trigger the anti-influenza response by inducing type 1 interferon, Th1, and cytotoxic responses and enhancing B-cell expansion and differentiation into CD27 high plasmablasts upon TLR7 stimulation (Douagi et al. 2009). pDCs are also necessary for optimal mucosal IgA and serum IgG production after primary, but not secondary, booster influenza vaccination, live attenuated virus vaccination, and inactivated whole virus or split virus vaccination. By contrast, pDCs are not needed to induce an immune response to a live virus (Koyama et al. 2010).

Upon infection of highly pathogenic influenza virus strains, Tip-DCs produce large concentrations of both tumor necrosis factor (TNF) and nitric oxide (NO), which results in tissue damage (Aldridge et al. 2009). However, controlled concentrations of NO induce TGF- β RII expression by B-cells, thereby enabling T-cell dependent IgA class switching. MyD88 signaling downstream of TLR2, 4, and/or 9, which is critical for the induction of iNOS, facilitates T-cell-independent IgA secretion in BAFF- and APRIL-dependent manners (Tezuka et al. 2007).

Late-activator APC (LAPC), a newly identified APC, may play an important role in the immune response several days after influenza virus infection. While the influenza virus activates DCs at approximately 3 days after infection and induces Th1-type responses, the LAPC is activated at approximately 8 days after infection. This results in the induction of a Th2-type response, production of IgA, IgG₁ and IgG₂ antibodies, and downregulation of the anti-viral Th1-type response (Yoo et al. 2010).

6 Mucosal Vaccine Design

Currently used seasonal influenza vaccines are produced based on the prediction of strains that might cause an epidemic in the following season. These vaccines are generally injected intramuscularly or subcutaneously, and are expected to reduce the severity of the disease caused by specific strains that are homologous to the vaccine strain. These vaccines neither induce cross-protection against the heterologous strain nor prevent infection because they largely induce neutralizing IgG antibodies in the serum. On the other hand, influenza vaccines currently under design aim to induce broader cross-protection and are referred to as 'universal influenza vaccines'. The more diverse and broader cross-protective immune response induced by natural infection than by current parenteral vaccinations suggests the induction of several possible immunological effectors that add to cross-protection. Furthermore, individuals of different genders, ages, and genetic backgrounds respond differently to vaccines; thus, they may rely on different immune mediators for their protection (Nayak et al. 2010; McKinstry et al. 2011).

While infection with the natural influenza virus is superior to vaccination in inducing cross-protection against infection by mutated viruses within a particular subtype of the A-type virus in humans (Hoskins et al. 1976, 1979; Couch and Kasel 1983), an inactivated whole virus particle vaccine has been shown to be more immunogenic than split vaccines. This is in agreement with the general view that the effectiveness and safety of vaccines are usually inversely correlated.

Both inactivated whole virion vaccines and split seasonal vaccines can induce protective immune responses against the homologous virus (Greenbaum et al. 2004). While heterosubtypic immunity is not observed after administration of an ether-split vaccine, an inactivated whole virion vaccine can induce broad heterosubtypic immunity (Takada et al. 2003). The stronger immunogenicity of the inactivated whole virion vaccine in mice is likely due to the stimulation of innate

immunity by genomic single-stranded RNA via TLR7 (Diebold et al. 2004; Lund et al. 2004). Since most viruses produce dsRNA during replication (Jacobs and Langland 1996), synthetic dsRNA can likely act as a partial molecular mimic of viral infection.

This has been confirmed in a previous study where intranasal administration of an ether-split vaccine from PR8 (a H1N1 influenza virus strain) and poly(I:C), a TLR3 agonist adjuvant, induced a strong anti-HA IgA and IgG response in nasal washes and serum, respectively, while vaccination without poly(I:C) induced a weak response. In addition, administration of either an A/Beijing (H1N1) or A/Yamagata (H1N1) vaccine, which are antigenically different from A/PR8, in the presence of poly(I:C) conferred complete protection against A/PR8 virus challenge in a mouse model of nasal infection, indicating that intranasal vaccination with poly(I:C) adjuvant confers cross-protection against variant viruses (Ichinohe et al. 2005). Safety issue of the adjuvant is very important. One of dsRNA poly(I:C₁₂U)(Ampligen) which are clinically safe were recently shown to be a potent inducer of innate immune responses (Caskey et al. 2011). This dsRNA, poly(I:C₁₂U)(Ampligen), was investigated as a dsRNA adjuvant for intranasal avian influenza vaccines (Ichinohe et al. 2009).

The stronger immunogenicity produced by the live virus than by the whole inactivated virus may be caused by a mechanism that does not involve stimulation of TLR7 or 3. For example, other receptors, or a different biodistribution or kinetic profile may be involved. For inactivated vaccine the former might be mimicked by using a ligand for TLRs as an adjuvant, the latter two might possibly be mimicked by the use of different carriers for the antigens that will influence kinetics as well as biodistribution (Bachmann and Jennings 2010).

While investigators continue to understand infections caused by the influenza virus, the ultimate goal is to produce a vaccine that can overcome natural infections. This might be achieved by carefully selecting highly conservative domains within influenza membrane proteins and using them as vaccines in combination with several adjuvants which could activate a broad spectrum of tissues and cells.

A recent clinical study reported that intranasal administration of a whole inactivated influenza virus without adjuvant but with a prime-booster induced high levels of nasal neutralizing antibodies that consisted primarily of polymeric IgA (Ainai et al. 2013). It is not clear whether the absence of adjuvant was not important for eliciting the antibody response in these subjects who would have had a cross-protective memory resulting from a history of infections and/or vaccinations.

In conclusion, the induction of IgA antibodies after vaccination can enhance the immune response by introducing a local immune response, which adds to cross-protection, balances pro-inflammatory responses, and increases the diversity of immunological memory. The fact that IgA antibodies alone cannot induce complete protection after heterosubtypic infection may be an advantage because partial protection by IgA antibodies can reduce the viral load and provide time for immune system priming. In this way, innate, humoral, and cellular responses are activated, resulting in the strongest renewal of immunological memory. This ensures the best possible preparedness for the next influenza virus encountered.

Acknowledgments E. van Riet is a recipient of a Postdoctoral Fellowship for Foreign Researchers from the Japan Society for the Promotion of Science. Our research is supported by grants from the Ministry of Health, Labour and Welfare, “H23-Shinkou-Ippan-015”. We also would like to acknowledge all the scientists whose work we could not cite due to restrictions on the number of references.

References

- Ainai A, Ichinohe T et al (2010) Zymosan enhances the mucosal adjuvant activity of poly(I:C) in a nasal influenza vaccine. *J Med Virol* 82(3):476–484
- Ainai A, Tamura S et al (2013) Intranasal vaccination with an inactivated whole influenza virus vaccine induces strong antibody responses in serum and nasal mucus of healthy adults. *Hum Vaccin Immunother* 9(9):1962–1970
- Aldridge JR Jr, Moseley CE et al (2009) TNF/iNOS-producing dendritic cells are the necessary evil of lethal influenza virus infection. *Proc Natl Acad Sci U S A* 106(13):5306–5311
- Amorij JP, Hinrichs W et al (2010) Needle-free influenza vaccination. *Lancet Infect Dis* 10(10):699–711
- Asahi-Ozaki Y, Yoshikawa T et al (2004) Secretory IgA antibodies provide cross-protection against infection with different strains of influenza B virus. *J Med Virol* 74(2):328–335
- Asahi Y, Yoshikawa T et al (2002) Protection against influenza virus infection in polymeric Ig receptor knockout mice immunized intranasally with adjuvant-combined vaccines. *J Immunol* 168(6):2930–2938
- Bachmann MF, Jennings GT (2010) Vaccine delivery: a matter of size, geometry, kinetics and molecular patterns. *Nat Rev Immunol* 10(11):787–796
- Bekeredjian-Ding I, Jegou G (2009) Toll-like receptors—sentinels in the B-cell response. *Immunology* 128(3):311–323
- Berkowska MA, Driessen GJ et al (2011) Human memory B cells originate from three distinct germinal center-dependent and -independent maturation pathways. *Blood* 118(8):2150–2158
- Bessa J, Jegerlehner A et al (2009) Alveolar macrophages and lung dendritic cells sense RNA and drive mucosal IgA responses. *J Immunol* 183(6):3788–3799
- Brandtzaeg P (2007) Induction of secretory immunity and memory at mucosal surfaces. *Vaccine* 25(30):5467–5484
- Caskey M, Lefebvre F et al (2011) Synthetic double-stranded RNA induces innate immune responses similar to a live viral vaccine in humans. *J Exp Med* 208(12):2357–2366
- Cerutti A (2008) The regulation of IgA class switching. *Nat Rev Immunol* 8(6):421–434
- Chen K, Cerutti A (2010) Vaccination strategies to promote mucosal antibody responses. *Immunity* 33(4):479–491
- Couch RB, Kasel JA (1983) Immunity to influenza in man. *Annu Rev Microbiol* 37:529–549
- Diebold SS, Kaisho T et al (2004) Innate antiviral responses by means of TLR7-mediated recognition of single-stranded RNA. *Science* 303(5663):1529–1531
- Douagi I, Gujer C et al (2009) Human B cell responses to TLR ligands are differentially modulated by myeloid and plasmacytoid dendritic cells. *J Immunol* 182(4):1991–2001
- Greenbaum E, Engelhard D et al (2004) Mucosal (SIgA) and serum (IgG) immunologic responses in young adults following intranasal administration of one or two doses of inactivated, trivalent anti-influenza vaccine. *Vaccine* 22(20):2566–2577
- Honjo T, Muramatsu M et al (2004) AID: how does it aid antibody diversity? *Immunity* 20(6):659–668
- Hoskins TW, Davies JR et al (1976) Influenza at Christ’s Hospital: March, 1974. *Lancet* 1(7951):105–108
- Hoskins TW, Davies JR et al (1979) Assessment of inactivated influenza-A vaccine after three outbreaks of influenza A at Christ’s Hospital. *Lancet* 1(8106):33–35

- Ichinohe T, Ainal A et al (2009) PolyI:polyC12U adjuvant-combined intranasal vaccine protects mice against highly pathogenic H5N1 influenza virus variants. *Vaccine* 27(45):6276–6279
- Ichinohe T, Pang IK et al (2011) Microbiota regulates immune defense against respiratory tract influenza A virus infection. *Proc Natl Acad Sci U S A* 108(13):5354–5359
- Ichinohe T, Tamura S et al (2007) Cross-protection against H5N1 influenza virus infection is afforded by intranasal inoculation with seasonal trivalent inactivated influenza vaccine. *J Infect Dis* 196(9):1313–1320
- Ichinohe T, Watanabe I et al (2005) Synthetic double-stranded RNA poly(I:C) combined with mucosal vaccine protects against influenza virus infection. *J Virol* 79(5):2910–2919
- Jacobs BL, Langland JO (1996) When two strands are better than one: the mediators and modulators of the cellular responses to double-stranded RNA. *Virology* 219(2):339–349
- Koyama S, Aoshi T et al (2010) Plasmacytoid dendritic cells delineate immunogenicity of influenza vaccine subtypes. *Sci Transl Med* 2(25):25ra24
- La Gruta NL, Kedzierska K et al (2007) A question of self-preservation: immunopathology in influenza virus infection. *Immunol Cell Biol* 85(2):85–92
- Lee BO, Rangel-Moreno J et al (2005) CD4 T cell-independent antibody response promotes resolution of primary influenza infection and helps to prevent reinfection. *J Immunol* 175(9):5827–5838
- Londrigan SL, Turville SG et al (2011) N-linked glycosylation facilitates sialic acid-independent attachment and entry of influenza A viruses into cells expressing DC-SIGN or L-SIGN. *J Virol* 85(6):2990–3000
- Lund JM, Alexopoulou L et al (2004) Recognition of single-stranded RNA viruses by Toll-like receptor 7. *Proc Natl Acad Sci U S A* 101(15):5598–5603
- MacLennan IC, Toellner KM et al (2003) Extrafollicular antibody responses. *Immunol Rev* 194:8–18
- Macpherson AJ, McCoy KD et al (2008) The immune geography of IgA induction and function. *Mucosal Immunol* 1(1):11–22
- Massacand JC, Kaiser P et al (2008) Intestinal bacteria condition dendritic cells to promote IgA production. *PLoS ONE* 3(7):e2588
- Matzinger P, Kamala T (2011) Tissue-based class control: the other side of tolerance. *Nat Rev Immunol* 11(3):221–230
- McKinstry KK, Strutt TM et al (2011) Hallmarks of CD4 T cell immunity against influenza. *J Intern Med* 269(5):507–518
- Nayak JL, Richards KA et al (2010) Analyses of the specificity of CD4 T cells during the primary immune response to influenza virus reveals dramatic MHC-linked asymmetries in reactivity to individual viral proteins. *Viral Immunol* 23(2):169–180
- Pang IK, Iwasaki A (2011) Inflammasomes as mediators of immunity against influenza virus. *Trends Immunol* 32(1):34–41
- Puga I, Cols M et al (2010) Innate signals in mucosal immunoglobulin class switching. *J Allergy Clin Immunol* 126(5): 889–895, quiz 896–887
- Renegar KB, Jackson GD et al (1998) In vitro comparison of the biologic activities of monoclonal monomeric IgA, polymeric IgA, and secretory IgA. *J Immunol* 160(3):1219–1223
- Renegar KB, Small PA Jr et al (2004) Role of IgA versus IgG in the control of influenza viral infection in the murine respiratory tract. *J Immunol* 173(3):1978–1986
- Rothausler K, Baumgarth N (2010) B-cell fate decisions following influenza virus infection. *Eur J Immunol* 40(2):366–377
- Seo SU, Kwon HJ et al (2010) MyD88 signaling is indispensable for primary influenza A virus infection but dispensable for secondary infection. *J Virol* 84(24):12713–12722
- Song W, Vaerman JP et al (1995) Dimeric and tetrameric IgA are transcytosed equally by the polymeric Ig receptor. *J Immunol* 155(2):715–721
- Stokes CR, Soothill JF et al (1975) Immune exclusion is a function of IgA. *Nature* 255(5511):745–746

- Takada A, Matsushita S et al (2003) Intranasal immunization with formalin-inactivated virus vaccine induces a broad spectrum of heterosubtypic immunity against influenza A virus infection in mice. *Vaccine* 21(23):3212–3218
- Tamura S, Funato H et al (1991) Cross-protection against influenza A virus infection by passively transferred respiratory tract IgA antibodies to different hemagglutinin molecules. *Eur J Immunol* 21(6):1337–1344
- Taylor HP, Dimmock NJ (1985) Mechanism of neutralization of influenza virus by secretory IgA is different from that of monomeric IgA or IgG. *J Exp Med* 161(1):198–209
- Tezuka H, Abe Y et al (2007) Regulation of IgA production by naturally occurring TNF α /iNOS-producing dendritic cells. *Nature* 448(7156):929–933
- Wang BZ, Xu R et al (2010) Intranasal immunization with influenza VLPs incorporating membrane-anchored flagellin induces strong heterosubtypic protection. *PLoS ONE* 5(11):e13972
- Weiser JN, Bae D et al (2003) Antibody-enhanced pneumococcal adherence requires IgA1 protease. *Proc Natl Acad Sci U S A* 100(7):4215–4220
- Yel L (2010) Selective IgA deficiency. *J Clin Immunol* 30(1):10–16
- Yoo JK, Galligan CL et al (2010) Identification of a novel antigen-presenting cell population modulating antiinfluenza type 2 immunity. *J Exp Med* 207(7):1435–1451
- Zaheen A, Martin A (2010) Induction and assessment of class switch recombination in purified murine B cells. *J Vis Exp* (42), pii: 2130. doi:[10.3791/2130](https://doi.org/10.3791/2130)