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Original Antigenic Sin: How Original? How Sinful?

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We review the phenomenon of "original antigenic sin" (OAS) in antibody responses to influenza A virus (IAV) infection or vaccination. OAS refers to the preferential induction of antibodies with higher affinity to priming versus boosting immunogens. We emphasize its mechanistic basis and origins in the basic immunobiology of B-cell responses to myriad immunogens. We tabulate 23 studies in animals and humans to show that the magnitude of OAS depends on many variables. We discuss a number of misconceptions about OAS, examine the extent to which OAS is sinful, and argue that OAS is evolutionary selected and not a deleterious by-product of selection for other features of the immune response. We end by raising questions regarding the mechanistic basis of OAS whose answers could contribute to improving influenza virus vaccines on the road to the holy grail of a "universal" influenza vaccine.

Human influenza A virus (IAV) was first isolated in 1933 at Mill Hill in London, by Wilson Smith (from his very own nose, no less), a 35-yr-old MD/virologist, who pioneered growing IAV in embryonated chicken eggs (Evans 1966). Mass human vaccination with formalin-inactivated egg-grown virus began a decade later. Humanity was in for a few surprises. First, even in the 1930s, it became clear that IAV isolates, unlike other viruses or bacterial pathogens known at the time, varied antigenically over time when tested with antisera derived from infected patients or animals. Second, by the mid-1940s, IAV had varied sufficiently to enable it to completely evade protection following vaccination with a 1934 strain. Third, in the early 1950s, it was found that individuals had a strong tendency to possess higher antibody (Ab) titers against IAV strains they were exposed to as children than to more recently circulating strains. Further, on vaccination with contemporary strains, they showed a greater increase in Ab titers to earlier strains than to the immunizing strain.

This phenomenon, brilliantly dubbed "original antigenic sin" (OAS) by Thomas Francis (Francis 1960), whose laboratory discovered it, has intrigued immunologists and virologists for decades. In no small part, this is because of its very name as well as two elegant immunochemical papers published in the 1960s by Fazekas de St Groth and Rob Webster with the imposing and unforgettable "Disquisitions on Original Antigenic Sin" in their titles (de St Groth and

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Webster 1966a,b) (only 19 other papers on PubMed have "disquisition" in the title). In reading these early studies, it is important to recognize that virtually nothing was known about the molecular nature of either Abs or their viral target molecules, the hemagglutinin (HA) and neuraminidase (NA). It is interesting to consider that 50 years from now influenza researchers will (hopefully) read contemporary papers with the same amazement about our insights despite our deep ignorance of how things "really are."

For reasons whose explication should provide employment for scientific historians and social psychologists for decades to come, the medical establishment grew complacent about human influenza viruses after the 1976 swine flu debacle, in which 45 million Americans were immunized against a potential pandemic strain that never became established in humans (although, ironically, the vaccination may have been effective three decades later, when swine-origin IAV did become pandemic). In the decades since, influenza (which include the influenza B viruses, which are only slightly cross-reactive antigenically with IAVs) has exacted a large toll on humanity because of the limited effectiveness of vaccination, which ranges from 10% to 65% on a yearly basis. Starting in 1997, the sporadic introduction of highly pathogenic avian IAV strains into human populations reinvigorated interest in IAV and critically in developing a "universal" influenza vaccine, which is now a priority of the United States government. According to the relevant National Institute of Allergy and Infectious Diseases web page (see niaid.nih.gov/diseases-conditions/universal-influenza-vaccine-research), the universal vaccine should be at least 75% effective against all human and zoonotic IAV strains for at least 1 yr following immunization in all age groups.

Developing such a vaccine is likely to require better understanding of the protective immune response to IAV, which in turn, will require a better understanding of OAS. The rekindled interest in OAS is reflected in the publication of at least 10 fine reviews in the past few years that deal with OAS in some detail, which the reader is encouraged to peruse (Cobey and Hensley 2017; Monto et al. 2017; Vatti et al. 2017; Henry et al. 2018; Lewnard and Cobey 2018; Devarajan and Swain 2019; Francis et al. 2019; Krammer 2019; Zhang et al. 2019; Zost et al. 2019).

Here, we emphasize immunological mechanistic aspects of the OAS phenomenon that have not been covered in detail by the previous reviews, discuss the evolutionary significance of OAS as a basic feature of the immune response, and identify critical questions for future research.

OAS MISCONCEPTIONS

An important problem in understanding OAS is its inconsistent use by different investigators. Here, we adhere to Francis's original definition: the induction of a more robust immune response to the priming "versus" a boosting immunogen that itself binds poorly, if at all, to Abs induced by the priming immunogen (Francis 1960). In an instructive example, the classic study of responses to the swine flu vaccine in 1978 (A/NJ/8/76; H1N1), individuals on average responded much more vigorously to the vaccine strain than to other H1 strains tested (including A/PR/8/34, H1N1 [PR8], which is fairly closely related to A/NJ/8/76) among all age groups tested (Noble et al. 1977). Based, however, on the clear (but lower) response to early H1 viruses, these investigators concluded that the vaccine induced an OAS response.

In part because of confusion about what constitutes OAS, misconceptions about OAS are common in the literature and among practicing viral immunologists and vaccinologists.

Misconception: OAS Is a Constant Feature of IAV Immunity

OAS is a robust phenomenon that has been observed in many clinical studies and is highly reproducible in animal models under controlled conditions. In Table 1, we summarize results from 16 studies in humans and in Table 2, nine studies in animal models. Altogether, OAS is observed in only about one-half of the studies.

Table 1. Summary of selected studies on the human recall antibody (Ab) responses following influenza virus exposure

Study	Study population	Immunogen	Adjuvant	Measured response	Findings	OAS?
Wrammert et al. 2008	10 subjects	Trivalent seasonal influenza vaccine	No	Plasmablast repertoire analysis and mAb responses by ELISA and functional assays	Influenza seasonal vaccination induced Ab responses highly specific to the immunizing antigen	No OAS
Moody et al. 2011	11 subjects	Trivalent seasonal influenza vaccine or experimental human H3N2 influenza virus exposure	No	Plasmablast repertoire analysis and mAb responses by ELISA and functional assays	Experimental human H3N2 influenza virus exposure was associated with increased frequency of OAS Abs, but less clonal expansion	OAS
Wrammert et al. 2011	9 subjects	Live human pH1N1 influenza virus exposure	No	Plasmablast repertoire analysis and mAb responses by ELISA and functional assays	Influenza pH1N1 infection resulted in increased cross-reactive Abs against epitopes in the HA stem and head	OAS Stem Abs
Li et al. 2012	24 subjects	Monovalent pH1N1 vaccine	No	Plasmablast repertoire analysis and mAb responses by ELISA and functional assays	Influenza pH1N1 vaccination predominantly induced a recall response targeting the HA stem	OAS Stem Abs
Li et al. 2013	54 subjects	Live human pH1N1 influenza virus exposure	No	Ab/mAb responses by ELISA and functional assays	Specificity of influenza pH1N1 Ab responses to cross-reactive epitopes on the HA head is modulated by preexposure history	OAS
Ellebedy et al. 2014	78 subjects	Trivalent seasonal influenza vaccine or monovalent H5N1 vaccine	No	Plasmablast repertoire analysis and Ab responses by ELISA and functional assays	First immunization with an heterologous (H5N1) influenza vaccine induced increased anti-HA stem Ab responses	OAS Stem Abs
O'Donnell et al. 2014	58 subjects	Monovalent pH1N1 vaccine	No	Ab response pre- and postvaccination by ELISA	Prior H1N1 influenza virus exposure did not impair immune response to pH1N1 vaccination	No OAS
Tan et al. 2014	3 subjects	Trivalent seasonal influenza vaccine	No	Plasmablast repertoire analysis and mAb responses by ELISA and functional assays	Influenza vaccination induced to immunizing antigen and historical influenza viruses	OAS mAbs uncommon

Continued

Table 1. *Continued*

Study	Study population	Immunogen	Adjuvant	Measured response	Findings	OAS?
Fonville et al. 2014	294 subjects	Live human H3N2 influenza virus exposure or trivalent seasonal influenza vaccine	No	Longitudinal Ab responses as well as pre- and postvaccination by functional assays	Back-boost Ab responses to historical human H3N2 viruses following influenza virus infection or vaccination	OAS uncommon in individual patients
Cortina-Ceballos et al. 2015	51 subjects	Trivalent seasonal influenza vaccine or monovalent pH1N1 vaccine	No	Plasmablast repertoire analysis and Ab responses by functional assays	Influenza pH1N1 vaccination induced a recall response to conserved epitopes marked by increased *IGHV1-69* usage	No OAS
Andrews et al. 2015a	21 subjects	Trivalent seasonal influenza vaccine or monovalent pH1N1 vaccine	No	Plasmablast repertoire analysis and mAb responses by ELISA and functional assays	First influenza pH1N1 exposure through vaccination induced a recall response targeting the HA stem	OAS observed after first pH1N1 exposure
Rajendran et al. 2017	152 subjects	Trivalent seasonal influenza vaccine	No	Ab response pre- and postvaccination by ELISA and functional assays	Anti-NA Ab responses increased with age and preferentially target influenza virus strains experienced early in life	OAS
Madsen et al. 2019	80 subjects	Monovalent pH1N1 vaccine	AS03 (squalene-based oil-in-water emulsion)	Ab response pre- and postvaccination by ELISA and functional assays	Despite prevaccination differences, all age groups reached similar levels of HA Ab levels	No OAS
Tesini et al. 2019	8 subjects	Live human H3N2 influenza virus exposure	No	Longitudinal memory B cell analysis and Ab responses by ELISA and functional assays	Infection resulted in increased Ab levels to infecting and historical H3 viruses	OAS in subset of individuals
Henry et al. 2019	39 subjects	Trivalent seasonal influenza vaccine or monovalent pH1N1 vaccine	No	Plasmablast repertoire analysis and mAb responses by ELISA and functional assays	Influenza vaccination in elderly subjects induced a recall response to conserved epitopes from memory B cells generated early in life	OAS in elderly
Ranjeva et al. 2019	706 subjects	Trivalent seasonal influenza vaccine	No	Longitudinal Ab responses by functional assays	Ab responses following influenza vaccination or infection shift focus with age to target cross-reactive epitopes	OAS in adults

Table 2. Summary of selected studies on recall responses in animal models following influenza virus exposure

References	Animal model	Immunogen	Adjuvant	Measured response	Findings	OAS?
de St Groth and Webster 1966b	Rabbit	Whole influenza virus vaccine	No	Ab response pre- and postvaccination by functional assays	Heterologous exposure to an antigenically related influenza virus induced a more robust Ab responses to the priming antigen	OAS
Webster 1966	Ferret	Live influenza virus exposure	No	Ab response pre- and post-influenza exposure by functional assays	Heterologous exposure to antigenically related influenza viruses induced a more robust Ab responses to the priming antigen	OAS
Virelizier et al. 1974a	Mouse	Monovalent influenza HA vaccine	No	Ab response pre- and post-influenza immunization by functional assays	Heterologous exposure to an antigenically related influenza virus induced a more robust Ab responses to the priming antigen	OAS
Jones and Ada 1987	Mouse	H2N2 live influenza virus exposure followed by homologous (H2N2) or H1N1 (heterologous) virus exposure	No	Antibody-secreting cells (ASCs) responses by ELISA and functional assays	Heterologous influenza virus exposure induced no increase in the anti-HA Ab responses to the priming antigen	No OAS
Kim et al. 2009	Mouse	Whole-inactivated influenza virus vaccine, live influenza virus exposure or HA-encoding DNA vaccine	No	Ab responses by functional assays	Different prime/boost immunization regimes induced OAS responses with the most profound effect observed following live influenza virus exposure	OAS

Continued

Table 2. *Continued*

References	Animal model	Immunogen	Adjuvant	Measured response	Findings	OAS?
Kim et al. 2012	Mouse	Whole-inactivated influenza virus vaccine or natural influenza virus exposure	*Bordetella pertussis* toxin, CpG or squalene-based oil-in-water nanoemulsion (NE)	Ab responses by functional assays	Use of adjuvants during primary or secondary exposure can mitigate or prevent OAS responses	No OAS
Linderman and Hensley 2016	Mouse	Whole influenza virus vaccine	No	Ab/mAb responses by ELISA and functional assays	Heterologous exposure to an antigenically related influenza virus induced a more robust Ab responses to the priming antigen. OAS Abs are protective	OAS
Li et al. 2013	Ferret	Natural human pH1N1 influenza virus exposure	No	Ab responses by ELISA and functional assays	Specificity of influenza pH1N1 Ab responses to cross-reactive epitopes on the HA head is modulated by preexposure history	OAS
O'Donnell et al. 2014	Ferret	Monovalent pH1N1 vaccine	No	Ab response pre- and postvaccination by ELISA	Prior H1N1 influenza virus exposure did not impair immune response to pH1N1 vaccination	No OAS

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Of particular note is the Fonville et al. (2014) "antibody landscape" study. Serum samples collected yearly for 6 years from 69 individuals were tested in hemagglutination inhibition (HI) assays for Abs that block viral attachment to host cells against 30 epidemic H3N2 viruses collected over a 41-yr period. Twelve of the individuals experienced laboratory-confirmed natural H3N2 infection, whereas 26 others were likely infected based on a large increase in serum Ab titers. There was enormous variability in the duration and induction of OAS Abs between individuals in an age-independent manner. Infection typically induced Abs to both contemporary and older strains, with only few individuals showing a strong OAS effect. At the same time, within several years postinfection, and often within a year, Ab titers to boosting strains typically waned, whereas response to older strains was maintained.

Fonville et al. (2014) also measured hemagglutination inhibiting (HI) Abs against the same panel of H3N2 viruses pre- and postimmunization in more than 100 patients in two vaccine trials with different seasonal vaccines. As with natural infection, there was enormous variation in the induction of OAS, which, on average, was not the predominant response.

In digesting and comparing information from various OAS studies, it is critical to consider the details.

What is the form of immunogen used for priming and boosting? Boosting with whole virus or infectious virus (which will generate whole virus or infected cell fragments with many HA copies) presents multivalent immunogens to B cells that favor activating B cells with Abs that bind to the boosting immunogen with low affinity. Boosting with infectious virus, however, will be modulated, sometimes extremely, by preexisting Abs, and possibly by cellular immune mechanisms as well, that limit viral replication and proportionally decrease the dose of boosting immunogen presented to the immune system. In general, limiting the amount of immunogen greatly exacerbates OAS, as the boosting antigen is sequestered by B cells specific for the priming immunogen. For experimental purposes to mechanistically dissect OAS (and perhaps, eventually, human vaccines), the influence of preexisting antibodies on the boosting immune response can be minimized by DNA/RNA immunization (Kim et al. 2009).

How are Ab responses measured? Anti-HA serum Abs from challenged individuals have been historically measured largely by functional assays—namely, hemagglutination inhibition and in vitro virus neutralization (VN), which are biased by the functional activities of antibodies specific for different HA regions. Abs specific for antigenic sites near the receptor binding site (RBS) typically show higher activities in these assays (i.e., they provide greater inhibition per occupancy) than those further down the HA spike (Angeletti et al. 2017), with Abs against the stem region showing no HI activity and limited VN activity in standard assays.

Such assays will therefore skew analysis toward Abs that bind the globular domain, and particularly Abs that show the highest functional activities, greatly underestimating Abs specific for the HA stem, as well as Abs whose epitopes are not exposed on native HA, rendering them invisible in functional assays. This factor is important in comparing findings from different OAS studies, as some studies, including the classical "Disquisitions" papers (de St Groth and Webster 1966a,b) and the original B-T–cell mechanistic studies of Virelizier et al. (1974a, b), were based on Ab binding assays. Direct Ab binding studies are much to be preferred in dissecting OAS mechanistically because they provide the most direct and comprehensive measure of anti-HA B cell responses. Further, it is becoming clear that Abs with weak or nondetectable HI or VN activities in vitro, including Abs specific for the stem or cryptic epitopes exposed by HA breathing (Yewdell et al. 1993; Watanabe et al. 2019), can exert antiviral activity in vivo via Fc-receptor (FcR)-based innate cellular immunity (DiLillo et al. 2016; He et al. 2016).

Data presentation can be critical in interpreting the magnitude and biological significance of OAS. HI and VN data are typically presented as logarithmic values. All things being equal, a twofold increase of a titer of 1/1000 represents a greater increase in Ab concentration than does a 10-fold increase in a starting

titer of 1/100. As twofold increases in HI and VN assays are often considered nonsignificant based on statistical analyses, this can skew interpretation of immune responses. Using more precise assays for Ab inhibition of attachment and infection in the form of plate-based attachment assays (using viral NA as a self-reporter) (Kosik and Yewdell 2017) and flow-based neutralization assays with fluorescent reporter IAVs (Kosik et al. 2019) would represent a major improvement in analyzing immunity to flu and with adaptations, other viruses as well.

Misconception: OAS Is Limited to Viruses within the Same Subtype

The recent recognition that HA stem-specific and, to a lesser extent, head-specific Abs can cross-react between subtypes within a group (and even between groups) has opened a new horizon in the OAS phenomenon (although there have been reports of unexplained cross-reactive Abs, including H2 and H3 HI+ Abs induced by vaccination with inactivated whole H1 virus [Noble et al. 1977]).

Inter-HA subgroup OAS appears to be of great importance in human "natural" protection against highly pathogenic zoonotic viruses. Gostic et al. (2016) reported that the severity and lethality of avian H5 and H7 virus infections are heavily biased by patient exposure history. Individuals first exposed as children to group 1 HA (H1 or H2) human viruses were preferentially protected against lethality from an avian group 1 HA virus (H5), whereas those initially exposed to a human group 2 HA virus (H3) were protected from an avian group 2 (H7) virus. Lifelong HA/NA subtype-specific susceptibility to severe infection resulting from first exposure(s) to viruses of the opposite subtype also appears to extend to H1N1 and H3N2 viruses (Arevalo et al. 2019; Gostic et al. 2019).

Misconception: OAS Generates Nonfunctional Abs

Many biologists conceive of Ab binding as a quantal event: Either an Ab binds an antigen or it does not. But interactions between substances are always scalar: At some level, every Ab binds every antigen, just with different affinity (or avidity for multivalent ligands). Primary antibodies induced by HA typically have dissociation constants (K_D) in the 1 to 10 nM range, increasing up to 100-fold after multiple rounds of somatic mutation induced by boosting (de St Groth and Webster 1966a,b; Webster 1966; Frank et al. 2015; Angeletti et al. 2019). Ab affinity for cross-reacting antigens will generally be lower than inducing immunogens, but even low-affinity interactions become meaningful at high concentrations of just one of the reactants. The binding of Abs with a $K_D > 0.1$ μM will likely not be detected in ELISA assays because of dissociation during the secondary labeling step. Such Abs will bind 50% of HAs, however, at a concentration of 0.1 μM, which is 15 μg/mL. For comparison's sake, the amount of Ab present in human serum specific for circulating strains is between 0.1 and 1 mg/mL (de St Groth and Webster 1966a), clearly sufficient for binding to "weakly" cross-reacting antigens.

Thus, it is entirely possible that OAS Abs that "do not bind" to challenge antigens based on overinterpreting in vitro assays can show biological activity in vivo. Complicating matters, the relationship between Ab virus in vitro neutralizing activity does not linearly predict in vivo protection or treatment activity. This is obviously true for stem binding Abs but even extends to head-specific Abs that block virus attachment (Mozdzanowska et al. 1997).

The induction of "nonbinding" OAS Abs is clearest at the level of monoclonal Abs (mAbs). This was first described by Gerhard (1978), who characterized mAbs secreted over a 30- to 40 d period by individual B-cell clones present in splenic fragments from irradiated mice that received limiting amounts of B cell from immunized mice (Klinman 1972). Gerhard reported that 70% of B cells stimulated in vitro with a 1946 strain produced mAbs that would not detectably bind (in a plate-based indirect radioimmunoassay) the 1934 strain used to prime the B cells. Importantly, in this splenic fragment system, activation of secondary B cells with priming virus required 100-fold less virus than activation of naive B cells (Braciale et al. 1976). This

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strongly implies the enhanced sensitivity of secondary B cells and accounts for their triggering by low-avidity antigens, underlying the OAS phenomenon.

This pioneering study was revisited by Linderman and Hensley (Linderman et al. 2014), who used a defined drift variant, S12a, that had been selected by sequential neutralization with 12 mouse mAbs to generate a large panel of new mAbs from mice immunized with PR8 and boosted with S12a. S12a differs from PR8 by 13 amino acids distributed among the five antigenic sites present in the H1 globular domain. This represents ~8 yr of typical antigenic drift in human H1 isolates. Using the standard measure of OAS, the HI assay, and also ELISAs to include Abs specific for epitopes distant from the RBS, they found that PR8 priming with S12a boosting recapitulated the classic findings of other animal studies. Boosting with inactivated virus at a dose similar to that typically used for human vaccination (note that immunogen does not scale with organism mass due to the nature of lymph drainage and delivery to lymph nodes) induced secondary Abs specific for the priming immunogen and primary Abs for the boosting immunogen. Importantly, adoptively transferred serum Abs induced by PR8-S12a immunization protected against S12a infectious challenge.

As expected, in ELISAs, the vast majority of PR8-elicited primary mAbs bound PR8 but not S12a, and ~90% of PR8 prime PR8 boosted mAbs were PR8-specific, with the rest cross-reacting strongly with S12a. After PR8 priming and S12a boosting, ~55% of mAbs were of the OAS phenotype in binding PR8 but not S12. Importantly, antigenic mapping of the mAbs, using a large panel of PR8 escape mutants with single amino acid substitutions covering the five major antigenic sites, revealed that the distribution of OAS Abs for binding to different antigenic sites was similar between OAS phenotype mAbs and PR8 specific mAbs, with Sb-site specific Abs dominating. Sequencing of several clonally related Sb-specific mAbs revealed that only a few amino acid substitutions in the heavy chain were required to increase binding to S12a by 20-fold.

Critically, protection experiments revealed that a mAb scored as S12a-"nonbinding" in a standard ELISA can protect mice against S12a infection as effectively as a mAb induced by S12a primary infection. As cautioned above, nonbinding is an operational term, and indeed, using higher concentrations of Abs, it was determined that the nonbinding Ab binds to S12a, but at ~200-fold lower avidity than to PR8 (K_D of 600 nM vs. 2 nM).

This important study hammers home the message that OAS phenotype Abs induced by vaccination can be biologically important on their own, and further, that under many circumstances they will be accompanied by primary Abs induced by the vaccine that will also be protective. At the same time, it is important to consider that there is solid evidence in animal models (Kim et al. 2009), and indeed in man (Gostic et al. 2016, 2019; Arevalo et al. 2019), that priming can weaken protection afforded by subsequent infection/vaccination with other IAVs.

Misconception: OAS Is Inevitable

First, as shown in Table 1, OAS was weak or absent in many studies and, as strikingly shown by Fonville et al. (2014) (the supplemental figures are well worth perusing), varies enormously among vaccinated or infected individuals.

Second, in the second Disquisition paper, Webster and Fazekas showed that increasing the dose of boosting virus in rabbits increased the relative Ab response to the boosting versus the priming virus. Increasing vaccine dose in humans had a similar effect (Webster et al. 1976). OAS is also mitigated in mice by multiple booster immunizations (Kim et al. 2012). This mechanism almost certainly contributes to the common finding that OAS is most extreme when conditions are such that boosting with infectious virus generates an infection that generates a limited amount of virus.

Third, Kim and colleagues found that including an adjuvant (including one similar to that used for human influenza vaccination) during either priming or boosting mice can mitigate

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OAS by increasing Abs to the boosting virus without decreasing the OAS Ab response.

Misconception: OAS Is a Peculiarity of Antiviral Immunity and Is Mechanistically Undefined

OAS has been described for at least four different virus groups: orthomyxoviruses (IAV), flaviviruses (dengue, zika), retroviruses (HIV), and picornaviruses (enterovirus) (Vatti et al. 2017). But the phenomenon is not limited to viruses. Remarkably, the general phenomenon of OAS in Ab responses to related immunogens was first reported in 1910 when Dreyer and Walker (1910) found that boosting rabbits primed with *Escherichia coli* with *Staphylococcus aureus* elicited a robust *E. coli* Ab response. Similar findings with antibacterial Ab responses were reported in several subsequent publications (see Dixon et al. 1954, for references).

Just a year after Davenport et al. (1953), Dixon et al. (1954) reported that after priming rabbits with bovine serum albumin (BSA), boosting with human serum albumin (HSA), which shares 75% sequence homology with BSA, results in an immediate (within 1 d) robust anti-BSA Ab response that is not cross-reactive with HSA. Cross-reactive and HSA-specific Abs begin to appear 6 d after boosting, with specific Abs at one-half the levels of cross-reactive Abs. (Interestingly, Ab responses were measured in absolute values, generally in the range of 100 to 500 μg/mL, or 0.67 to 3.3 μM. Such absolute quantitation of Ab responses, which was standard in the field, was somehow lost in the 1970s, to the detriment of science.)

Extending the OAS phenomenon to the haptens DNP and TNP (bound to a carrier protein), Eisen et al. (1969) showed via serum Abs that TNP can activate DNP-primed B cells that do not respond to TNP priming, and vice versa. Similarly, at the level of single antibody-secreting B cells detected via the Jerne plaque assay (Jerne and Nordin 1963), Deutsch and colleagues showed an extremely robust OAS response to a different hapten pair (Deutsch and Bussard 1972; Deutsch et al. 1973). Using isoelectric focusing to separate abundant oligo/monoclonal serum Abs that bind radiolabeled antigens, Cramer and Braun (1973) showed that vaccination with a heat-killed whole cell group A streptococcal vaccine elicited a strong secondary Ab response to a mutant strain with a distinct cell wall polysaccharide that would only detectably bind the priming polysaccharide.

The nature of OAS Abs at the level of Ab sequences was first characterized by Fish et al. (1989), who studied Ab responses to the Ars hapten in a mouse strain generating Abs encoded by a dominant single Vh gene segment. Immunization with sulfated Ars (Sulf) did elicit Abs with this Vh segement unless B cells were primed by Ars. The affinities of Sulf-elicited OAS Abs were, with few exceptions, 10-fold to >200-fold lower to Sulf versus Ars. This is too weak to activate naive B cells, explaining Sulf's low immunogenicity in primary responses. Notably, Sulf is able to activate only a small fraction of Ars memory cells, likely those with somatic mutations that increase Sulf binding.

To summarize, we know the following.

1. OAS is a universal phenomenon that reflects the basic working of the immune system in mice, rabbits, ferrets, humans, and likely most, if not all, jawed vertebrates (and perhaps jawless vertebrates as well [Altman et al. 2015]).

2. OAS is based on memory B cells being triggered by much lower antigen concentrations than naive B cells. Consequently, memory B cells are triggered by weakly cross-reacting immunogens to produce OAS Abs. This mechanism was first inferred by de St Groth and Webster (1966b) based on the rapid increase in OAS Abs and resistance to γ-irradiation, both typical of memory Ab responses.

3. OAS is exacerbated by boosting with limited amounts of immunogens, because memory B cells sequester the immunogens compromising the activation of naive B cells, despite the latter having higher affinity Abs for the immunogen.

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4. Although OAS Abs have low avidity for their activating immunogen, they still can exert antiviral activity if present at high enough concentrations.

IS OAS A BUG ? A FEATURE? BOTH?

The universality of OAS in B-cell immunity begs the question of its evolution. There is no apparent reason a priori that secondary B cells should be triggered by lower-affinity antigens than primary B cells. The increase in B-cell precursors in creating the memory B-cell pool alone would confer a memory phenotype. For invariant natural immunogens, the high sensitivity of secondary B cells that underlies OAS would seem to provide obvious advantages, as in most cases it would generate a rapid boost in effective Abs to proven dangerous non-self immunogen. A contributing factor to the evolution of hair-triggered-memory B cells may be the presence of Abs that reduce the effective dose of immunogens available for triggering.

If advantages conferred by highly sensitive triggering of memory B cells is the primary selection pressure for OAS, then its negative effects on B cells responses to antigenically variant viruses could be an unavoidable and acceptable cost. But are OAS Abs deleterious? As discussed above, low-avidity Abs can be effective in vivo. If so, their rapid increase as occurs in OAS might confer major evolutionary benefits. OAS may be particularly beneficial for protection against viruses with multiple cocirculating serotypes (e.g., rhinoviruses that were not selected based on contemporary immune escape). It is also important to remember that under most conditions, a typical primary response to the OAS challenge immunogen occurs in the face of the rapid increase in OAS Abs—in other words, a win–win scenario.

At the same time, the recent findings of Gostic et al. (2016) strongly imply that initial exposure to a given IAV incurs a lifelong cost in decreasing protection from severe infection against viruses with HAs from different groups. This is very likely to be based on B-cell responses, as T-cell responses are far more cross-reactive between IAV gene products, including the glycoproteins (although somewhat less cross-reactive than the internal proteins, which are far less variable).

Better accounting for the costs and benefits of OAS will require deeper understanding of basic immunological mechanisms underlying OAS including B-cell signaling, T-cell help, and accessory cell function, as well as carefully measuring the protective value of low-avidity Abs primed by cross-reactive immunogens. The success of cancer immunotherapy after decades of skepticism about the relevance of tumor immunosurveillance provides a shining example of the benefits of systematic and methodical investigation of basic immune mechanisms.

CONCLUSION: WHAT DO WE NEED TO KNOW ABOUT OAS?

A number of critical questions need to be addressed regarding the immunobiology and clinical relevance of OAS.

How do preexisting Abs affect OAS? By definition, OAS occurs in the presence of preexisting Abs. In mice, mAb Fabs and polyclonal Abs can selectively suppress memory responses to their cognate antigenic site, in the latter case, despite the presence of memory B cells derived from the primary responding B cells that produce the Abs (Angeletti et al. 2017). Thus, Abs can reverse the OAS phenomenon (original antigenic suppression), even overcoming the normal advantages of memory versus primary B cells in responding to challenge. Ab-mediated suppression appears to occur in humans, in whom higher Ab titers correlate with lower vaccine responses (Sasaki et al. 2008; Fonville et al. 2014). It also may contribute to the observation that human Ab titers are higher to strains in trivalent vaccines that have varied the most over the years of repeated vaccination (and natural exposure) (Andrews et al. 2015b; Skowronski et al. 2017).

Central to understanding anti-IAV Ab function is a detailed characterization of the composition of Abs in body fluids. Remarkable progress has been made in mass spectrometric–based detection and quantitation of affinity-pu-

rified Abs (Georgiou et al. 2014). In an astonishing study of the anti-IAV Ab repertoire of a single 57-yr-old (to start) patient, Lee et al. (2019) found that over a 5-yr period a single Ab species constituted ~20% of all Abs against a circulating strain, reaching 50% immediately after vaccination. Twenty-four Abs accounted for 65%–85% of the repertoire at any one time. The pauci-clonal nature of the response, despite repeated vaccination, raises many questions, including how common is this limited repertoire among different age groups, how can the immune system be persuaded to recruit new B-cell clones, and, not least, does this enable immune escape and account for antigenic drift?

How do adjuvants modulate OAS? Adjuvants are well known to enhance Ab responses to influenza vaccines resulting from myriad alterations in numerous innate and adaptive cells. Kim et al.'s (2012) findings show that in mice adjuvants can mitigate OAS both at the priming and boosting stages. The priming effect suggests a major role for Th cells in the phenomenon. This can be tested by adoptive transfer experiments with purified B and $CD4^+$ T-cell populations, and possibly $CD4^+$ T cell-clones/lines/TCR transgenic cells. Kim and colleagues observed that although three adjuvants tested overrode OAS in secondary responses, one of the three failed in primary responses, providing another jumping-off point for additional experiments. To relate these findings to humans, it should be possible to compare OAS Ab responses in samples from some of the many clinical trials establishing the efficacy of adjuvanted vaccines. Indeed, Madsen et al. (2019) recently reported that adjuvanted human vaccination failed to induce OAS Abs.

How important are weakly binding Abs in influenza immunity? The protective effect of such Abs is well established in mice (Linderman et al. 2014), but difficult to address in humans. In flavivirus infections, such Abs are well established to be harmful, based on enhancing infection of FcR-bearing cells. In IAV, there is no evidence for this effect, consistent with the limited ability of IAV to replicate in nonrespiratory epithelial cells. Protective effects might be inferred from correlating clinical outcomes on an individual patient basis using Ab landscapes (Fonville et al. 2014). This question is ultimately intimately linked with the larger issue of how anti-HA Abs protect against human IAV, breaking down the relative contributions of blocking viral attachment, fusion, release, and enhancing FcR-based innate cellular antiviral mechanisms.

How important is imprinting in IAV pathogenesis and how can it be modulated? Epidemiological studies clearly show that initial exposure to IAV improves the clinical outcome decades in the future to infection with strains that possess HA from the same group (Gostic et al. 2016, 2019; Arevalo et al. 2019). To what extent is this a positive effect on homotypic immunity versus a negative effect on heterotypic immunity and are there any effects on infection with influenza B viruses or even more distantly related viruses? Is the group-specific nature of imprinting due to priming of HA stem-specific B cells (Tesini et al. 2019)? If so, can it be overcome by immunogens designed to activate only B cells specific for the nonimprinted stem or avoided entirely by including group I and group II immunogens in the first vaccine a child receives (perhaps, immunizing with each immunogen in different sites to avoid competition in individual lymph nodes [Angeletti et al. 2019]).

Why is it so difficult to generate durable Ab responses to drifted viruses in a sizable fraction of the population after vaccination or even infection? Fonville et al. (2014) observed this in a large number of individuals of all ages, pointing to a defect in generating long-lived bone marrow plasma cells, which are the principal source of durable serum Ab responses. This likely is a major factor in IAV vaccine ineffectiveness. Because noninfluenza vaccines, with few exceptions, generate durable responses in a high fraction of individuals over many age groups, it appears that immunological memory interferes with this process. A critical question is the extent to which this is specific for IAV or rather, as seems much more likely, generally occurs with closely related immunogens. This may be the true sin in OAS and is ripe for testing in animal models, which could provide essential insight regarding the underlying mechanism and how it might be overcome in humans.

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