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Brief Reviews

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Original Antigenic Sin: How First Exposure Shapes Lifelong Anti–Influenza Virus Immune Responses

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The term "original antigenic sin" (OAS) was first used in the 1960s to describe how one's first exposure to influenza virus shapes the outcome of subsequent exposures to antigenically related strains. In the decades that have passed, OAS-like responses have been shown to play an integral role in both protection from and susceptibility to infections. OAS may also have an important deterministic role in the differential efficacy of influenza vaccine responses observed for various age cohorts across seasons. In this article, we review how the understanding of OAS has progressed from its initial description and highlight important outstanding questions in need of further study. ***The Journal of Immunology*****, 2019, 202: 335–340.**

Original antigenic sin (OAS) describes the phenomenon whereby the development of immunity against pathogens/Ags is shaped by the first exposure to a related pathogen/Ag.

The original "original antigenic sin"

The term was first coined by Thomas Francis in 1960 (1). In his seminal study, Francis observed that hemagglutination inhibition assay titers were highest against seasonal influenza strains to which specific age cohorts had first been exposed (2). These observations were supported by serum absorption experiments, which confirmed that the vast majority of anti–influenza virus Abs in a population were cross-reactive against the pioneer strain of that age group (3).

Taken together, these data led Francis to postulate that subsequent infections with similar influenza virus strains preferentially boost the Ab response against the original strain (2). Although OAS has often historically been depicted as a problematic response, recent data have demonstrated that, in certain contexts, eliciting OAS may also be beneficial.

The critical role of primary exposure in shaping the composition of the Ab repertoire was not only observed in humans after influenza virus infections; this phenomenon was also observed in animal models and in the context of other infectious agents. For example, additional serum absorption experiments in ferrets infected in succession with three different influenza virus strains demonstrated that nearly all of the host Abs after the infection series were reactive against the first strain, only a fraction of serum Abs could be absorbed by the secondary virus, and fewer yet by the tertiary virus (3). These results could be replicated using sera of human donors who had been vaccinated against various influenza virus strains (3–6) and also using sera from rats that had been serially infected (7). In addition to influenza virus infections, OAS has also been reported in children who were exposed to sequential dengue virus infections (8). In all cases, the manifestation of OAS is fundamentally dependent upon the relatedness of Ags between primary and secondary infections as this phenomenon is not observed in the context of sequential exposure to distantly related (or unrelated) Ags (9).

Recent refinements

Although the hierarchical nature of the Ab response against influenza virus was initially described over half a century ago, there has continued to be substantial interest in developing a more detailed understanding of the influence of OAS on subsequent infections and vaccinations using modern cohorts. To this end, in 2012, a large cross-sectional study of Ab responses against H3N2 viruses that circulated between 1968 and 2008 in southern China clearly reaffirmed the strong correlation between Ab titers and age of encounter to particular influenza virus strains. Individuals reliably had the highest titers of neutralizing Abs against those strains that circulated within the first decade of life and progressively lower titers of Abs against strains that circulated later (10).

Cohort-based studies have undoubtedly provided valuable insight into the population structure of the Ab response as a consequence of OAS. However, a lack of longitudinal data has hindered a detailed understanding of how hierarchical Ab responses develop within a given host over long periods of time. To address this gap, our group obtained serum samples gathered over a 20-y period from 40 individuals enrolled in the Framingham Heart Study and measured changes in their Ab

Michael G. DeGroote Institute for Infectious Disease Research, McMaster Immunology Research Centre, Department of Biochemistry and Biomedical Sciences, McMaster University, Hamilton, Ontario L8S 4K1, Canada

ORCID: 0000-0003-3426-5069 (M.S.M.).

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Address correspondence and reprint requests to Dr. Matthew S. Miller, Michael G. DeGroote Institute for Infectious Disease Research, McMaster Immunology Research Centre, Department of Biochemistry and Biomedical Sciences, McMaster University, Hamilton, ON L8S 4K1, Canada. E-mail address: mmiller@mcmaster.ca

Abbreviations used in this article: bnAb, broadly neutralizing Ab; HA, hemagglutinin; OAS, original antigenic sin.

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titers against H1, H2, and H3 viruses in ∼5-y intervals (11). We observed that exposure to strains encountered later in life "back-boosted" the Ab response to strains of the same subtype encountered earlier in life. Thus, the strains of a given subtype encountered earliest in life experienced the greatest number of back-boosting events, leading them to be consistently maintained at the highest Ab titers (Fig. 1A) (11). The way in which infections and vaccinations affect Ab titers to strains encountered earlier in life has also been elegantly studied and described by the Smith Laboratory (12). Importantly, their work using "Ab landscapes" demonstrated that antigenically advanced viruses were capable of both boosting Abs against antecedent strains and inducing Abs against antigenically advanced strains. Recent high-throughput analyses of plasmablast repertories induced by vaccination have also supported the supposition that many Abs are derived from memory B cells specific for previously encountered strains (13–15).

Effects of OAS on protection from infection

Clearly, one of the most profound implications of OAS is the influence it exerts on an individual's or cohort's relative protection against infections. A well-known recent example of a situation in which OAS conferred protection later in life occurred during the 2009 H1N1 "Swine Flu" pandemic. During this pandemic, older individuals who had been exposed to the 1918 H1N1 "Spanish Flu" (and closely related strains) experienced substantially lower relative mortality rates than those usually observed for an advanced age group (16–19). Exposure to earlier H1 viruses has also been proposed as an explanation for the relatively low mortality observed by older individuals during the 1918 Spanish Flu pandemic (20–23).

In recent years, there has been a substantial renewal of interest in understanding how and when OAS and pre-existing immunity affect incidence and mortality of influenza virus infections (24). A detailed examination of age-specific mortality during the 1957 H2N2 "Asian Flu" pandemic led Ma and colleagues to conclude that "antigenic imprinting," or OAS, was the most parsimonious explanation for the observed age-related trends in mortality (25). In 2016, Gostic et al. (26) demonstrated that individuals imprinted with H1N1 viruses in childhood were protected against avian-origin H5N1 infection later in life, whereas those imprinted with H3N2 viruses in childhood received similar protection against avian-origin H7N9 infections. OAS-like responses have also been shown to affect the boosting of memory B cell responses that produce broadly neutralizing Abs (bnAbs) specific to the hemagglutinin (HA) stalk/stem domain (27). Several studies have demonstrated that early-life exposure to seasonal H1N1 viruses resulted in boosting of bnAbs against group 1 HAs upon exposure to the antigenically distinct 2009 pandemic H1N1 virus or subsequent to vaccination against H5N1 (11, 15, 28–31). Similarly, vaccination of healthy adults (who would presumably have been exposed early in life to H3N2 viruses) with an H7N9 vaccine induced bnAbs against group 2 HAs (32). Titers of these Abs, which can be measured by ELISA or microneutralization assay, have been shown to correlate with protection in animal models (31–34).

A hallmark of OAS-like Ab responses is that these Abs typically bind strongly to the founding strain of virus against which they were initially elicited but bind poorly to drifted variants. Despite this, cross-reactive OAS Abs were found to bind to the same general regions of HA as those generated during a primary exposure. These Abs were often clonally related and remained capable of protecting against challenge from antigenically drifted strains in vivo (35). Thus, OAS responses can offer protective benefits during secondary exposures to drifted viruses as well.

Effects of OAS on susceptibility to infection

Although the effects of OAS can in certain cases be beneficial by enhancing an individual's or cohort's relative protection against future infections when the strains are antigenically related, it follows that this phenomenon can be problematic when strains are distantly related, leading to increased susceptibility to later infections. Using single year of age data, our group reanalyzed the infamous "W-shaped" mortality curve caused by the 1918/19 H1N1 Spanish Flu pandemic. We reported that those born in 1890, the year of the H3Nx "Russian Flu" pandemic, experienced an unusual peak in mortality (23). This phenomenon recurred when we examined the single-year mortality data from the 2009 H1N1 Swine Flu pandemic and found an unexpected peak in mortality for those born during the 1957 H2N2 Asian Flu pandemic (36). This led us to hypothesize that early-life imprinting by pandemic strains of influenza virus might increase susceptibility during subsequent pandemics caused by antigenically distinct strains.

OAS-like responses were also problematic during the 2013–2014 influenza season, when H1N1 viruses acquired a mutation in an HA epitope that was the primary target of the Ab response mounted by middle-aged individuals. The cohort generated a focused Ab response against this epitope during early life exposure to seasonal H1N1 viruses that circulated in the 1970s. As reported by the Hensley laboratory, this epitope was conserved in the original 2009 H1N1 pandemic strain. However, the drifted H1N1 strain that emerged in 2013–2014 contained a mutation in this region of HA that resulted in poor Ab binding and subsequently unusually high mortality for middle-aged individuals (37, 38).

Impact of OAS on vaccine effectiveness

The effectiveness of influenza virus vaccines is known to vary by season, by age group, and by vaccination history. However, understanding the contribution of each of these variables is complicated by the diverse methods used to arrive at efficacy estimates. These complexities have been thoroughly discussed in an excellent recent review by Lewnard and Cobey (39). We therefore limit our discussion in this article to only those studies most directly related to the impact of OAS on vaccine efficacy.

In 1999, Smith and colleagues first proposed the "antigenic distance hypothesis," which postulated that differences in vaccine efficacy were due to the relative antigenic relatedness of the past vaccine strains, current vaccine strains, and the circulating epidemic strains (40). When the antigenic distance between all three strains is close, efficacy is predicted to be high. However, if the antigenic distance between past and current vaccine strains is close but more distant from the circulating epidemic strain, then efficacy suffers. Importantly,

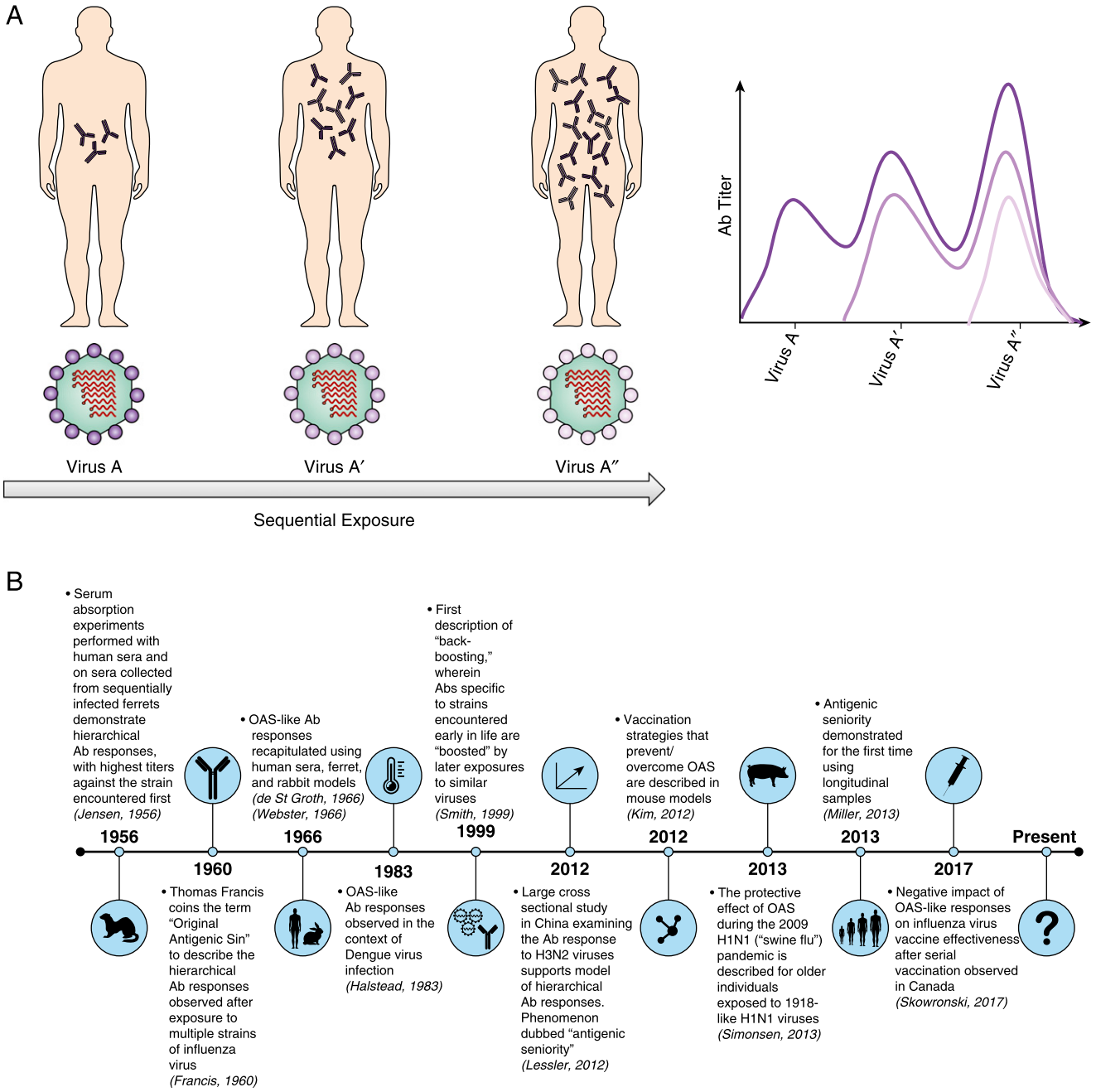

FIGURE 1. OAS: key findings and mechanistic insights. (**A**) An individual's first exposure to influenza virus can shape the humoral immune response to subsequent infection and vaccination, a phenomenon first described in 1960 by Thomas Francis. Subsequent studies have demonstrated that Abs specific to strains encountered earlier in life are often back-boosted by later exposures to viruses with related antigenicity. A hierarchical Ab response is then generated and maintained because the strains encountered earliest are back-boosted the greatest number of times. Back-boosted Abs may be less effective at neutralization of circulating strains of virus whose antigenicity has changed as a result of antigenic drift. In contrast, when directed against conserved, protective epitopes (such as the HA stalk/stem domain), back-boosting may be essential to achieving protection. (**B**) Although several important advances related to the mechanistic basis and consequences of OAS have been made since its initial description over 60 y ago, many important questions remain unanswered.

this model does not rely exclusively upon the "original" Ag but can be influenced by any previous exposure.

The "negative interference" predicted by the antigenic distance hypothesis was proposed as an explanation for the unusual observation that vaccine efficacy against H3N2 was apparently reduced in repeat vaccinees when compared with first-time vaccinees during the 2012–2013 influenza season in Canada (41). Recent studies performed by repeat vaccination of ferrets with H3 strains confirmed the occurrence of OAS responses but observed a progressive increase in Ab cross-reactivity and avidity upon repeated exposures (42). During the 2014–2015 influenza season, the circulating H3N2 strain acquired a glycosylation site that was not present in the egg-grown vaccine because of mutations acquired during vaccine production. This led to an extremely low vaccine effectiveness in that season (43). Adults who would have been previously exposed to strains lacking the glycosylation site early in their childhood mounted particularly strong responses against the vaccine strain, which would likely have offered little protection against infection (44, 45).

Finally, a striking age-associated drop in vaccine efficacy was also observed for older and middle-aged adults during the H1N1-dominated 2015–2016 influenza season. During that season, the circulating H1N1 strains also acquired a new glycosylation site (39). Skowronski et al. (46) reported that efficacy was particularly low in repeat vaccinees. These age-associated reductions in vaccine efficacy were consistent with OAS-like responses (47).

Mechanistic studies of OAS

Although the hierarchical Ab responses that typify OAS have been reported consistently in the literature since Francis' initial description of the phenomenon, the mechanisms that govern OAS-like responses have remained elusive and controversial (Fig. 1B). One of the greatest sources of controversy has stemmed from the inconsistency with which OAS responses are induced by different types of influenza virus exposures (i.e., infections versus vaccinations). This issue was explored directly in a 2009 paper by the Jacob group (48). OAS was observed in mice sequentially vaccinated with HA-encoding DNA vaccines as well as in mice that were sequentially infected. However, OAS was not observed in mice sequentially vaccinated with formalin-inactivated viruses. In the infected group, replication of the secondary virus was limited because of immunity elicited by the primary infection. Therefore, differences in the amount of available Ag during the secondary infection might explain the lower Ab titers elicited against that virus. The authors proposed that OAS might occur because of competition between naive and memory B cells for common epitopes, with memory responses dominating because of higher cell frequency and/or lower activation threshold (48). In a later study, the same group showed that OAS could be prevented by the administration of adjuvants with the first or second immunization or by repeated boosting with the secondary immunogen (49). Together, these findings are also consistent with the notion that the magnitude of the immune response elicited by a given influenza virus exposure is a critical determinant of OAS-like responses.

In line with this hypothesis, our group found that severe infections, such as those caused by pandemic strains, might be capable of "reprogramming" the hierarchical Ab response caused by earlier imprinting with less virulent strains of the virus. For example, early serological studies showed that individuals born between ∼1863 and 1890 (the year of the H3Nx Russian Flu pandemic) all had high titers of Abs against the virus that caused the 1968 H3N2 "Hong Kong Flu" and were roughly equally protected from mortality (50). This suggests that exposure to the 1890 H3Nx pandemic strain was able to "override" the imprint of earlier seasonal strains to which those born decades before the 1890 pandemic (i.e., from 1863 onwards) would have been exposed.

In 2015, the Wilson Laboratory performed a clonal analysis on Abs isolated from plasmablasts of individuals who received the influenza virus vaccine over two consecutive seasons. The authors reported that roughly half of the clones analyzed were activated in both seasons. This was almost certainly an underestimate because of the methodology employed to perform the analysis. However, the study provided important confirmation that Ab responses against influenza virus in previously exposed individuals are strongly biased by contributions from the memory compartment.

Using mathematical modeling, Ndifon recently proposed that OAS may be explained by the activity of regulatory T cells whose elicitation is proportional to magnitude of the immune response stimulated during a given exposure (51). The model suggested that regulatory T cells activated by the original Ag reduce the amount of Ag loaded by dendritic cells upon exposure to the second Ag, which consequently results in less pronounced activation of naive lymphocytes. Another modeling study has demonstrated that "epitope masking" by preexisting Abs may explain many of the features of OAS (52). Both hypotheses require further empirical validation.

Unanswered questions

The understanding of OAS has progressed substantially since its initial description in 1960, yet many questions remain unanswered. Among the most poorly understood aspects of OAS is the extent to which different types of exposures (e.g., natural infection, inactivated vaccine, live-attenuated vaccine, DNA vaccine, etc.) are capable of priming and/or eliciting OAS-like responses. Similarly, whether certain types of exposures are capable of overriding or reprogramming the preexisting hierarchical Ab response, and if so, in what context, requires further elucidation. These issues have profound implications when considering the design of influenza virus vaccines, wherein OAS responses might be desirable in certain cases (e.g., when targeting memory responses to conserved epitopes) and unwanted in others (e.g., when targeting novel neutralizing epitopes on drifted virus strains).

The vast majority of OAS studies have been focused on B cell/Ab responses against HA. As a result, the extent to which other cell types (e.g., T cells) and viral Ags might be subject

Table I. Definitions of terminology used to describe hierarchical nature of Ab response against influenza virus

Terminology	Definition
OAS	Coined by Thomas Francis to describe hierarchical Ab response observed after sequential exposure to antigenically related influenza virus strains. Often associated with negative outcomes because of connotation with sin, although it is not clear that this was Francis' intent.
Antigenic imprinting	Used to describe the observation that one's first exposure to influenza virus leaves an immunological "imprint" that shapes the outcome of subsequent exposures. Tends to encompass all factors that affect the adaptive immune response (i.e., not only B cell/Ab response). Agnostic as to outcome (positive or negative) of the imprinting.
Back-boosting	Refers specifically to the ability of secondary exposures to influenza virus to boost titers of Abs against previously encountered strains. A mechanistic explanation to explain hierarchical Ab responses against influenza virus.
Negative interference	Describes the hypothesis that Abs specific to the original/primary strain of influenza virus "interfere" with the induction of equal Ab responses against subsequently encountered strains.
Antigenic seniority	An agnostic variation on OAS that describes the hierarchical nature of the Ab response against influenza virus, without connotation of negative outcomes often associated with sin.

to OAS remains largely uncertain. Recent work from the Krammer Laboratory has provided compelling evidence for OAS-like responses against neuraminidase (53). Meanwhile, many of the dominant epitopes recognized by T cells are derived from the more conserved internal proteins of influenza virus. How T cell specificity for internal viral proteins relative to viral surface glycoproteins influences the likelihood of developing OAS-like responses and what the consequences of such responses might be on the outcome of infection or efficacy of vaccination also require further study.

Conclusions

Despite the persistent connotation of "sin" as a negative attribute, it is clear that OAS-like responses are neither inherently "good" nor "bad." The desirability of OAS responses is instead context dependent. A plethora of terminology has emerged to describe OAS-like phenomena, all of which vary subtly in the presumptive consequence (positive, negative, agnostic) of these responses (Table I). Selective elicitation of OAS may be beneficial for the induction of broad immunity against conserved epitopes for which there is pre-existing immunity, such as the HA stalk/stem domain. However, it seems clear that OAS can also be detrimental when the boosting of memory responses to conserved, but nonprotective, epitopes comes at the expense of generating new responses against protective, but antigenically drifted, epitopes.

Many have questioned the teleological basis of OAS-like responses in global protection against pathogens. Although answers to questions such as these in the context of evolution are inherently speculative, it at least seems clear that the profound selective benefits offered by the development of immunological memory outweigh the potentially negative consequences associated with favoring the memory response in the context of pathogens whose antigenicity can rapidly evolve.

Recently, there has been a major expansion in research and funding dedicated to the development of better and more broadly protective influenza virus vaccines. This, in turn, has catalyzed substantial renewed interest in defining the mechanisms governing OAS and the influence that pre-existing immunity exerts on subsequent vaccine responses. Although many specific instances wherein OAS-like responses have been observed, both experimentally and during natural influenza virus epidemics, have been studied in great detail, a holistic and predictive model of the situations in which OAS responses occur is far from being realized. To accomplish this goal, considerable effort will need to be directed to understanding the complex interactions between Abs, cell types, and Ags that ultimately determine response outcomes.

Disclosures

The authors have no financial conflicts of interest.

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