Original antigenic sin

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Fate-mapping antibodies to study sinful immune dynamics

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Antibody dynamics resulting from sequential immunization are complex, limiting the study of concepts such as 'original antigenic sin'. Here, molecular fate-mapping defines an 'addiction' of boosted antibodies to primary clones, and OAS-like suppression of new clones, to a degree inversely related to boosting antigenic distance.

Booster immunizations are a hallmark of most vaccination schedules, including against highly mutative viruses such as influenza and SARS-CoV-2. These pathogens evade immunity via rapid antigenic evolution at key protective epitopes; thus, in addition to elevating or 'refreshing' levels of pre-existing protective immune mediators (such as specific antibodies and T cells), boosters also aim to 'update' or diversify the adaptive immune repertoire against novel strains, to broaden protection. However, despite the established utility of this approach, concerns exist regarding the potential for 'original antigenic sin' (OAS)[1], a phenomenon wherein primary antibody responses may interfere with the quality and magnitude of secondary responses, reducing the overall efficacy of booster immunizations and/or predisposing to secondary infection. In this issue of *Nature*, Schiepers et al.[2] develop a molecular fate-mapping 'K-tag' mouse model to deconvolute this phenomenon, specifically by unravelling serum OAS-like antibody dynamics in mice following sequential exposure to homologous and drifted viral antigens.

The concept of OAS was first proposed by Thomas Francis Jr in the mid-1950s to explain particular patterns of influenza-reactive antibody titers among cohorts of different ages[1]. Specifically, Francis noted that antibodies specific for influenza 'families' encountered first during childhood characterize individuals throughout their life, and are successively boosted by subsequent exposure to drifted variants. Today, immunologists consider this to fit within the scope of 'antigenic imprinting', a broader term that accounts for childhood-derived memory biases irrespective of implication (such as biases that could either modulate or not modulate future de novo antibody induction, and/or lead to either protection against or susceptibility to future infection)[3]. To date, there have been several reports describing the beneficial effects of imprinted memory responses to influenza (see, for example, refs. 3–5) and, more recently, SARS-CoV-2[6]. However, Francis also posited that this early-life bias could negatively affect novel antibody responses to drifted variants[1]. Although examples of OAS-like immune bias have been observed[7], this 'sinful' aspect of imprinting has been difficult to study under experimental conditions. In part, this has been due to technical limitations in discriminating antibodies derived from initial versus subsequent antigen exposures, independent of other attributes such as specificity, isotype or affinity maturation.

Recently, advancements in mouse genomics such as the development of inducible Cre-*lox* systems have helped lift this barrier, allowing researchers to investigate the contribution of primary versus secondary antibody responses following variant viral antigen exposure. In this vein, Schiepers et al.[2] modified the mouse kappa antibody light chain gene to encode a Cre-excisable Flag tag, with a Strep tag further downstream. Without intervention, these $Igk^{Tag/Tag}$ mice produce Flag-tagged antibodies. However, when they are bred with germinal center (GC) B cell-restricted tamoxifen-inducible Cre mice, immunized or infected, and given tamoxifen over a restricted timeframe (on days 4, 8 and 12), the Flag insert is removed and Strep-tagged antibodies are produced instead (Fig. 1a). In this way, cellular progeny and antibodies derived from the 'primary cohort' of GC B cells are identifiable as $Strep^+$. Subsequently, upon cessation of tamoxifen, B cells activated as part of any secondary or tertiary immunization are instead identifiable as $Flag^+$. Using this approach, the authors demonstrate that, in the context of homologous immunizations (that is, repeated immunization with the same antigen), de novo boost-derived serological antibody responses are inhibited, concurrent with an 'addiction' to prime-derived clones. This suppression of de novo responses approached a substantial magnitude of 55-fold in the context of homologous wild-type SARS-CoV-2 mRNA vaccination (Fig. 1b). In contrast, boosting with immunogens of sufficient antigenic distance, such as increasingly drifted influenza A hemagglutinins (HAs) or SARS-CoV-2 variant spike mRNAs, allowed more room for both new (boost-derived) and old (prime-derived) antibodies; de novo suppression was mitigated to <4-fold (Fig. 1b,c). Finally, the authors used deep mutational scanning to classify epitopes targeted by primary ($Strep^+$) and secondary ($Flag^+$) antibodies following heterologous prime boost (sequential SARS-CoV-2 spike wild-type and BA.1 mRNA), finding that primary cohort antibodies tended to target conserved regions of the glycoprotein, while de novo secondary $Flag^+$ antibodies targeted BA.1-specific antigenic sites.

Concerns about the impact of OAS are founded on the idea that pre-existing immunity elicited by initial exposure to one antigenic variant may suppress de novo responses to new epitopes on drifted variants of the same molecule. With regard to influenza, it is estimated that individuals experience 1–4 immunogenic H3N2 infections per decade, with higher rates in childhood[8]. Although substantial, this is almost certainly an underestimate of total influenza infections, in that H1N1 and influenza B were not assessed. Furthermore, annual vaccination against circulating strains is recommended for individuals aged six months or older in many countries. In aggregate, human exposure to rapidly evolving influenza antigens such as HA and neuraminidase begins to stimulate the immune system early in life and continues throughout. The consequence of this is evidenced by a recent observation that 95% of pre-existing B cells reactive to an infecting

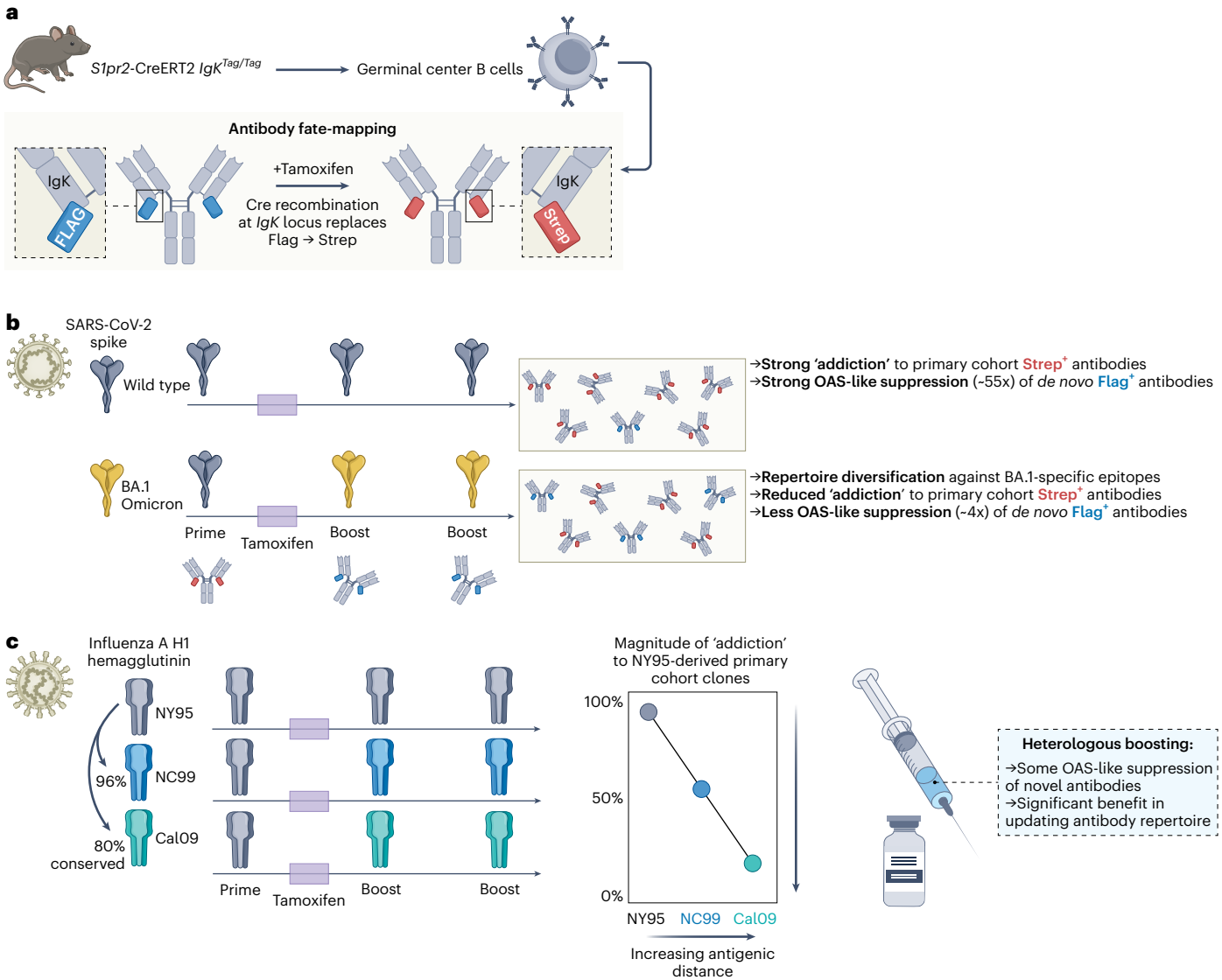

Fig. 1 | Molecular fate-mapping to decipher OAS. a, Schiepers et al.[2] designed a mouse model wherein the administration of tamoxifen after primary immunization or infection labels all antibodies derived from the primary germinal center B cells with a Strep tag. Upon cessation of tamoxifen treatment, any antibodies produced by subsequent secondary or tertiary immunization will be tagged with Flag. **b**,**c**, Using this model, the authors showed that sequential immunization with SARS-CoV-2 spike mRNA vaccines (**b**) and influenza hemagglutinins (**c**) elicits an 'addiction' to clones from the primary cohort, and a suppression of de novo secondary clonal activation. Although this effect was strong in the case of homologous antigen exposure (**b**,**c**, top row), immunization with drifted variants both updated the antibody repertoire and largely, but not completely, mitigated de novo suppression (**b**,**c**, bottom row(s)). These findings suggest that booster vaccines for viral variants serve a crucial function in diversifying the antibody repertoire, but that new secondary antibody clones are in fact inhibited in an OAS-like manner, to a degree dependent on antigenic distance. Figure created using BioRender (biorender.com).

H3N2 influenza strain cross-reacted to past strains, with most binding to non-protective but conserved epitopes[7]. Thus, if an OAS-like phenomenon can limit de novo responses against drifted antigenic variants, efforts to optimize antibody immunity against relevant circulating influenza strains may be continually hindered by a lifetime of past exposures.

In dissecting this phenomenon at the molecular level, the authors[2] shed light on the utility of booster vaccines in the context of pre-existing immunity. Initially, the group observed a strong, suppressive OAS-like phenomenon for secondary serum antibodies elicited by homologous prime-boost. These data are in line with previous observations of reduced serum antibody responses in a cohort of people immunized with identical versus variant influenza vaccine strains over multiple years[9]. However, this is likely of evolutionary advantage, given that for conserved antigens and slowly evolving pathogens, it is logically of greater value to prioritize reactivation of memory B cells (a rapid pre-matured response) rather than re-engagement of naive B cells (a slower immature response)

if the latter provides no qualitative benefit to the antibody repertoire. Thus, although this observation fits the bill for OAS, it is of little concern in terms of the efficacy of homologous boosters. In comparison, as boosting antigens became antigenically divergent, the magnitude of this suppression was substantially mitigated. Some suppression of de novo responses did remain (~4-fold for drifted HA (10% amino acid difference) and SARS-CoV-2 spike (2% difference), versus ~55-fold for homologous antigen). Importantly, a large proportion of the Flag$^+$ BA.1-specific titers elicited were not cross-reactive to wild-type spike, and specifically bound drifted BA.1 epitopes. Thus, while confirming that booster immunizations perform their intended function in updating the immune repertoire, these data also show that novel-variant-reactive clonotypes can indeed be suppressed by pre-existing immunity, in line with the classical model of OAS. Although marginal compared to that seen with homologous boosting, the ~4-fold suppression observed for variant boosting (if translatable to humans) may be of concern, specifically in the context of vaccinating individuals with immune deficiencies or comorbidities.

Fate-mapping of serum antibodies represents an important technological step forward in our ability to understand serological phenomena such as OAS. Previously, analyses had largely been restricted to the tracking of antigen-specific B cells and antibody clonotypes. In earlier work, naive-derived clones were observed to dominate recall GC responses over memory-derived clones in the context of both homologous and drifted HA boosting[10]. Extrapolating this to the serum antibody level and interpreting it through the lens of OAS, one might expect the resulting boost-elicited antibodies to derive from this secondary naive B cell cohort (that is, be primarily Flag$^+$ here) and might expect to find negligible 'primary addiction' or de novo suppression. However, the predominance of Strep$^+$ antibodies seen here after boosting contradicts this idea. In comparison, these findings are in line with the predisposition of boosted serum influenza-specific antibodies to maintain a high degree of clonotypic stability over time[11] and to derive mainly from pre-existing memory B cell clones during variant influenza exposures[7]. Although this may explain the observation of 'primary addiction', additional context is required to understand how this relates to de novo suppression, which is likely to involve phenomena such as epitope masking by recalled antibody clones and/or rapid absorption of re-encountered antigens via immune complex uptake[12]. Overall, this work[2] is the first to extend polyclonal antibody analyses beyond specificity and isotype to origin, and in doing so, provides clear experimental evidence in mice for the existence of a surprisingly potent OAS-like phenomenon at the serological level.

Moving forward, antibody fate-mapping and the precise quantification of de novo suppression could assist the development of optimized vaccines against antigenically variable pathogens such as influenza, SARS-CoV-2 and HIV. Although it is clear that variant-specific serum antibodies can be suppressed by pre-existing immunity, it will be of interest to ask whether boost-derived clones targeting individual variant epitopes are suppressed in a uniform or biased manner. If the latter, there may be benefit in formulating preclinical vaccine candidates to shepherd novel antibody responses toward less suppressed sites. In addition, understanding whether different vaccination strategies (mRNA, split, recombinant, diverse adjuvants) can modulate the degree of suppression may guide the future use and evolution of specific vaccine platforms. In answering these and similar questions, antibody fate-mapping represents a useful new tool to help mitigate the pernicious influence of OAS in viral vaccine development.

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