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The use of longitudinal cohorts for studies of dengue viral pathogenesis and protection

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Abstract

In this review, we describe how longitudinal prospective community-, school-, and household-based cohort studies contribute to improving our knowledge of viral disease, focusing specifically on contributions to understanding and preventing dengue. We describe how longitudinal cohorts enable measurement of essential disease parameters and risk factors; provide insights into biological correlates of protection and disease risk; enable rapid application of novel biological and statistical technologies; lead to development of new interventions and inform vaccine trial design; serve as sentinels in outbreak conditions and facilitate development of critical diagnostic assays; enable holistic studies on disease in the context of other infections, comorbidities, and environmental risk factors; and build research capacity that strengthens national and global public health response and disease surveillance.

Introduction

Although the most well-known prospective cohort studies have focused on predictors of chronic disease [1], cohort studies are also important for understanding infectious diseases. In this study design, individual-level baseline characteristics are measured in a healthy population followed over time as participants naturally acquire disease, thus enabling identification of factors associated with or protective against disease risk. For example, two key findings of such studies include identification of distinct transmission rates of influenza A and B viruses among humans [2] and differential gender-based HIV transmission rates in discordant couples [3]. Prospective community-, school-, and household-based cohort studies are particularly useful to study acute viral diseases such as dengue. Dengue virus is comprised of four serotypes, DENV1–4. Infection with one serotype provides long-term protection against disease upon re-infection with the same serotype. However, prior immunity can protect against or enhance disease during secondary heterotypic DENV infection, which is the greatest risk factor for severe dengue, Dengue Hemorrhagic Fever/ Dengue Shock Syndrome (DHF/DSS). DHF/DSS is thought to be caused in part by

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antibody-dependent enhancement (ADE): sub-neutralizing antibody titers enhance viremia [4] by enabling infection of monocytes and macrophages via Fcγ receptors [5,6]; this instigates pathologic immune cell activation and elevated NS1 secretion that result in vascular leak and shock [7]. Because immune history is critical for understanding subsequent disease risk and protection, cohort studies are invaluable for studying protection against and pathogenesis of dengue disease.

Here, we discuss the full value of longitudinal cohorts for: measuring basic determinants and immunological and virological characteristics of dengue disease in populations, estimating correlates of protection and disease risk, providing critical and timely information during outbreaks, enabling rapid development of new assays for diagnosis and surveillance, informing vaccine trial design, studying disease in a broader population context, building research capacity, and informing local and international policy-making (Table 1).

Review of dengue cohort studies

We used PubMed to download all articles with the term 'dengue' in the title and 'cohort' in either the title or abstract (n=283, January 4, 2018). Titles and abstracts were screened to identify prospective cohort studies of dengue in healthy populations (some reviewed previously in [8–10]; we do not review infant cohorts here). We identified 28 cohort studies from 1964 to the present (Table 2).

Incidence, burden, and risk factors

Incidence

Dengue cohort studies are used to estimate DENV infection and disease incidence in a given population. Symptomatic disease is measured by active surveillance for febrile illness and testing of acute and convalescent blood samples with molecular biological, virological and/or serological methods. Inapparent infections are measured by rise in antibody titers between pre- and post-epidemic or annual blood samples. Dengue disease incidence ranges from 0.3 to 4.6 per 100 person-years, exhibiting substantial heterogeneity by year and location. Cohort studies have shown that incidence of symptomatic dengue is higher in Asia than Latin America and that a larger fraction of dengue cases require hospitalization in Asia [11–13]. DENV-attributable incidence among febrile cases was measured in Thailand and accounted for 15% of DALYs attributable to febrile illness [14,15]. Estimates of dengue disease incidence in cohort studies have been compared to national-level surveillance data, enabling determination of expansion factors (e.g., from 4.7 to 22 cases identified by active surveillance for every 1 case identified by passive surveillance) [16–19] and estimation of national and global incidence, burden, and mortality [20–22]. DENV infection incidence ranges from 3 to 39.4 per 100 person-years [12,23–25], with the ratio of symptomatic to inapparent infections (S:I) varying dramatically in cohorts within epidemics, across years [12], and by geographic area (e.g., nearby schools) [13,26,27]. Analysis has shown that years with high S:I ratio (more symptomatic infections) are often followed by years with low S:I ratio (more inapparent infections) [12,28].

Primary versus secondary infections

The first dengue cohort studies found that DHF/DSS cases were only observed in individuals who had anti-DENV antibodies in pre-infection samples [8,29]. Larger cohort studies proved that pre-existing immunity is a strong risk factor (odds ratio 6.5 in one study, relative risk >50 in another) for DHF/DSS, and DENV2 was most strongly associated with DSS [30–32]. Cohort studies also showed that the probability of symptomatic disease is lower during primary than secondary DENV infection, particularly when the secondary DENV infection occurred >1 year after primary infection [33].

Age and sex

Cohort studies have not consistently shown differences in DENV infection or symptomatic dengue by sex [12,13,24,25], although differences in DSS by sex have been observed [8]. Age is related to both the probability of exposure and disease incidence. First, younger children have more undifferentiated fever caused by DENV, possibly because they do not describe symptoms as easily as older children [34]. Second, older age is associated with probability of DENV infection, likely due to increased mobility [12,35] and body surface area [36,37]. Third, age of secondary DENV infection is associated with higher probability of severe disease [38], while age of acquisition of post-secondary infection immunity is associated with reduced probability of serologically detectable DENV infection given exposure [24,29]. Finally, older age is associated with greater probability of disease, even controlling for anti-DENV antibody titer and number of previous infections [39].

Spatial heterogeneity

Dengue cohort studies have revealed spatial heterogeneity of circulating serotypes and genetic diversity of viral strains circulating in a given population, including extensive gene flow from larger urban centers into more rural populations as well as between nearby schools [40–42]. Spread of a novel serotype, DENV3, in Iquitos, Peru, was correlated with high pre-existing community seroprevalence, suggesting certain areas had higher risk of transmission [27,43].

Force of infection

Cohort studies collect age-stratified seroprevalence data, enabling estimation of the force of infection – the rate at which naïve individuals become infected in a population. Age-stratified seroprevalence data from cohort studies have been used to estimate average historical and annual differences in the force of infection, and where serotype-specific neutralizing antibody titers were measured, serotype-specific force of infection [24,44–46].

Correlates of protection and disease risk

The value of cohort studies for measuring immune correlates

While hospital-based studies are critical for identifying prognostic indicators in acute-phase samples for progression to severe dengue [47] or viral determinants [48] associated with severe dengue outcomes, they are limited in that they can only examine individuals who are

already sick. Cohorts are essential for evaluating how *pre-existing* immunity correlates with infection and disease outcome.

Correlates of protection and risk

Most studies of correlates of protection and risk have examined neutralizing antibody or ADE titers in non-random subsets of dengue cohorts using classical serological assays such as the plaque reduction neutralization test (PRNT) or ADE assays, although some use newer tools [27,32,35,49,50]. In a Thai cohort, neutralizing antibodies distinguished non-severe dengue fever (DF) cases from DHF/DSS cases infected with DENV3, but not DENV2 [51]. In the same cohort, antibody-dependent cellular cytotoxicity was found to correlate with neutralizing antibody titers and IgG_1 levels as well as viremia in DENV3 patients, but not with disease severity [52]. Another Thai cohort confirmed that PRNT titer does not perfectly correlate with protection, as individuals with high PRNT titers could still acquire symptomatic DENV infections [53]. Other cohort studies have compared inapparent to symptomatic DENV infections and found that the magnitude [50,54] and breadth [35,55] of neutralizing antibody titers correlate with reduced probability of symptomatic disease. The Dengvaxia Phase 3 clinical trial data demonstrated that neutralizing antibody titers are associated with vaccine efficacy, although with differences by age and/or pre-vaccination immune status [56].

Efforts to relate ADE titers to severe dengue probability have been less successful in cohort studies, possibly due to issues with how ADE is measured *in vitro*. One early success measured enhancement capacity in primary human monocytes [57], but other studies using different cell lines were not able to find an association between ADE level *in vitro* and disease severity [58]. Recently, it has been shown that specific titers of pre-existing anti-DENV binding antibodies are associated with elevated risk of DHF/DSS, controlling for other covariates, in the Nicaraguan cohort [39].

Flavivirus interactions

Cohort studies are ideal for estimating the effect of prior dengue immunity on Zika disease, and will ultimately enable measurement of the effect of ZIKV infection on dengue. The historical finding that anti-Japanese Encephalitis Virus neutralizing antibodies are associated with elevated probability of symptomatic DENV infection is potentially relevant to the current Zika epidemic and the impact it may have on future dengue disease risk [59].

Antibody longevity

Another major value of cohorts is longitudinal sampling that enables measurement of durability and decay of key antibody populations and biomarkers over long periods of time. Hospital-based studies reveal antibody decay out to 6–12 months post-infection, followed by stability or increases in titer at >1 year [60–62]. Studies of cohort samples also show stability or increases in breadth >1 year after primary DENV infection, with less clear trends following secondary infection [27,49,50,63]. Further analyses of the dynamics of antibodies for all individuals in cohorts will improve our understanding of the durability of antibodies following primary, secondary, and post-secondary DENV infection. Systems serology

approaches, as used for evaluation of HIV trial samples, are also promising, especially when high-throughput methods are available [64].

Full cohort analyses

Promising but under-utilized potential immune correlates include the antibody titers (often binding antibodies) measured regularly on all cohort participants. It has now been shown that anti-DENV binding antibodies correlate with protection from symptomatic dengue at high titer and with elevated risk of DHF/DSS at a specific range of low titers [39], suggesting these antibody measurements are more useful than previously thought. Because it is not feasible to run all assays on all cohort participants, a research priority is sampling cohort participants for in-depth analysis in a way that accounts for how well they represent the population as a whole to allow inference of cohort-level effects.

Infection histories

Measuring the rise in anti-DENV binding antibodies [12,13,24,55] and/or neutralizing antibodies [27,49,65], between paired samples has enabled identification of the sequence and number of previous DENV infections. However, cross-reactivity between serotypes, which can be assay-dependent, makes inferring the exact history of infections difficult, and serological responses resembling homologous re-exposures may have been erroneously called heterotypic infections [66,67]. There are now efforts to call infection histories with fewer assumptions and in a more data-driven way, accounting for potential homotypic infections [66,67] and for boosts [50,68] that do not reach the 4-fold rise antibody titer requirement for inapparent infections. For influenza, accounting for measurement error makes it possible to meaningfully interpret 2-fold rises in Hemagglutinin Inhibition titer [69], and a similar approach is in process for dengue (H.Salje, personal communication). Another approach is to estimate timing of infections based on differential antibody decay to distinct antigens, as has been done for malaria [70].

Viral antigenic diversity

Substantial antigenic diversity by genotype and clade exists, both within and between serotypes [71]. In Peru, prior DENV1 immunity differentially neutralized DENV2 genotypes, resulting in mild disease instead of the expected severe dengue epidemic when DENV2 was introduced for the first time [72,73]. A recent study in the same population suggests that immunity to one DENV2 genotype may not protect against another DENV2 genotype [67]. A key finding from the Nicaraguan cohort study was that a particular relationship existed between prior serotype-specific immunity and susceptibility to severe disease with different clades of the secondary infecting serotype [74]. Importantly, there is concern about the effect of genotypic variation on homologous protection – some type-specific antibodies may be so specific that they do not protect against other genotypes of the same serotype [67,75,76], which could affect vaccine efficacy [77].

Novel immunological and virological techniques

Antibodies

Natural infections are critical for understanding vaccine-mediated protection. Tools such as antibody depletions followed by neutralization tests [78,79] and chimeric viruses in which key epitopes are swapped between serotypes enable identification of particular antibody populations and epitopes associated with protection for each serotype [80–82]. A recent study used chimeric viruses and samples from hospital and cohort studies to show that variable proportions of DENV3 neutralizing activity in natural primary DENV3 infections were attributable to the quaternary type-specific epitope 5J7 across individuals, indicating that other DENV3 type-specific epitopes remain to be identified [63]. Investigating whether cross-neutralizing epitopes other than the potently neutralizing multitypic EDE epitope [83,84] exist is also a major priority.

B cells

Multiple studies have shown significantly more plasmablasts during DENV infection than other febrile illnesses [85], in some cases associated with secondary dengue and disease severity [86]. B cells were found to be type-specific early after primary infection but became more cross-reactive later (6 months), while cross-reactive B cell responses were seen both early and late after secondary infection, often with highest specificity to a serotype other than the secondary infecting serotype [86,87]. A sequencing study of peripheral blood cells from dengue cases across time revealed a convergent antibody signature in the B cell complementarity-determining region 3 enriched in acute-phase samples [88]. Ongoing work is measuring the specificity of B cells to all four DENV serotype plus ZIKV at a single-cell level in the context of a cohort study where prior DENV infection history is known [89,90].

T cells

Early studies suggested that during secondary infection, DENV-specific $CD4^+$ and $CD8^+$ T cells were mostly heterotypic and became activated, leading to a “cytokine storm”, and then underwent programed cell death, potentiating severe dengue disease [91,92]. However, more recent studies point to a protective role for T cells. A nested case-control in the Kampheng Phet cohort found higher levels of TNFα-, IFNγ-, and IL-2-producing T cells in those who developed inapparent as compared with symptomatic DENV infection [93]. Other studies used population-based estimates of T cell responses and associated these with previously identified HLA alleles found to be protective in case-control hospital studies [94]. Further T cell profiling of pre-infection samples in cohorts is needed [95], as it is difficult to establish the direction of causality of T cell functionality in acute-phase samples.

Immune profiling

Comprehensive immune profiling of individuals with severe versus non-severe dengue versus inapparent DENV infection is an important area of future research. A recent study identified viremic but asymptomatic individuals using an index cluster design and found that asymptomatic individuals have feedback mechanisms that regulate activation of the adaptive

immune response that facilitates viral clearance as compared to those with symptomatic or severe dengue [96].

Informing vaccine trial design and evaluation

Estimates of DENV infection and dengue incidence from cohort studies enabled sample size and power calculations for Phase 3 dengue vaccine efficacy trials, while an understanding of the patterns of serotype circulation allowed vaccine trials to be designed in locations to ensure sufficient representation of serotypes for estimating serotype-specific vaccine efficacy [10].

Additionally, four critical observations from cohort studies were borne out in recent Phase 3 clinical trials of a dengue vaccine. First, decent vaccine efficacy was estimated up to 12 months after final vaccine dose (25 months post-first dose) [97,98], but elevated risk of hospitalized dengue was observed thereafter in young vaccine recipients [99]. Cohort studies have observed heterotypic protection against symptomatic secondary dengue <2 years after a primary infection but elevated risk of symptomatic and severe dengue ≥2 years after primary infection [49,100,101]. Second, the observation of dramatically different vaccine efficacy against symptomatic dengue between individuals with prior dengue immunity and individuals who were dengue-naïve [97,98] is consistent with the observation of differential risk of dengue following primary versus secondary DENV infection [32] as well as differences in the composition of neutralizing antibodies after primary versus secondary infections [79]. Third, serotype-specific vaccine efficacy correlated with prevalence of type-specific neutralizing antibodies to that serotype [78], consistent with natural infection studies showing quaternary type-specific potently neutralizing antibodies after primary infection [79]. However, heterotypic neutralizing antibodies at sufficiently high titers can protect against symptomatic DENV infection in both natural infections and vaccine trials [50,56]. Finally, elevated incidence of severe dengue was observed for seronegative vaccinees compared to placebo controls and seropositive vaccinees >12 months after vaccination [99,102], an effect strongly suggestive of classical ADE [4,103], as observed in cohort studies [39].

Added value of longitudinal cohort studies

Sentinels in outbreak conditions

Cohort studies have proven highly valuable during outbreaks of emerging pathogens, pivoting quickly to address new emerging disease threats. Cohort study personnel, especially in coordination with national health systems, serve as critical human resources for identifying and characterizing emerging pathogens as well as measuring key determinants of novel infectious diseases. Such real-time high-quality data are very useful for public health decision-making by national authorities and international agencies, as well as for the scientific community. For example, the Nicaraguan cohort captured the first pandemic H1N1 case in 2009 in Nicaragua [104], and chikungunya [105] and Zika were added to the cohort study as the respective epidemics emerged. Because the Nicaraguan cohort study is embedded within the Ministry of Health, this enabled analyses of Zika in Nicaragua that furnished actionable data in real time, including studies of age-stratified seroprevalence,

spatial risk factors, the effect of prior DENV immunity on Zika, and outcomes of ZIKV infection in pregnancy.

Diagnostic assay development

Well-characterized samples are an essential resource for development of new diagnostic assays for public sector laboratories, academic studies, and companies. For example, when ZIKV arrived in the Nicaraguan cohort, existing pre-exposure DENV samples and post-exposure ZIKV samples enabled extensive diagnostic assay development and adaptation of existing methods to Zika, including multiplex realtime RT-PCR [106], NS1-based BOB ELISA [107], and Zika IgM-capture and inhibition ELISAs [108]. This enabled the Nicaraguan Ministry of Health to rapidly implement Zika diagnostics and disease surveillance during the epidemic. Samples were also shared widely, enabling multiple novel assays to be developed by other groups [109–112].

Use for other pathogens and risk factors

Long-running cohort studies can be expanded into "multiple use" cohorts, as the infrastructure and design for monitoring one acute viral disease is amenable to studies of other pathogens (e.g., dual dengue-influenza cohort studies; cohorts to study multiple arboviruses) [113,114]. Additionally, a broader conception of health and disease can link upstream determinants (e.g., household and environmental risk factors, socioeconomic status, geospatial data [115–117]) with individual biosignatures (e.g., microbiome, novel antibody repertoire profiling, metabolomics, GWAS, eQTL, viral sequencing) and reveal stable features that are associated with documented outcomes of specific infectious and chronic diseases and overall health patterns. These analyses in turn should suggest actionable interventions between the upstream determinants, the stable biosignatures, and downstream health outcomes.

Research capacity-building

Longitudinal cohort studies are also models of international collaborations that combine local expertise on disease, assay development, and surveillance with international scientific expertise, new technologies, and support [118,119]. Such programs require trust built over time as groups work side-by-side on research that benefits both the local population and the international scientific community. Such collaborations incorporate training opportunities for local students and health professionals [118] and technology transfer (biological as well as information technologies) to enable national and global public health departments to conduct the highest quality infectious disease research and surveillance. Robust involvement and communication with local Ministries of Health are critical so that key information about the diseases in question can be used for public health decision-making and evidence-based policy.

9. Concluding thoughts

Overall, longitudinal cohort studies have provided critical insights into dengue epidemiology, immune correlates, and pathogenesis. Importantly, there are many possibilities for digging deeper into understanding immunity and disease in populations and

impacting public health more broadly, by leveraging and expanding long-running cohorts studying endemic and emerging viral pathogens.

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Highlights

- Cohort studies measure disease rates, transmission parameters, and risk factors
- Cohorts identify correlates of protection and risk and inform vaccine trial design
- They provide critical data in outbreaks and enable development of diagnosis assays
- Cohorts build research capacity and inform local and international policy-making

Table 1

Ten ways cohort studies promote scientific research and infectious disease control

1	Estimate basic infection and disease incidence, transmission parameters, and risk factors
2	Identify correlates of protection and disease risk
3	Enable scientific studies of well-characterized samples with advanced scientific techniques
4	Provide longitudinal samples to study kinetics of antibody and biomarker levels
5	Inform vaccine trial design and evaluation
6	Serve as sentinels during outbreaks to inform local and international policy decision-making
7	Collect high-quality samples for diagnostic assay development
8	Enable holistic studies of multiple diseases and environmental and socioeconomic factors
9	Increase understanding of individual and intrinsic differences that drive immunity to pathogens
10	Foster infectious disease infrastructure, research, and control in disease-affected countries in close collaboration with Ministries of Health

Table 2

Community-, school-, or household-based longitudinal prospective cohort studies of dengue (in chronological order)

Location	Years
Bangkok, Thailand [120]	1962–1964
Koh Samui Island, Thailand [121]	1966–1967
Rayong, Thailand [32]	1980–1981
Bangkok, Thailand [31,32]	1980–1981
Yangon, Myanmar [30]	1984–1988
Iquitos, Peru [73]	1993–1996
Yogyakarta, Indonesia [25]	1995–1996
Kamphaeng Phet I, Thailand [15,23,26]	1998–2002
Iquitos, Peru (2 studies during this period) [27]	1999–2005
West Java, Indonesia [122]	2000–2004
Maracay, Venezuela [123]	2001–2002
Managua, Nicaragua [124]	2001–2003
Kamphaeng Phet II, Thailand [125–128]	2004–2006
Long Xuyen, Vietnam [129]	2004–2007
Managua, Nicaragua [12,113,130]	2004–present
Ratchaburi, Thailand [131–133]	2005–2009
Iquitos, Peru [35,44]	2006–2010
West Java, Indonesia [134]	2006–2009
Kampong Cham, Cambodia, [135]	2006–2008
Patillas, Puerto Rico [136]	2007–2008
Iquitos, Peru [67,137]	2007-present
Colombo, Sri Lanka [55,138,139]	2008–2010
Preparatory studies for CYD-TDV Phase III vaccine trials: Indonesia, Malaysia, Philippines, Thailand Vietnam [140]	2010–2011
Preparatory studies for CYD-TDV Phase III vaccine trials: Brazil, Colombia, Puerto Rico, Mexico [141]	2010–2011
Medellin, Colombia [14]	2010–2011
CYD-TDV Phase III vaccine trial placebo controls [11]	2011–2014
Dhaka, Bangladesh [142]	2012
Cebu City, Philippines [17,24]	2012-present