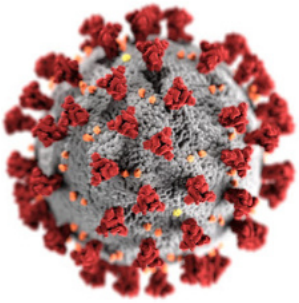

COVID-19 Action Newsletter

UT Southwestern Department of Internal Medicine
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The Situation: Epidemic Continues to Surge in Texas

In the world as of July 2, 2020, 10,719,286 cases of Covid-19 and 516,786 deaths have been confirmed. In the United States, there have been 2,686,587 cases, the most in the world followed in order by Brazil, Russia, India, the United Kingdom, Peru, Chile, Spain, Italy, Iran, Mexico, Pakistan, France, Turkey, Germany, Saudi Arabia, South Africa, Bangladesh, Canada, Columbia, Qatar and China.[1] Deaths in the U.S. through July 2 have been estimated at 128,062.[2] For the first time, Texas is seeing over 8,000 new cases per day, and Harris County has filled all ICU beds. Over the past 6 weeks the number of new cases per day In Dallas has doubled.[3]

From March 10 through June 30, there have been 21,882 confirmed cases of Covid-19 reported from Dallas County with 380 confirmed deaths, over one-third of these from long-term care facilities.[3] Of hospitalized cases in Dallas County, more than two-thirds have been under 65 years of age, and about half have not had any high risk chronic health conditions. After June 1, more than one-half of cases have been in the age group, 18-39 years of age. Diabetes mellitus has been seen in about one-third of all hospitalized patients. More men than women have died. The age-adjusted rates of confirmed Covid-19 cases in non-hospitalized patients have been highest among Hispanics (667.4 per 100,000), Asians (187.4 per 100,000) and Blacks (136.4 per 100,000). These rates have been higher than Whites (43.8 per 100,000). Over 60% of overall Covid-19 cases to date have been Hispanic.[3]

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Feature Article

Vaccines for Covid-19

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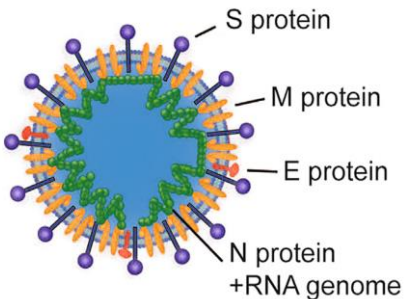
The huge impact of the Covid-19 pandemic, affecting billions of people across the world, has led to an ongoing search for an exit strategy. While there has been progress in potential therapies for SARS-CoV-2, there is still no effective "cure" for Covid-19. A safe and effective vaccine represents the most viable long-term solution to the pandemic. Current estimates are that approximately 70% of the population would have to become immune to achieve desired herd immunity for Covid-19.[1]

There is reasonable rationale as to why a vaccine would be successful for this disease. The majority of patients with Covid-19 improve in the absence of therapeutic intervention, suggesting that natural immunity is sufficient to control the disease. Short-term protective immunity has been demonstrated following infection in animal challenge models and associated with anamnestic B and T cell immune responses.[2] The hope is that a vaccine can recapitulate such a protective response. Prior immunologic studies from the related viruses of SARS and MERS have identified that the spike protein (or S protein) is a key target for eliciting a protective

neutralizing antibody response.[3] Although there has been evidence of genetic drift in the virus, these mutations to date have not led to significant alterations in S protein.



Many of the vaccine candidates targeting a single antigen of the virus have targeted S protein or fractions of S protein. S protein, located in the viral membrane, is one of 4 structural proteins of the virus (**Figure**). It is a trimeric protein that mediates cell entry via binding to the host cell ACE2 receptor. Each monomer of trimeric S protein contains two subunits, S1 and S2, mediating attachment and membrane fusion, respectively.[4] As alluded to above, the development of neutralizing antibody responses to homologue spike proteins of SARS and MERS were found to be protective in animal models, leading to cautious optimism for its use as a target in SARS-CoV-2.

Immune correlates of protection for SARS-CoV-2 are still being understood and are likely derived from a combination of innate and adaptive immune responses. Neutralizing antibody towards S protein or fragments of S protein represent the most promising approach. Titers of neutralizing antibody to the receptor-binding domain of the spike protein were found to be significantly higher in patients who have recovered from Covid-19 compared to healthy controls.[5] In addition, a monoclonal antibody CB6 developed from a patient who had recovered from Covid-19 was able to prevent infection and protect from disease in a non-human primate model.[6] A comprehensive characterization of T cell responses using full genome-spanning peptide pools from the La Jolla Institute for Immunology demonstrated increased frequency of SARS-CoV-2-specific T cells in patients recovered from Covid-19 versus unexposed individuals.[7] In addition, in a fraction of those unexposed individuals, the authors were able to show T cell reactivity to SARS-CoV-2 peptides, suggesting a level of cross-reactivity of immune responses derived from circulating endemic coronaviruses (e.g. 229E, NL63, OC43 and HKU1) that typically cause a mild illness.

Given the duration of time needed and vaccine candidate attrition that is typically seen in vaccine development, the approach to a vaccine for Covid-19 has focused on a huge number of parallel attempts. At the time of writing, there are over 125 vaccine candidates in pre-clinical development with 13 in Phase 1, 2 or 3 clinical trials (**Table**). A wide range of scientific approaches have been taken for Covid-19 vaccine development

Vaccine	Developer	Type	Design	Stage
mRNA-1273	Moderna and NIAID	mRNA	Pre-fusion S protein	Phase 2
BNT162	BioNTech and Pfizer	mRNA	Different mRNA formats (S and RBD)	Phase 1/2
INO-4800	Inovio Pharmaceuticals	DNA	S protein	Phase 1
AZD1222	Univ of Oxford and AstraZeneca	Adenovirus vector	Chimp Adenovirus vector (+ S protein)	Phase 2b/3
Ad5-nCoV	CanSino Biologics	Adenovirus vector	Human Adenovirus (5) vector (+ S protein)	Phase 2
Unnamed	Wuhan Inst. of Biological Products and Sinopharm	Inactivated	-	Phase 1/2
Unnamed	Beijing Inst. of Biological Products/Sinopharm	Inactivated	-	Phase 1/2
PiCoVacc	Sinovac	Inactivated + adj	β-propiolactone inactivation	Phase 1
Unnamed	Inst. of Medical Biology and Chinese Academy of Medical Sciences	Inactivated	-	Phase 1
NVX-CoV2373	Novavax	Protein Subunit	Recombinant S + with Matrix M	Phase 1/2
Gam-Covid-Vac Lyo	Gamaleya Research Institute	Adenovirus vector	Human Adenovirus serotypes 5 and 6	Phase 1
Unnamed	Imperial College London	Self amplifying RNA	S protein	Phase 1
Unnamed	Curevac	mRNA	S protein	Phase 1

including traditional inactivated and subunit vaccines, to more novel approaches such as nucleic acid-based vaccines.

Inactivated vaccines take whole virus and use chemical or physical inactivation with the goal of preserving immunogenicity. Preclinical data for an inactivated vaccine PiCoVacc was recently published showing ability to generate antibody responses to S protein in a mouse model and to confer protection in a non-human primate model.[8]

Protein subunit vaccines are derived from a recombinant protein expression system to produce the antigen of interest (e.g. S protein). This is currently the most popular approach for Covid-19 vaccine candidates with over 50 vaccine attempts in pre-clinical or clinical development.

More novel approaches include the use of nucleic acid-based vaccines (mRNA and DNA) where genetic material is directly injected allowing host cells to produce the protein of interest and viral vector vaccines where an unrelated virus (e.g., adenovirus) is used to "shuttle" DNA encoding for protein of interest into the cells, allowing *in vivo* synthesis of the intended antigen.[9]

All of these approaches will produce different flavors of immunologic responses and the hope is that several will prove successful to accommodate the range of population needing to be vaccinated (e.g. immune-compromised, children, elderly), and to accommodate the global population need.

Despite the huge progress in vaccine development that has been made in a short time, it is anticipated that there will be several challenges as vaccines progress to larger Phase 3 trials that test field efficacy. Safety is an essential component of all approved vaccines and will be evaluated rigorously. Based on prior animal model data and studies of vaccines of related coronaviruses, theoretical concerns exist about the potential for antibody-dependent enhancement and cellular immunopathology.[10] So far this has not emerged as a concern in SARS-CoV-2. In addition, meaningful endpoints for clinical trials will have to be decided, such as protection from acquisition of infection, protection from severe disease, or a combination of these. Perhaps the largest challenge will be whether masking and social distancing will suppress viral transmission in populations enough to deny clinical trials sufficient cases to achieve valid results. Phase 3 trials will each require several thousands of subjects to be rapidly enrolled and require considerable flexibility in trial designs including the potential to depart from the traditional individual randomized controlled setup.

In summary, despite the tremendous progress that has been made in vaccine development, there are still several hurdles to overcome, the most important being the large-scale field trials of safety and efficacy. The rest of 2020 will be a critical phase in understanding how soon we may have a vaccine for Covid-19.

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Pathogenesis

Why do few children get Covid-19: implications for opening schools

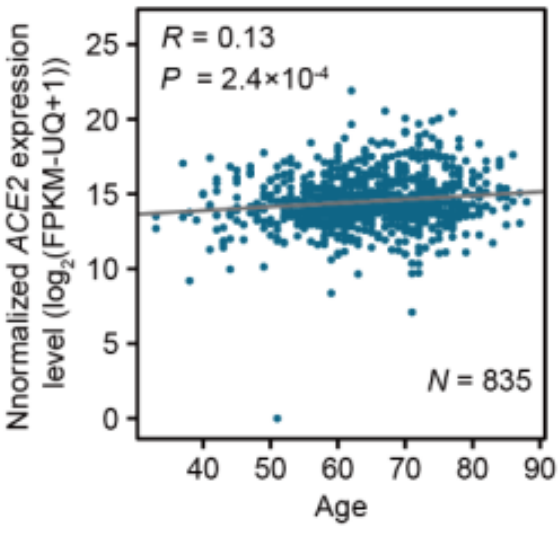
One of the most intriguing mysteries of the Covid-19 pandemic is why infants and children rarely develop Covid-19 illness. Vital statistics, epidemiologic studies and clinical descriptions all agree that fewer than 2 percent of cases and deaths from the disease occur in kids below 15 years of age. While children can become infected, they are far more likely to remain asymptomatic than adults.

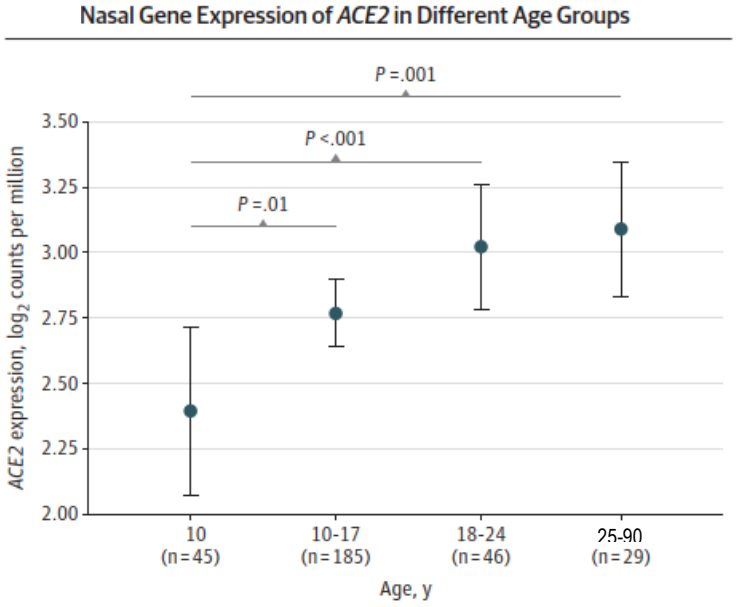
The reason for the sparing of children has been elusive. Speculation centered early on the ACE2 receptor to which the SARS-CoV-2 virus binds to enter human cells. The concentration of ACE2 receptors appears to determine the systems most affected by illness. Studies comparing the number of ACE2 receptors in children and adults have been inhibited by the difficulty of measuring the concentration of these receptors in large numbers of living people of all ages.

Now the first two studies to address this question have appeared. The first was the preprint of an unpublished manuscript, by Chen et al., that appeared in late February reporting an analysis of ACE2 RNA in lung tissue from 835 patients with lung adenocarcinoma in The Cancer Genome Atlas (TCGA) public database.[1] They first demonstrated that ACE2 expression was unaffected by tumorigenesis, allowing findings in cancer genomic databases to be generalized to the population without cancer. Their analysis found a strong association of ACE2 receptor RNA level with age in human lung tissue (**Fig. 1)**; note that the vertical axis shows ACE2 receptor RNA level on a $\log_2$ scale, which obscures the relatively steep slope of the regression line. Overall the ACE2 receptor expression increased by 20% per decade of life. A major limitation of the study was that the age range was limited to ages 30 to 90 years, reflecting the age distribution of adenocarcinoma of the lung, thus not addressing the expression levels in children. Also a peer-reviewed version of the paper has not been published.

In the second study, which appeared as a research letter in JAMA on June 16, Bunyavanich et al. presented ACE2 receptor RNA levels from cytology brushings of nasal epithelium of 305 people of all ages from 4 to 60 years.[2] These data supported the prior speculation that ACE2 receptor RNA levels were substantially lower in children than adults and lowest in children 10 years of age or younger (**Fig. 2**). Again notice that the ACE2 expression on the vertical axis is measured on the $\log_2$ scale, tending to underplay the difference between children and adults.

Fig. 1. Lung cell ACE2 by age group





The far lower infection risk with Covid-19 in children, likely related to the lower mass of ACE2 receptors, contrasts sharply with the considerable transmission risk of other respiratory viruses, particularly influenza, in which children are disproportionately affected and rapid transmission in schools is the major source amplifying

epidemics in adults and deaths in the aged. In fact, the analogy with influenza was the main force driving the closure of schools across the country as Covid-19 initially spread through the U.S.

Whether infected children are less likely than infected adults to transmit the infection to others remains an open question; however, cities that did not close schools this spring did not seem to have greater epidemic spread, and Covid-19 case clusters in day care centers appear mostly related to transmission among adult staff or staff-to-child transmission.

From the growing knowledge of Covid-19, the American Academy of Pediatrics has just released a new guideline strongly advocating the reopening of schools. The new guideline emphasizes reliance on masking and handwashing by staff and students and physical distancing of only 3 feet instead of 6. Their overriding rationale, considering the total wellbeing of children, is that the academic loss from less effective online learning and other problems of staying home, such as increased child abuse and food insecurity, more than outweigh what appears to be minimal risk of SARS-CoV-2 transmission.

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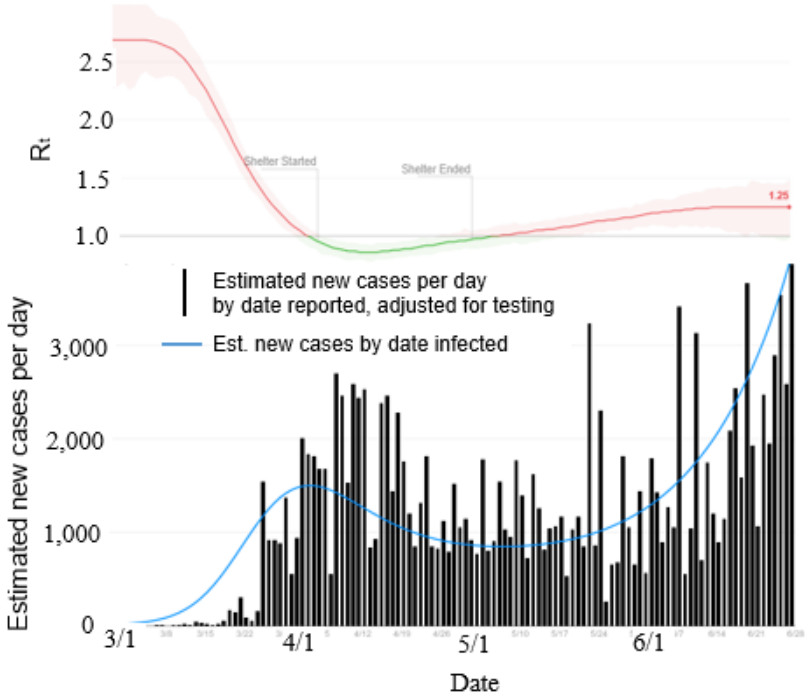
Epi Corner

Impact of R_0 and case burden on epidemic growth

The epidemiologic website *rt.live* provides plots of the Effective Reproduction Number (Rt, or the R_0 over time), estimating the changes in the number of secondary cases caused by each case. Recall that R_0=1.0 indicates that the epidemic will continue at the same rate; R_0>1.0 that it will grow; and R_0<1.0 that it will decline. The **figure** juxtaposes the longitudinal trend in the Texas statewide Rt with the epidemic curve of the number of new confirmed Covid-19 cases over the course of the state's epidemic.

The epidemic curve (black bars) presents the estimated numbers of cases each day adjusted for the amount of testing that was done. Since testing was more severely constrained by supply problems early in the epidemic, the adjustment inflated the number of early reported cases compared to the later ones. The estimated number of new cases by date infected (blue line) projects the reported cases to the estimated time of infection acquisition (shifts the curve to the left).



Notice that an R_0>1.0 had a greater amplifying impact later in the epidemic when there were more cases to begin with than early with few cases around. Increasingly strong epidemiologic evidence (see *C.A.N.* vol. 1, No. 10, 6/19/20) indicates that a statewide masking mandate would force the R_0 below 1.0 and help to end the state's epidemic.

In the News

Goldman Sachs: national mask mandate will save $1T

According to a new *Forbes Magazine* report, a study by Goldman Sachs shows that a national mask mandate would increase mask wearing sufficiently and reduce Covid-19 spread enough to obviate the need for strict lockdown measures to stem the current surge in the U.S. epidemic, particularly in states like Texas and Florida where masks are currently not required. The study estimated that the measure would avoid a 5%, or $1 trillion, drop in the GDP from lockdowns that would otherwise be required to control the current explosive spread.

Further evidence masked protests did not spread CoV

In the June 19 issue of the *Newsletter*, we reported that tests in large numbers of people who participated in protests in New York City, Boston and Seattle were finding only a 1 percent positivity rate for SARS-CoV-2, no different than the background rate in those populations, suggesting that being outdoors and near universal masking had overcome the risks from the crowding and shouting. Now New York City's Department of Health reports that in the weeks following their city's demonstrations, the number of new cases per day have progressively declined from the 700s to the 300s over the following three weeks. Another study reported that 12 of the 13 cities where large protests were held saw no subsequent increases in the daily case counts; the one exception was Phoenix, AZ, where the state's governor had ended the stay-at-home mandate and opened bars and restaurants simultaneously with the protests.

U.S. considering pooled testing to stretch capacity

The practice of pooling samples from multiple patients to screen for diseases such as tuberculosis and syphilis is an old idea that has recently been under study for the current situation. Yesterday Adm. Brett Giroir, a former member of the UT Southwestern faculty and now deputy secretary of health and human services in charge of national testing strategy, announced that the government would soon advocate pooled testing for screening large numbers of asymptomatic people, such as employees of a business or students of a school or a university. It has recently been used to advantage in China, Germany, Israel and Thailand, and is of great interest to infectious disease specialists in this country. It works by having a laboratory technician combine nasal swabs or aliquots of saliva samples from multiple subjects—usually 5 to 10—and keep the remainder of the samples for later testing if needed. The combined sample is then tested for SARS-CoV-2, and only if it is positive would the individual saliva samples be tested. The approach, which can extend testing resources by 70 percent, works well in populations with positivity rates of 1 to 2 percent, but not in special settings like meat packing plants where rates are 10 percent or higher and thus too many pooled samples would be positive. National recommendations and FDA approval are expected in August possibly in time for school openings.

From the Editors

The editors thank Dr. Arasaratnam for his article on vaccines for Covid-19.

The aim of this weekly newsletter is to serve as a source of information for the UT Southwestern community which can lead to better understanding and control of a new disease (Covid-19) caused by the pandemic spread of an emerging viral pathogen (SARS-CoV-2). We welcome questions, comments, and suggestions for topics and authors.