RSV respiratory syncytial virus (RSV) was discovered in 1956 and was initially dubbed “chimpanzee coryza agent.” In 1957, it was found to be the cause of bronchiolitis. It is now known that RSV infects everyone by 3 years of age and repeatedly infects people every few years throughout life. Although most infections result in mild or no disease, as many as 2 to 3% of infants are hospitalized with RSV during their first year of life, which makes RSV a leading cause of hospitalization in children younger than 5 years of age. It is also a major cause of death, rivaling seasonal influenza, among frail older adults. In an attempt to address the disease burden, especially in infants younger than 6 months of age, vaccine-development efforts began within a few years after the virus was discovered.

At the time, the whole-inactivated polio vaccine developed by Jonas Salk was a new and promising intervention. It had been licensed in 1955, and by the early 1960s, cases of paralytic polio in the United States had dropped from tens of thousands to a few dozen per year. Informed and encouraged by that success, researchers developed a whole-inactivated RSV vaccine and conducted four large studies in 1965 and 1966. The youngest cohort of children was immunized before 6 months of age and before their first RSV infection. During the winter of 1966–1967, there was an outbreak of RSV in the youngest age group, and of the 31 vaccinated infants, 20 were infected, 16 were hospitalized, and 2 died.1 This tragedy put an end to RSV vaccine development for decades.

As new technologies became available, they were incrementally applied in RSV research, providing a new set of tools and information to guide vaccine development. The viral genome sequence became available in 1982, and mouse monoclonal antibodies in 1983. Surface glycoprotein and fusion glycoprotein (F) were isolated from the virus in 1984, recombinant proteins were derived from poxvirus vectors in 1986, and recombinant F protein was derived from insect cells in 1989. Small-animal models of RSV infection were also developed during this decade and were used to show that passively administered neutralizing antibody could protect against RSV infection, supporting the finding from clinical studies that the level of maternally derived serum neutralizing activity in infants correlated with protection from severe RSV disease.

Small-animal models were also used in the 1990s to study the role of T cells in RSV pathogenesis and to begin elucidating the immunologic determinants of RSV–vaccine–associated enhanced respiratory disease. In the whole-inactivated RSV vaccine studies of the 1960s, disease enhancement was not as prominent in children in the older age cohorts who were immunized after they had natural RSV infection, and in the 1970s it was shown that live-virus vaccines could be given safely to infants who had not previously been exposed to RSV antigen.

After nearly 30 years of work to elucidate the immunologic events that resulted in RSV-vaccine–enhanced disease, vaccine development efforts were cautiously reinitiated. Greater understanding of how to avoid RSV-vaccine–enhanced disease, along with the newly established ability to pro-

The Journey to RSV Vaccines — Heralding an Era of Structure-Based Design

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duce RSV infectious molecular clones, led to the exploration of multiple vaccine approaches, including live virus, chimeric viruses, vector-based delivery, subunit proteins, peptides, virus-like particles, and nanoparticle display.

Five phase 3 trials were conducted using F protein in the postfusion conformation, which was known to be a target of neutralizing antibodies. Two trials used virus-derived protein, and three were based on recombinant proteins, one from insect cells and two from Chinese hamster ovary cells. None of these candidate vaccines boosted neutralizing activity by more than a factor of five, and field trials did not achieve their primary efficacy objectives. In vitro studies conducted by Melero and colleagues suggested that antibodies specific for the prefusion form of F (preF) may be responsible for the majority of neutralizing activity in human serum. Collectively, these studies suggested that the protein structure of F could be a key factor in the antigenicity of RSV.

The first atomic-level structure of a class I fusion protein to be solved was that of influenza hemagglutinin, by Wiley and colleagues in 1981. Whereas hemagglutinin remains relatively stable in its prefusion conformation until it is exposed to a low pH, trimeric fusion proteins from most other enveloped viruses are metastable, and it wasn’t until 2006 that Lamb and colleagues published the first atomic-level structure of a paramyxovirus F protein in its prefusion conformation. Shortly after that, a program to determine the atomic-level structure of RSV F was initiated at the Vaccine Research Center of the National Institute of Allergy and Infectious Diseases, beginning with individual epitopes and culminating in full structures of postfusion F (postF) and preF.

Obtaining RSV preF crystals was difficult, because the unmodified and untethered protein spontaneously rearranges into its highly stable postfusion conformation (see illustration). Therefore, to capture the desired prefusion trimer conformation, the molecule had to be constrained at both ends. Ultimately, a trimerization domain derived from the fibrinogen protein from T4 phage, foldon, was used at the C-terminal domain, and antibody Fab fragments that could form a complex by binding the apex of preF were used to stabilize the trimer. These antibodies were identified by screening monoclonal antibodies (mAbs) that could neutralize virus but did not bind postF.

One of these mAbs was discovered by screening hybridomas from immunized mice, and the other two human mAbs were found in the patent database, originally discovered by Beaumont and colleagues with the use of an unbiased method for propagating clones of peripheral-blood memory B cells. The structure of RSV preF was determined with the use of the human mAb D25 that is the precursor of the highly potent nirsevimab that has been shown to prevent severe RSV disease for up to 150 days when administered to infants at birth. Thus, solving the RSV preF structure revealed a new site of vulnerability and neutralization sensitivity, indicating that it may be a target for vaccine development.

Introducing disulfide and cavity-filling mutations stabilized F in the preF conformation. Proline substitutions and other mutations have also been found to stabilize preF. Having preF as a reagent allowed detailed mapping of the antigenic surface of F in all its conformations, defining of the contribution of each epitope to virus neutralization, and isolation of hundreds of new human monoclonal antibodies. Defining the epitope-specific repertoire and B-cell phenotypes that compose the immune response to RSV showed that antibodies that exclusively bind preF have much greater neutralizing potency than antibodies that bind both preF and postF. Subsequently, it was shown that preF is much more immunogenic than postF for boosting RSV neutralizing activity, and now several studies have shown that preF is effective when used as a vaccine during pregnancy or in older adults. A decades-long process of applying new technologies to basic RSV research gradually yielded the fundamental knowledge about the virology and pathogenesis that was needed to design and evaluate effective vaccines.

Notably, the success of structure-based vaccine design for RSV informed the rapid response to Covid-19. Outbreaks of Middle East respiratory syndrome coronavirus were occurring while the RSV preF structure was being analyzed. At that time, we did not know the atomic-level structure of any coronavirus protein, but over the next few years, spike protein structures were solved, and ultimately stabilizing mutations were identified to maintain the prefusion spike conformation and improve protein expression levels. These studies were the basis for coronavirus pandemic preparedness planning that included public–private and academic collaborations that were operative in 2019. Because of the technical advances in biomedical science and the RSV blueprint, work that had taken decades in RSV research was compressed into just a few
The Effect of Respiratory Syncytial Virus (RSV) Fusion Glycoprotein (F) Structure on Antigenicity.

The trimeric RSV F protein in its prefusion state (middle) is anchored on the viral envelope by a transmembrane domain at the C-terminus. At the apex of the prefusion F protein, there is an epitope (denoted in red) targeted by antibodies with high neutralizing activity. When F protein rearranges into the postfusion form (left), either spontaneously on the viral membrane or after creating a fusion pore with the host-cell membrane, the epitope is lost. Stabilizing mutations can be introduced on the interior of the protein (right, small circled areas) to hold it in the prefusion conformation and preserve neutralization-sensitive epitopes at the apex for use as a vaccine antigen. The ectodomain of RSV F vaccines can be delivered as a soluble trimeric protein (right) by constraining the C-terminus (right, large circled area) or, if expressed by gene delivery, the membrane of the protein can be anchored by retaining the transmembrane domain.

weeks for SARS-CoV-2, providing sequences, structures, and reagents needed to rapidly develop safe and effective vaccines and therapeutic mAbs.

With Covid-19 vaccines now approved for clinical use and RSV vaccines shown to be effective and awaiting approval, we have entered an era of precision antigen design based on protein engineering guided by atomic-level structure. I hope these advances will lead to future successes in addressing unmet needs and combating threats from emerging pathogens.

Disclosure forms provided by the author are available at NEJM.org.

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Looking After Our Own

Richard M. Boulay, M.D.

If you follow me, Dr. Boulay, she’s in ICU-10,” the intensivist began, walking me down the labyrinthine corridors. “She remains delirious, but her rigidity is improving. Her head CT, bloodwork, and tox screen are normal. We just sent an LP. She’s still tachy to 180, but her pressure is holding. She’s not any worse in the few hours she’s been here, but she’s no better. Her husband...