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Facemasks in the COVID-19 era: A health hypothesis

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Abstract

Many countries across the globe utilized medical and non-medical facemasks as non-pharmaceutical intervention for reducing the transmission and infectivity of coronavirus disease-2019 (COVID-19). Although, scientific evidence supporting facemasks' efficacy is lacking, adverse physiological, psychological and health effects are established. Is has been hypothesized that facemasks have compromised safety and efficacy profile and should be avoided from use. The current article comprehensively summarizes scientific evidences with respect to wearing facemasks in the COVID-19 era, providing prosper information for public health and decisions making.

Keywords: Physiology, Psychology, Health, SARS-CoV-2, Safety, Efficacy

Introduction

Facemasks are part of non-pharmaceutical interventions providing some breathing barrier to the mouth and nose that have been utilized for reducing the transmission of respiratory pathogens [1]. Facemasks can be medical and non-medical, where two types of the medical masks primarily used by healthcare workers [1], [2]. The first type is National Institute for Occupational Safety and Health (NIOSH)-certified N95 mask, a filtering face-piece respirator, and the second type is a surgical mask [1]. The designed and intended uses of N95 and surgical masks are different in the type of protection they potentially provide. The N95s are typically composed of electret filter media and seal tightly to the face

Table 1

Physiological and Psychological Effects of Wearing Facemask and Their Potential Health Consequences.

Physiological Effects	Psychological Effect	Health Consequences
• Hypoxemia • Hypercapnia • Shortness of breath • Increase lactate concentration • Decline in pH levels • Acidosis • Toxicity • Inflammation • Self-contamination • Increase in stress hormones level (adrenaline, noradrenaline and cortisol) • Increased muscle tension • Immunosuppression	• Activation of "fight or flight" stress response • Chronic stress condition • Fear • Mood disturbances • Insomnia • Fatigue • Compromised cognitive performance	• Increased predisposition for viral and infection illnesses • Headaches • Anxiety • Depression • Hypertension • Cardiovascular disease • Cancer • Diabetes • Alzheimer disease • Exacerbation of existing conditions and diseases • Accelerated aging process • Health deterioration • Premature mortality

In addition to hypoxia and hypercapnia, breathing through facemask residues bacterial and germs components on the inner and outside layer of the facemask. These toxic components are repeatedly rebreathed back into the body, causing self-contamination. Breathing through facemasks also increases temperature and humidity in the space between the mouth and the mask, resulting a release of toxic particles from the mask's materials [1], [2], [19], [26], [35], [36]. A systematic literature review estimated that aerosol contamination levels of facemasks including 13 to 202,549 different viruses [1]. Rebreathing contaminated air with high bacterial and toxic particle concentrations along with low O_2 and high CO_2 levels continuously challenge the body homeostasis, causing self-toxicity and immunosuppression [1], [2], [19], [26], [35], [36].

A study on 39 patients with renal disease found that wearing N95 facemask during hemodialysis significantly reduced arterial partial oxygen pressure (from PaO_2 101.7 to 92.7 mm Hg), increased respiratory rate (from 16.8 to 18.8 breaths/min), and increased the occurrence of chest discomfort and respiratory distress [35]. Respiratory Protection Standards from Occupational Safety and Health Administration, US Department of Labor states that breathing air with O_2 concentration below 19.5% is considered oxygen-deficiency, causing physiological and health adverse effects. These include increased breathing frequency, accelerated heartrate and cognitive impairments related to thinking and

of the wearer, whereas surgical masks are generally loose fitting and may or may not contain electret-filtering media. The N95s are designed to reduce the wearer's inhalation exposure to infectious and harmful particles from the environment such as during extermination of insects. In contrast, surgical masks are designed to provide a barrier protection against splash, spittle and other body fluids to spray from the wearer (such as surgeon) to the sterile environment (patient during operation) for reducing the risk of contamination [1].

The third type of facemasks are the non-medical cloth or fabric masks. The non-medical facemasks are made from a variety of woven and non-woven materials such as Polypropylene, Cotton, Polyester, Cellulose, Gauze and Silk. Although non-medical cloth or fabric facemasks are neither a medical device nor personal protective equipment, some standards have been developed by the French Standardization Association (AFNOR Group) to define a minimum performance for filtration and breathability capacity [2]. The current article reviews the scientific evidences with respect to safety and efficacy of wearing facemasks, describing the physiological and psychological effects and the potential long-term consequences on health.

Hypothesis

On January 30, 2020, the World Health Organization (WHO) announced a global public health emergency of severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) causing illness of coronavirus disease-2019 (COVID-19) [3]. As of October 1, 2020, worldwide 34,166,633 cases were reported and 1,018,876 have died with virus diagnosis. Interestingly, 99% of the detected cases with SARS-CoV-2 are asymptomatic or have mild condition, which contradicts with the virus name (*severe* acute respiratory syndrome-coronavirus-2) [4]. Although infection fatality rate (number of death cases divided by number of reported cases) initially seems quite high 0.029 (2.9%) [4], this overestimation related to limited number of COVID-19 tests performed which biases towards higher rates. Given the fact that asymptomatic or minimally symptomatic cases is several times higher than the number of reported cases, the case fatality rate is considerably less than 1% [5]. This was confirmed by the head of National Institute of Allergy and Infectious Diseases from US stating, "the overall clinical consequences of COVID-19 are similar to those of severe seasonal influenza" [5], having a case fatality rate of approximately 0.1% [5], [6], [7], [8]. In addition, data from hospitalized patients with COVID-19 and general public indicate that the majority of deaths were among older and chronically ill individuals, supporting the possibility that the virus may exacerbates existing conditions but rarely causes death by itself [9], [10]. SARS-CoV-2 primarily affects respiratory system and can cause complications such as acute respiratory distress syndrome (ARDS), respiratory failure and death [3], [9]. It is not clear however, what the scientific and clinical basis for wearing facemasks as protective strategy, given the fact that facemasks restrict breathing, causing hypoxemia and hypercapnia and increase the risk for respiratory complications, self-contamination and exacerbation of existing chronic conditions [2], [11], [12], [13], [14].

Of note, hyperoxia or oxygen supplementation (breathing air with high partial O_2 pressures that above the sea levels) has been well established as therapeutic and curative practice for variety acute and chronic conditions including respiratory complications [11], [15]. It fact, the current standard of care practice for treating hospitalized patients with COVID-19 is breathing 100% oxygen [16], [17], [18]. Although several countries mandated wearing facemask in health care settings and public areas, scientific evidences are lacking supporting their efficacy for reducing morbidity or mortality associated with infectious or viral diseases [2], [14], [19]. Therefore, it has been hypothesized: 1) the practice of

wearing facemasks has compromised safety and efficacy profile, 2) Both medical and non-medical facemasks are ineffective to reduce human-to-human transmission and infectivity of SARS-CoV-2 and COVID-19, 3) Wearing facemasks has adverse physiological and psychological effects, 4) Long-term consequences of wearing facemasks on health are detrimental.

Evolution of hypothesis

Breathing Physiology

Breathing is one of the most important physiological functions to sustain life and health. Human body requires a continuous and adequate oxygen (O_2) supply to all organs and cells for normal function and survival. Breathing is also an essential process for removing metabolic byproducts [carbon dioxide (CO_2)] occurring during cell respiration [12], [13]. It is well established that acute significant deficit in O_2 (hypoxemia) and increased levels of CO_2 (hypercapnia) even for few minutes can be severely harmful and lethal, while chronic hypoxemia and hypercapnia cause health deterioration, exacerbation of existing conditions, morbidity and ultimately mortality [11], [20], [21], [22]. Emergency medicine demonstrates that 5–6 min of severe hypoxemia during cardiac arrest will cause brain death with extremely poor survival rates [20], [21], [22], [23]. On the other hand, chronic mild or moderate hypoxemia and hypercapnia such as from wearing facemasks resulting in shifting to higher contribution of anaerobic energy metabolism, decrease in pH levels and increase in cells and blood acidity, toxicity, oxidative stress, chronic inflammation, immunosuppression and health deterioration [24], [11], [12], [13].

Efficacy of facemasks

The physical properties of medical and non-medical facemasks suggest that facemasks are ineffective to block viral particles due to their difference in scales [16], [17], [25]. According to the current knowledge, the virus SARS-CoV-2 has a diameter of 60 nm to 140 nm [nanometers (billionth of a meter)] [16], [17], while medical and non-medical facemasks' thread diameter ranges from 55 μm to 440 μm [micrometers (one millionth of a meter), which is more than 1000 times larger [25]. Due to the difference in sizes between SARS-CoV-2 diameter and facemasks thread diameter (the virus is 1000 times smaller), SARS-CoV-2 can easily pass through any facemask [25]. In addition, the efficiency filtration rate of facemasks is poor, ranging from 0.7% in non-surgical, cotton-gauze woven mask to 26% in cotton sweeter material [2]. With respect to surgical and N95 medical facemasks, the efficiency filtration rate falls to 15% and 58%, respectively when even small gap between the mask and the face exists [25].

Clinical scientific evidence challenges further the efficacy of facemasks to block human-to-human transmission or infectivity. A randomized controlled trial (RCT) of 246 participants [123 (50%) symptomatic)] who were allocated to either wearing or not wearing surgical facemask, assessing viruses transmission including coronavirus [26]. The results of this study showed that among symptomatic individuals (those with fever, cough, sore throat, runny nose ect…) there was no difference between wearing and not wearing facemask for coronavirus droplets transmission of particles of >5 μm. Among asymptomatic individuals, there was no droplets or aerosols coronavirus detected from any participant with or without the mask, suggesting that asymptomatic individuals do not transmit or infect other people [26]. This was further supported by a study on infectivity where 445 asymptomatic individuals were exposed to asymptomatic SARS-CoV-2 carrier (been positive for

SARS-CoV-2) using close contact (shared quarantine space) for a median of 4 to 5 days. The study found that none of the 445 individuals was infected with SARS-CoV-2 confirmed by real-time reverse transcription polymerase [27].

A *meta*-analysis among health care workers found that compared to no masks, surgical mask and N95 respirators were not effective against transmission of viral infections or influenza-like illness based on six RCTs [28]. Using separate analysis of 23 observational studies, this *meta*-analysis found no protective effect of medical mask or N95 respirators against SARS virus [28]. A recent systematic review of 39 studies including 33,867 participants in community settings (self-report illness), found no difference between N95 respirators versus surgical masks and surgical mask versus no masks in the risk for developing influenza or influenza-like illness, suggesting their ineffectiveness of blocking viral transmissions in community settings [29].

Another *meta*-analysis of 44 non-RCT studies (n = 25,697 participants) examining the potential risk reduction of facemasks against SARS, middle east respiratory syndrome (MERS) and COVID-19 transmissions [30]. The *meta*-analysis included four specific studies on COVID-19 transmission (5,929 participants, primarily health-care workers used N95 masks). Although the overall findings showed reduced risk of virus transmission with facemasks, the analysis had severe limitations to draw conclusions. One of the four COVID-19 studies had zero infected cases in both arms, and was excluded from *meta*-analytic calculation. Other two COVID-19 studies had unadjusted models, and were also excluded from the overall analysis. The *meta*-analytic results were based on only one COVID-19, one MERS and 8 SARS studies, resulting in high selection bias of the studies and contamination of the results between different viruses. Based on four COVID-19 studies, the *meta*-analysis failed to demonstrate risk reduction of facemasks for COVID-19 transmission, where the authors reported that the results of *meta*-analysis have low certainty and are inconclusive [30].

In early publication the WHO stated that "facemasks are not required, as no evidence is available on its usefulness to protect non-sick persons" [14]. In the same publication, the WHO declared that "cloth (e.g. cotton or gauze) masks are not recommended under any circumstance" [14]. Conversely, in later publication the WHO stated that the usage of fabric-made facemasks (Polypropylene, Cotton, Polyester, Cellulose, Gauze and Silk) is a general community practice for "preventing the infected wearer transmitting the virus to others and/or to offer protection to the healthy wearer against infection (prevention)" [2]. The same publication further conflicted itself by stating that due to the lower filtration, breathability and overall performance of fabric facemasks, the usage of woven fabric mask such as cloth, and/or non-woven fabrics, should only be considered for infected persons and not for prevention practice in asymptomatic individuals [2]. The Central for Disease Control and Prevention (CDC) made similar recommendation, stating that only symptomatic persons should consider wearing facemask, while for asymptomatic individuals this practice is not recommended [31]. Consistent with the CDC, clinical scientists from Departments of Infectious Diseases and Microbiology in Australia counsel against facemasks usage for health-care workers, arguing that there is no justification for such practice while normal caring relationship between patients and medical staff could be compromised [32]. Moreover, the WHO repeatedly announced that "at present, there is no direct evidence (from studies on COVID-19) on the effectiveness face masking of healthy people in the community to prevent infection of respiratory viruses, including COVID-19"[2]. Despite these controversies, the potential harms and risks of wearing facemasks were clearly acknowledged. These including self-contamination due to hand practice or non-replaced when the mask is wet, soiled or damaged, development of facial skin lesions, irritant dermatitis or worsening acne and psychological discomfort.

Vulnerable populations such as people with mental health disorders, developmental disabilities, hearing problems, those living in hot and humid environments, children and patients with respiratory conditions are at significant health risk for complications and harm [2].

Physiological effects of wearing facemasks

Wearing facemask mechanically restricts breathing by increasing the resistance of air movement during both inhalation and exhalation process [12], [13]. Although, intermittent (several times a week) and repetitive (10–15 breaths for 2–4 sets) increase in respiration resistance may be adaptive for strengthening respiratory muscles [33], [34], prolonged and continues effect of wearing facemask is maladaptive and could be detrimental for health [11], [12], [13]. In normal conditions at the sea level, air contains 20.93% O_2 and 0.03% CO_2, providing partial pressures of 100 mmHg and 40 mmHg for these gases in the arterial blood, respectively. These gas concentrations significantly altered when breathing occurs through facemask. A trapped air remaining between the mouth, nose and the facemask is rebreathed repeatedly in and out of the body, containing low O_2 and high CO_2 concentrations, causing hypoxemia and hypercapnia [35], [36], [11], [12], [13]. Severe hypoxemia may also provoke cardiopulmonary and neurological complications and is considered an important clinical sign in cardiopulmonary medicine [37], [38], [39], [40], [41], [42]. Low oxygen content in the arterial blood can cause myocardial ischemia, serious arrhythmias, right or left ventricular dysfunction, dizziness, hypotension, syncope and pulmonary hypertension [43]. Chronic low-grade hypoxemia and hypercapnia as result of using facemask can cause exacerbation of existing cardiopulmonary, metabolic, vascular and neurological conditions [37], [38], [39], [40], [41], [42]. Table 1 summarizes the physiological, psychological effects of wearing facemask and their potential long-term consequences for health.

coordination [36]. A chronic state of mild hypoxia and hypercapnia has been shown as primarily mechanism for developing cognitive dysfunction based on animal studies and studies in patients with chronic obstructive pulmonary disease [44].

The adverse physiological effects were confirmed in a study of 53 surgeons where surgical facemask were used during a major operation. After 60 min of facemask wearing the oxygen saturation dropped by more than 1% and heart rate increased by approximately five beats/min [45]. Another study among 158 health-care workers using protective personal equipment primarily N95 facemasks reported that 81% (128 workers) developed new headaches during their work shifts as these become mandatory due to COVID-19 outbreak. For those who used the N95 facemask greater than 4 h per day, the likelihood for developing a headache during the work shift was approximately four times higher [Odds ratio = 3.91, 95% CI (1.35–11.31) p = 0.012], while 82.2% of the N95 wearers developed the headache already within ≤10 to 50 min [46].

With respect to cloth facemask, a RCT using four weeks follow up compared the effect of cloth facemask to medical masks and to no masks on the incidence of clinical respiratory illness, influenza-like illness and laboratory-confirmed respiratory virus infections among 1607 participants from 14 hospitals [19]. The results showed that there were no difference between wearing cloth masks, medical masks and no masks for incidence of clinical respiratory illness and laboratory-confirmed respiratory virus infections. However, a large harmful effect with more than 13 times higher risk [Relative Risk = 13.25 95% CI (1.74 to 100.97) was observed for influenza-like illness among those who were wearing cloth masks [19]. The study concluded that cloth masks have significant health and safety issues including moisture retention, reuse, poor filtration and increased risk for infection, providing recommendation against the use of cloth masks [19].

Psychological effects of wearing facemasks

Psychologically, wearing facemask fundamentally has negative effects on the wearer and the nearby person. Basic human-to-human connectivity through face expression is compromised and self-identity is somewhat eliminated [47], [48], [49]. These dehumanizing movements partially delete the uniqueness and individuality of person who wearing the facemask as well as the connected person [49]. Social connections and relationships are basic human needs, which innately inherited in all people, whereas reduced human-to-human connections are associated with poor mental and physical health [50], [51]. Despite escalation in technology and globalization that would presumably foster social connections, scientific findings show that people are becoming increasingly more socially isolated, and the prevalence of loneliness is increasing in last few decades [50], [52]. Poor social connections are closely related to isolation and loneliness, considered significant health related risk factors [50], [51], [52], [53].

A *meta*-analysis of 91 studies of about 400,000 people showed a 13% increased morality risk among people with low compare to high contact frequency [53]. Another *meta*-analysis of 148 prospective studies (308,849 participants) found that poor social relationships was associated with 50% increased mortality risk. People who were socially isolated or fell lonely had 45% and 40% increased mortality risk, respectively. These findings were consistent across ages, sex, initial health status, cause of death and follow-up periods [52]. Importantly, the increased risk for mortality was found comparable to smoking and exceeding well-established risk factors such as obesity and physical inactivity [52]. An

umbrella review of 40 systematic reviews including 10 *meta*-analyses demonstrated that compromised social relationships were associated with increased risk of all-cause mortality, depression, anxiety suicide, cancer and overall physical illness [51].

As described earlier, wearing facemasks causing hypoxic and hypercapnic state that constantly challenges the normal homeostasis, and activates "fight or flight" stress response, an important survival mechanism in the human body [11], [12], [13]. The acute stress response includes activation of nervous, endocrine, cardiovascular, and the immune systems [47], [54], [55], [56]. These include activation of the limbic part of the brain, release stress hormones (adrenalin, neuro-adrenalin and cortisol), changes in blood flow distribution (vasodilation of peripheral blood vessels and vasoconstriction of visceral blood vessels) and activation of the immune system response (secretion of macrophages and natural killer cells) [47], [48]. Encountering people who wearing facemasks activates innate stress-fear emotion, which is fundamental to all humans in danger or life threating situations, such as death or unknown, unpredictable outcome. While acute stress response (seconds to minutes) is adaptive reaction to challenges and part of the survival mechanism, chronic and prolonged state of stress-fear is maladaptive and has detrimental effects on physical and mental health. The repeatedly or continuously activated stress-fear response causes the body to operate on survival mode, having sustain increase in blood pressure, pro-inflammatory state and immunosuppression [47], [48].

Long-Term health consequences of wearing facemasks

Long-term practice of wearing facemasks has strong potential for devastating health consequences. Prolonged hypoxic-hypercapnic state compromises normal physiological and psychological balance, deteriorating health and promotes the developing and progression of existing chronic diseases [23], [38], [39], [43], [47], [48], [57], [11], [12], [13]. For instance, ischemic heart disease caused by hypoxic damage to the myocardium is the most common form of cardiovascular disease and is a number one cause of death worldwide (44% of all non-communicable diseases) with 17.9 million deaths occurred in 2016 [57]. Hypoxia also playing an important role in cancer burden [58]. Cellular hypoxia has strong mechanistic feature in promoting cancer initiation, progression, metastasis, predicting clinical outcomes and usually presents a poorer survival in patients with cancer. Most solid tumors present some degree of hypoxia, which is independent predictor of more aggressive disease, resistance to cancer therapies and poorer clinical outcomes [59], [60]. Worth note, cancer is one of the leading causes of death worldwide, with an estimate of more than 18 million new diagnosed cases and 9.6 million cancer-related deaths occurred in 2018 [61].

With respect to mental health, global estimates showing that COVID-19 will cause a catastrophe due to collateral psychological damage such as quarantine, lockdowns, unemployment, economic collapse, social isolation, violence and suicides [62], [63], [64]. Chronic stress along with hypoxic and hypercapnic conditions knocks the body out of balance, and can cause headaches, fatigue, stomach issues, muscle tension, mood disturbances, insomnia and accelerated aging [47], [48], [65], [66], [67]. This state suppressing the immune system to protect the body from viruses and bacteria, decreasing cognitive function, promoting the developing and exacerbating the major health issues including hypertension, cardiovascular disease, diabetes, cancer, Alzheimer disease, rising anxiety and depression states, causes social isolation and loneliness and increasing the risk for prematurely mortality [47], [48], [51], [56], [66].

Conclusion

The existing scientific evidences challenge the safety and efficacy of wearing facemask as preventive intervention for COVID-19. The data suggest that both medical and non-medical facemasks are ineffective to block human-to-human transmission of viral and infectious disease such SARS-CoV-2 and COVID-19, supporting against the usage of facemasks. Wearing facemasks has been demonstrated to have substantial adverse physiological and psychological effects. These include hypoxia, hypercapnia, shortness of breath, increased acidity and toxicity, activation of fear and stress response, rise in stress hormones, immunosuppression, fatigue, headaches, decline in cognitive performance, predisposition for viral and infectious illnesses, chronic stress, anxiety and depression. Long-term consequences of wearing facemask can cause health deterioration, developing and progression of chronic diseases and premature death. Governments, policy makers and health organizations should utilize prosper and scientific evidence-based approach with respect to wearing facemasks, when the latter is considered as preventive intervention for public health.

CRediT authorship contribution statement

Baruch Vainshelboim: Conceptualization, Data curation, Writing - original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Immunization with SARS Coronavirus Vaccines Leads to Pulmonary Immunopathology on Challenge with the SARS Virus

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Abstract

Background: Severe acute respiratory syndrome (SARS) emerged in China in 2002 and spread to other countries before brought under control. Because of a concern for reemergence or a deliberate release of the SARS coronavirus, vaccine development was initiated. Evaluations of an inactivated whole virus vaccine in ferrets and nonhuman primates and a virus-like-particle vaccine in mice induced protection against infection but challenged animals exhibited an immunopathologic-type lung disease.

Design: Four candidate vaccines for humans with or without alum adjuvant were evaluated in a mouse model of SARS, a VLP vaccine, the vaccine given to ferrets and NHP, another whole virus vaccine and an rDNA-produced S protein. Balb/c or C57BL/6 mice were vaccinated IM on day 0 and 28 and sacrificed for serum antibody measurements or challenged with live virus on day 56. On day 58, challenged mice were sacrificed and lungs obtained for virus and histopathology.

Results: All vaccines induced serum neutralizing antibody with increasing dosages and/or alum significantly increasing responses. Significant reductions of SARS-CoV two days after challenge was seen for all vaccines and prior live SARS-CoV. All mice exhibited histopathologic changes in lungs two days after challenge including all animals vaccinated (Balb/C and C57BL/6) or given live virus, influenza vaccine, or PBS suggesting infection occurred in all. Histopathology seen in animals given one of the SARS-CoV vaccines was uniformly a Th2 type immunopathology with prominent eosinophil infiltration, confirmed with special eosinophil stains. The pathologic changes seen in all control groups lacked the eosinophil prominence.

Conclusions: These SARS-CoV vaccines all induced antibody and protection against infection with SARS-CoV. However, challenge of mice given any of the vaccines led to occurrence of Th2-type immunopathology suggesting hypersensitivity to SARS-CoV components was induced. Caution in proceeding to application of a SARS-CoV vaccine in humans is indicated.

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Introduction

Severe acute respiratory syndrome (SARS) emerged in Guangdong, People's Republic of China, in late 2002, and spread to other countries in Asia and to Canada in the ensuing months [1–3]. Infection control efforts brought the infection under control by mid-2003 [4]. More than 8000 cases, including almost 800 deaths, were reported during the outbreak period [4]. Increasing age and comorbidity were risk factors for severe disease and death [5,6,7]. Since 2003, only sporadic cases have been reported; however, the possibility that SARS outbreaks could reemerge naturally or be deliberately released is a public health concern.

SARS is caused by a Coronavirus (SARS-CoV) [8,9]. Limited data are available about the ecology of SARS-CoV, but bats are thought to be the animal reservoir for the virus which may be transmitted to small mammals with exposure to these small animals as the source of human infections [10]. The clinical disease is similar to other severe acute respiratory infections, including influenza; the SARS case definition includes clinical, epidemiologic, and laboratory criteria [11,12]. A number of therapeutic efforts were employed for the disease in Asia and in Canada; however, no treatment of clear value was identified. Animal models were developed using mice, hamsters, ferrets and

nonhuman primates, and efforts to identify useful treatments and effective vaccines are ongoing.

Vaccine candidates for preventing SARS have been developed by various groups and include inactivated whole virus, spike (S) protein preparations, virus-like particles (VLPs), plasmid DNA and a number of vectors containing genes for SARS-CoV proteins [13–28]. Phase I studies in humans have been conducted with a whole virus vaccine and a DNA vaccine [29–30].

An early concern for application of a SARS-CoV vaccine was the experience with other coronavirus infections which induced enhanced disease and immunopathology in animals when challenged with infectious virus [31], a concern reinforced by the report that animals given an alum adjuvanted SARS vaccine and subsequently challenged with SARS-CoV exhibited an immunopathologic lung reaction reminiscent of that described for respiratory syncytial virus (RSV) in infants and in animal models given RSV vaccine and challenged naturally (infants) or artificially (animals) with RSV [32,33]. We and others described a similar immunopathologic reaction in mice vaccinated with a SARS-CoV vaccine and subsequently challenged with SARS-CoV [18,20,21,28]. It has been proposed that the nucleocapsid protein of SARS-CoV is the antigen to which the immunopathologic reaction is directed [18,21]. Thus, concern for proceeding to humans with candidate SARS-CoV vaccines emerged from these various observations.

The studies reported here were conducted to evaluate the safety, immunogenicity, and efficacy of different SARS-CoV vaccines in a murine model of SARS.

Materials and Methods

Tissue Cultures and Virus

Vero E6 tissue cultures [obtained from The American Type Culture Collection (ATCC), CRL:1586] were grown in Dulbecco's modified minimum essential medium (DMEM) supplemented with penicillin (100 units/ml), streptomycin (100 µg/ml), 0.2% sodium bicarbonate and 10% fetal bovine serum (FBS). The Urbani strain of SARS-CoV was obtained from T.G. Ksiazek at the Centers for Disease Control and Prevention (Atlanta, GA), and a working stock of this virus was prepared by serially passaging a portion of the seed virus three times (p3) in Vero E6 cultures. The culture fluid from infected cells was clarified by low-speed centrifugation, filtered through a 0.45 µm filter, aliquoted, and stored at −80°C.

Vaccines

Four different SARS-CoV vaccines were evaluated in these studies (Table 1). Two whole virus vaccines were evaluated; one was prepared in Vero tissue cultures, zonal centrifuged for purification, and double-inactivated with formalin and UV irradiation, the DI vaccine (DIV); it was tested with and without alum adjuvant [16]. The other whole virus vaccine was prepared in Vero cells, concentrated, purified, inactivated with beta propiolactone and packaged with alum adjuvant (BPV) [13]. A recombinant DNA spike (S) protein vaccine (SV) was produced in insect cells and purified by column chromatography was tested with and without alum adjuvant [17]. The fourth vaccine (the VLP vaccine) was a virus-like particle vaccine prepared by us as described previously; it contained the SARS-CoV spike protein (S) and the Nucleocapsid (N), envelope (E) and membrane (M) proteins from mouse hepatitis coronavirus (MHV) [20].

Animals

Six- to eight-week-old, female Balb/c and C57BL/6 mice (Charles River Laboratory, Wilmington, MA), were housed in cages covered with barrier filters in an approved biosafety level 3 animal facility maintained by the University of Texas Medical Branch (UTMB) at Galveston, Texas. All of the experiments were performed using experimental protocols approved by the Office of Research Project Protections, Institutional Animal Care and Use Committee (IACUC), University of Texas Medical Branch and followed National Institutes of Health and United States Department of Agriculture guidelines.

Study Design

Three different experiments, performed for comparing different vaccines, are reported here. Adjuvanted (alum) and non-adjuvanted (PBS) vaccines were obtained from the NIH/BEI resource. Groups of mice (N = 12–13 per group) were administered various dosages of each vaccine intramuscularly (IM) on days 0 and 28; mice given only PBS, alum, trivalent inactivated influenza vaccine or live SARS-CoV were included as controls in various experiments. On day 56, five mice from each group were sacrificed for assessing serum neutralizing antibody titers and lung histopathology; the remaining seven or eight mice in each group were challenged with 10^6TCID$_{50}$/60 µl of SARS-CoV intranasally (IN). Challenged mice were euthanized on day 58 for determining virus quantity and preparing lung tissue sections for histopathologic examination.

Neutralizing Antibody Assays

Mice were anesthetized with isoflurane and then bled from the retro-orbital sinus plexus. After heat inactivation at 56°C for 30 minutes, sera were stored at −80°C until tested. Assays for virus-specific neutralizing antibodies were performed on serial 2-fold diluted samples of each serum using 2% FBS-DMEM as the diluent in 96-well tissue culture plates (Falcon 3072); the final volume of the serially diluted samples in each well was 60 µl after addition of 120 TCID$_{50}$ of SARS-CoV in 60 µl into each well. The beginning dilution of serum was 1:20. The dilutions were incubated for 45–60 minutes at room temperature; then 100 µl of each mixture was transferred into duplicate wells of confluent Vero E6 cells in 96-well microtiter plates. After 72 hours of incubation, when the virus control wells exhibited advanced virus-induced CPE, the neutralizing capacity of individual serum samples were assessed by determining the presence or absence of cytopathic effect (CPE). Neutralizing antibody titers were expressed as the reciprocal of the last dilution of serum that completely inhibited virus-induced CPE.

Collection and Processing of Lungs for Histology and Virus Quantity

Two days post SARS-CoV challenge, mice were euthanized and their lungs were removed. Lung lobes were placed in 10% neutral buffered formalin for histological examination and immunohistochemistry (IHC), as described previously [34,35]. For virus quantitation, the remaining tissue specimen was weighed and frozen to −80°C. Thawed lung was homogenized in PBS/10% FBS solution using the TissueLyser (Qiagen; Retsch, Haan, Germany). The homogenates were centrifuged and SARS-CoV titers in the clarified fluids were determined by serial dilution in quadruplicate wells of Vero E6 cells in 96-well plates. Titers of virus in lung homogenates were expressed as TCID$_{50}$/g of lung ($\log_{10}$); the minimal detectable level of virus was 1.6 to 2.6 $\log_{10}$ TCID$_{50}$ as determined by lung size.

Table 1. Experimental Groups for Evaluation of SARS Coronavirus Vaccines.

Group	Exp 1[1] Vaccine Comparisons	Exp 2[1] Higher SV Dosage plus DIV and BPV Comparisons	Exp 3[1,3] Mouse and Vaccine Specificity
1	DIV/1 μg[2]	PBS	PBS-PBS
2	DIV/0.5 μg	Live virus	PBS
3	DIV/0.25 μg	SV/9 μg	Live virus
4	DIV/0.125 μg	SV/3 μg	Flu vaccine
5	DIV/1 μg + alum	SV/1 μg	DIV/1 μg
6	DIV/0.5 μg + alum	SV/9 μg + alum	DIV/1 μg + alum
7	DIV/0.25 μg + alum	SV/3 μg + alum	BPV/undil + alum
8	DIV/0.125 μg + alum	SV/1 μg + alum	PBS-PBS
9	SV/2 μg[2]	DIV/1 μg	PBS
10	SV/1 μg	DIV/0.25 μg (50 μl)	Live virus
11	SV/0.5 μg	DIV/1 μg + alum	Flu vaccine
12	SV/0.25 μg	DIV/0.25 μg + alum (50 μl)	DIV/1 μg
13	SV/2 μg + alum	BPV/undil + alum[2]	DIV/1 μg + alum
14	SV/1 μg + alum	BPV/undil + alum (25 μl)	BPV/undil + alum
15	SV/0.5 μg + alum		
16	SV/0.25 μg + alum		
17	VLP/2 μg[2]		
18	VLP/2 μg + alum		
19	Alum		
20	PBS		

[1]Design = All experiments in Balb/c mice except as noted in Exp 3. Each group contained 12–13 mice; all were given 100 μl of vaccine IM at dosages with or without alum as indicated on days 0 and 28 except as noted. Five mice in each group were sacrificed on day 56 for serum antibody; remaining mice were given 10^6 $TCID_{50}$ of SARS-CoV intranasal on day 56 and sacrificed on day 58 for virus and lung histology.
[2]DIV/dosage = Vaccine DIV = Zonal centrifuge purified doubly inactivated (formalin and UV) whole virus SV/dosage = Vaccine SV = Recombinant baculovirus expressed S glycoprotein of SARS-CoV VLP/dosage = Vaccine VLP = Virus-like particles containing SARS-CoV S glycoprotein and E, M, and N proteins from mouse hepatitis coronavirus BPV/dosage = Vaccine BPV = Purified beta propiolactone inactivated whole virus plus alum.
[3]Experiment 3 = Groups 1 to 7 were Balb/c mice; groups 8 to 14 were C57BL/6 mice. Flu vaccine was licensed trivalent 2009-10 formulation of high dosage vaccine (60 μg of HA of each strain). Groups 1 and 8 were given PBS (placebo) and challenged with PBS; all others were challenged with live SARS-CoV.
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Histopathology

Evaluations for histopathology were done by pathologists masked as to the vaccine/dosage of each specimen source; numeric scores were assigned to assess the extent of pathologic damage and the eosinophilic component of the inflammatory infiltrates.

Statistical Analysis

Neutralizing antibody titers, lung virus titers, histopathologic lesion score and eosinophilic infiltration scores were averaged for each group of mice. Comparisons were conducted using parametric and nonparametric statistics as indicated.

Results

Experiments

The three experiments performed, vaccines and dosages used and controls for each experiment are shown in Table 1. The vaccines were evaluated for immunogenicity and efficacy; however, because of the previous report of immunopathology on challenge of ferrets and nonhuman primates that had been vaccinated with a whole virus adjuvanted vaccine and mice that had been vaccinated with a VLP vaccine, the primary orientation was to assess for immunopathology among animals in relation to type of vaccine, dosage, serum antibody responses, and virus infection. The vaccine preparations were made for human trials so identifying a preparation that was likely to be both safe and protective in humans was desired. The rationale for each experiment is described.

Comparison of Vaccines (Experiment 1). To differentiate between vaccines, three vaccine preparations were simultaneously evaluated, the double-inactivated (formalin and UV) whole virus vaccine (DIV), the rDNA-expressed S protein vaccine (SV), and the previously evaluated chimeric viral-like particle vaccine (VLP) that had led to immunopathology with virus challenge [16,17,20].

Geometric mean serum neutralizing antibody titers for each group on day 56 are shown in figure 1A. Geometric mean titers for those given a nonadjuvanted or alum adjuvanted vaccine were not different for the double-inactivated whole virus vaccine (DIV), and the VLP vaccine, ($p>0.05$, student's t-test), but were different for the S protein vaccine (SV) ($p = 0.001$, student's t test). Geometric mean titers for the different dosage groups given the DI vaccine (DIV) with alum and those for the groups given the S protein vaccine (SV) with or without alum were significantly different ($p = 0.007$, $p = 0.028$, and $p = 0.01$, respectively, Kruskall-Wallis) while the geometric means for those dosage groups given the DI vaccine (DIV) without alum were not ($p>0.05$, Kruskall-Wallis). In a multiple regression analysis, postvaccination titers for the DI vaccine (DIV) were significantly increased by both alum and higher dosage (for alum, $p = 0.012$, for dosage, $p < 0.001$); for the S protein vaccine (SV), only alum increased responses ($p = 0.001$).

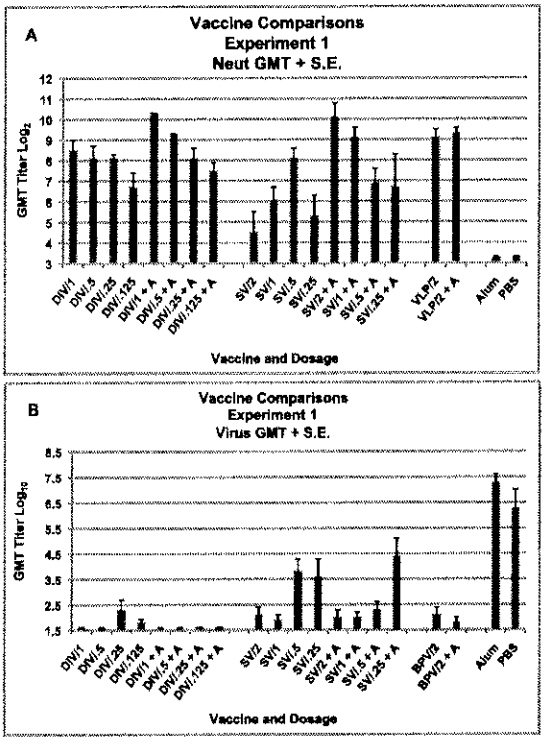

Figure 1. Vaccine Comparisons of Three SARS-CoV Vaccines, Experiment 1. Serum neutralizing (neut) antibody and lung virus titers for each vaccine dosage group. A. Geometric mean serum antibody titer as log_2 and standard error of the mean (S.E.) on day 56 for each vaccine dosage group. Seven to eight mice per group. Vaccines: double inactivated whole virus (DIV), recombinant S protein (SV), viral-like particle vaccine (VLP), with alum (+A). Five mice per group were given 0.1 ml of vaccine intramuscularly on days 0 and 28. B. Geometric mean virus titer (log_{10} $TCID_{50}$/g) and standard error of the mean (S.E.) in lungs on day 58 (two days after SARS-CoV challenge) for each vaccine dosage group. Analyses: A. GMT with compared to without alum: DIV $p>.05$, VLP $p>.05$, SV $p=.001$. GMT for different vaccine dosage: DIV with alum $p=.007$, DIV without alum $p>.05$, SV with alum $p=.028$, SV without alum $p=.01$. Multiple regression: GMT increased for alum $p=.012$ and dosage $p<.001$, for SV alum only $p=.001$. B. GMT for all DIV groups not different $p>.05$, GMT for SV group without alum p .008 and with alum p .023. GMT for VLP group is not different $p>.05$.
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Two days after challenge, lungs were obtained from all animals for virus quantitation and histology. CoV titers are shown in figure 1B. Geometric mean lung titers in the alum and PBS control groups were $10^{7.3}$ and $10^{6.3}$ $TCID_{50}$/g, respectively. All vaccine groups exhibited lower titers or no detectable virus on day two after challenge. None of the animals given any of the alum-adjuvanted DI vaccine (DIV) dosages and only an occasional animal in the lower dosages of nonadjuvanted vaccine yielded virus (Kruskall-Wallis and Mann Whitney U tests, $p>0.05$ for all comparisons). All groups given the S protein vaccine (SV) yielded virus after challenge and the differences between groups were significant ($p=0.002$ for all groups, $p=0.023$ for alum and $p=0.008$ for no adjuvant, Kruskall-Wallis); also, geometric mean titers were higher for the groups given lower vaccine dosages. Geometric mean titers for the VLP vaccine groups were similar ($p>0.05$).

In the vaccine comparison experiment, lung lesion scores for histopathology were graded for individual animals on a scale of 0 to 4 where 0–2 represented degree of cellular infiltration and 3–4 represented the degree of bronchiolar epithelial cell necrosis and airway cellular debris (figure 2A). As shown, all animals exhibited pathologic changes after challenge including those animals with no measurable virus on day two suggesting virus infection had occurred but was not detectable on day two because of a short duration of infection or neutralization of virus by antibody in the lung during processing. The higher scores (>3) in some groups related primarily to the fact that virus infection had induced inflammatory infiltrates and epithelial cell necrosis with desquamation of the epithelium and collection of cellular debris in airways of these animals. Mean score differences were noted among the various vaccines ($p=<0.001$, Anova). Those groups given the DI vaccine (DIV) without alum had higher mean scores than did those given DI vaccine (DIV) with alum ($p=0.001$, Mann-Whitney U); similarly, the group given the VLP vaccine without alum had a higher mean score than for those given VLP vaccine with alum ($p=0.008$, Mann-Whitney U). Post hoc comparisons for the three different vaccines indicated that the DI vaccine (DIV) group overall had lower lesion scores than either the S protein vaccine (SV) group or the alum and PBS control groups ($p=0.001$ comparing the DI and S protein vaccines (DIV and SV) and $p<0.001$ for DIV vs. control groups, Tukey HSD and Dunnett t, respectively), but not the VLP vaccine group ($p>0.05$, Tukey HSD). The S protein vaccine group (SV) was also lower overall than the control groups ($p=0.048$, Dunnett t).

When the characteristics of the infiltrates were compared, animals given alum or PBS exhibited epithelial cell necrosis and peribronchiolar and perivascular mononuclear cell infiltrates consistent with epithelial cell infection and an inflammatory response seen in viral infections. In addition to mononuclear cells, however, infiltrates among vaccinated animals contained neutrophils and eosinophils that were not seen in the lesions of the animals that had been previously given PBS or alum only (figure 2B) suggesting a T helper cell type 2 hypersensitivity reaction; increased eosinophils are a marker for a Th2-type hypersensitivity reaction. Percent eosinophils was lower in these vaccinated animals (mean 1–3.2%) than had been seen in animals given VLP vaccines in the earlier study (mean $13.2\pm9.6\%$ and $22\pm9.9\%$ of cells for VLP with PBS or alum, respectively in that study) but no (0%) eosinophils were seen in the lung infiltrates of control animals in this experiment. This pattern of excess eosinophils in cellular infiltrates seen in lung sections from animals given vaccine and not in control animals was as seen in the earlier study with VLP vaccine and those later with other vaccines although the percent eosinophils was lower in this study.

The mean percent eosinophils differed between groups ($p<0.001$, Anova). Overall, the percent was lower for the groups given the DI and S protein alum adjuvanted vaccines than for the corresponding nonadjuvanted group ($p=0.049$ for DIV and 0.001 for SV, Mann-Whitney U). For the vaccines, the eosinophil mean percentages were lower for the S protein vaccine (SV) than for either the DI vaccine (DIV) or VLP vaccine (DIV vs. SV, $p=0.002$; VLP vs. SV, $p=<0.001$, Tukey HSD). Additionally, eosinophil percentages for all three vaccines, including the S protein vaccine, were significantly greater than the controls (SV, DIV and VLP vaccine, $p<0.001$ for each, Tukey HSD).

Higher Dosages of the S Protein Vaccine Plus the bp Inactivated Whole Virus Vaccine, Experiment 2. This experiment was conducted to verify the findings in the initial

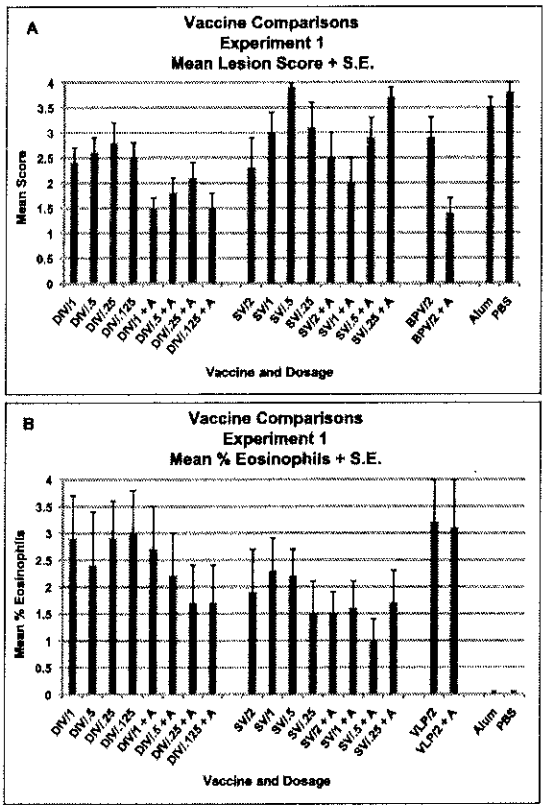

Figure 2. Vaccine Comparisons of Three SARS-CoV Vaccines, Experiment 1. Mean lung cellular infiltration/lesion pathology and percent eosinophils in infiltrates for each vaccine dosage group two days after challenge with SARS-CoV. A. Mean lesion score and standard error of the mean (S.E.) for each vaccine dosage group. All mice exhibited lung histopathology. Scores are mean of scores for seven to eight mice per group. Scoring. 0 – no pathology, 1 and 2 – (1) minimal (2) moderate peribronchiole and perivascular cellular infiltration, 3 and 4 – 1 and/or 2 plus minimal (3) or moderate (4) epithelial cell necrosis of bronchioles with cell debris in the lumen. B. Mean percent eosinophils on histologic evaluation for seven to eight mice in each vaccine dosage group. Mean for each mouse is the mean percent eosinophils on five separate microscopy fields of lung sections. Analyses: A. Mean lesion scores were different $p<.001$. DIV without alum greater than with alum $p=.001$, VLP without alum greater than with alum $p=.008$. Posthoc comparisons: DIV lower than SV $p=.001$ and controls $p<.001$ but not VLP $p>.05$. SV lower than controls p .048. B. Mean percent eosinophils were different $p<.001$. Mean percent eosinophils lower for DIV with alum than without alum $p=.049$ and lower for SV with alum than without alum $p=.001$. Mean percent eosinophils lower for SV than DIV $p=.002$ or VLP. $P=<.001$. Mean percent eosinophils greater than controls for DIV, SV and VLP, all three vaccines $p<.001$.
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experiment of a hypersensitivity immunopathologic-like reaction after SARS-CoV challenge of vaccinated animals, to determine if a higher dosage of the S protein vaccine (SV) would suppress infection and still exhibit a similar reaction, and whether the original β propiolactone inactivated whole virus vaccine (BPV) that had shown an immunopathologic-like reaction after challenge of vaccinated ferrets and nonhuman primates exhibited a similar immunopathologic reaction in the mouse model [13,14]. Additionally, a live virus "vaccination" group was added in this experiment for comparison of challenge results following vaccinations with inactivated vaccines to those following earlier infection.

Serum neutralizing antibody responses are shown in figure 3A. The bp inactivated vaccine (BPV), was only available at one dosage with alum so a smaller volume (25 μl) was given to one group for a dosage comparison. Geometric mean titers for the groups given the alum adjuvanted version of the DI and the S protein vaccines were greater than for the unadjuvanted vaccine (DIV $P=0.014$, SV $p<0.001$, student's t test). In multiple regression analysis, titers were also significantly increased after both the DI and S protein vaccines with use of alum ($p\leq0.01$); no dosage effect was noted. The geometric mean neutralizing antibody titers of the two bp inactivated vaccine groups (BPV) were different ($p=0.039$, Mann-Whitney U).

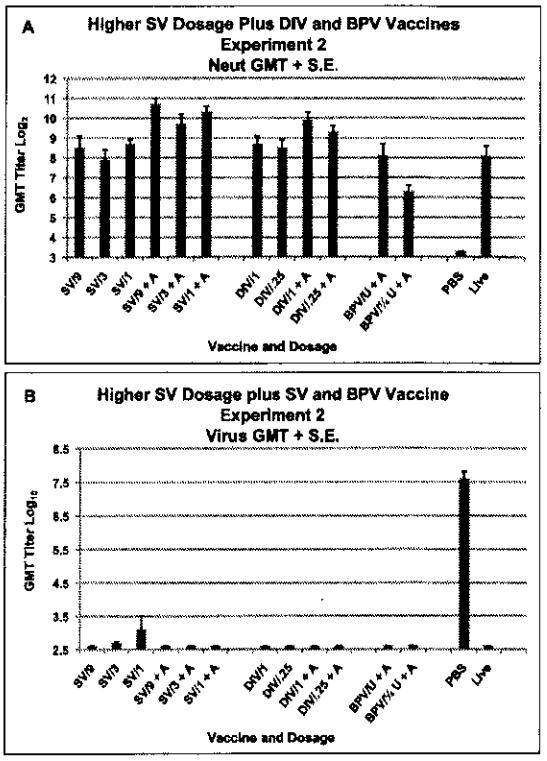

Figure 3. Higher Dosages of SV Vaccine plus DIV and BPV Vaccine Comparisons, Experiment 2. Serum neutralizing (neut) antibody and lung virus titers for each vaccine dosage group. A. Geometric mean serum antibody titer and standard error of the mean (S.E.) on day 56 for each vaccine dosage group. Five mice per group given 0.1 ml of vaccine intramuscularly on days 0 and 28. B. Geometric mean virus titer (log_{10} $TCID_{50}$/g) and standard error of the mean (S.E.) in lungs on day 58 (two days after SARS-CoV challenge) for each vaccine dosage group. Seven to eight mice per group. Vaccines: double inactivated whole virus (DIV), recombinant S protein (SV), β propiolactone inactivated whole virus (BPV) with alum (+A). Analyses: A. GMT with alum greater than without alum: SV $p<.001$, DIV $p=.014$. GMT for the two BPV groups are different $p=.039$. Multiple regression: DIV and SV increased with alum $p\leq.01$, no dosage effect $p>.05$.
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Two days after challenge with 10^6 $TCID_{50}$ of SARS-CoV, titers in mice given PBS varied between $10^{7.0}$ and $10^{8.0}$ $TCID_{50}$ per g of tissue; one vaccinated animal in the group given the S protein vaccine (SV) at the 3 μg and the 1 μg dosage without alum yielded virus but all other animals in all other groups were culture negative for virus (figure 3B).

Shown in figure 4A are the mean lesion scores on histologic evaluations. The scoring system for experiments two and three were developed by a replacement pathologist who preferred a scale of 0 to 3 which corresponded to a judgment of mild, moderate or severe (figure 4A). Mean lesion scores for this grading system overall were significantly different from each other ($p<0.001$, Anova) and scores were lower for the S protein vaccine than for either of the whole virus vaccines (SV versus DIV and BPV, $p<0.001$ and $p=0.006$, respectively, Tukey HSD). Of interest is that those given live virus and then challenged with live virus two months later exhibited an infiltrative disease severity comparable to the PBS and vaccinated groups despite no detectable virus on day two, again suggesting some degree of infection may have occurred earlier.

The mean eosinophil scores for the lung infiltrations were lower for the S protein vaccine groups [SV vs. DIV $p<0.001$; SV vs. BPV, $p<0.001$, Tukey HSD]; however, they were clearly greater than seen in those given PBS or live virus earlier ($p<0.001$, Tukey HSD) (figure 4B).

Representative photo micrographs of lung sections from mice in this experiment two days after challenge with SARS-CoV are shown in figure 5. The pathologic changes were extensive and similar in all challenged groups (H & E stains). Perivascular and peribronchial inflammatory infiltrates were observed in most fields along with desquamation of the bronchial epithelium, collections of edema fluid, sloughed epithelial cells, inflammatory cells and cellular debris in the bronchial lumen. Large macrophages and swollen epithelial cells were seen near lobar and segmental bronchi, small bronchioles and alveolar ducts. Necrotizing vasculitis was prominent in medium and large blood vessels, involving vascular endothelial cells as well as the tunica media, and included lymphocytes, neutrophils, and eosinophils in cellular collections. Occasional multinucleated giant cells were also seen. The eosinophil component of infiltrates was very prominent in animals vaccinated with the experimental vaccine preparations when compared to animals mock-vaccinated using PBS, or those exposed earlier to live virus (figure 6); few to no eosinophils were seen in those lung sections. Thus, while pathology was seen in sections from the control mice, the hypersensitivity-type pathologic reaction with eosinophils was not seen. The morphological identification of eosinophils in H&E stains was supported by using Giemsa stain to highlight intracytoplasmic granules in selected lung sections (not shown), and confirmed by immunostaining with antibodies against mouse eosinophil major basic protein (provided by the Lee Laboratory, Mayo Clinic, Arizona) [36].

The different groups of vaccinated animals showed similar trends in severity of pathology and of eosinophils in inflammatory infiltrates; however, the DIV and BPV preparations at high dosage tended to produce a greater infiltration with eosinophils.

Mouse and Vaccine Specificity (Experiment 3). Experiment 3 was performed to evaluate vaccine and mouse strain specificity. SARS-CoV vaccines used were the DI vaccine (DIV) with and without alum and the bp inactivated vaccine (BPV), which contains alum, at the highest dosage. For mouse strain specificity, Balb/c mice were included for consistency between experiments; C57BL/6 mice were given the same vaccines and dosages as Balb/c mice for comparison as C57BL/6 mice do not exhibit a bias for Th2 immunologic responses as do

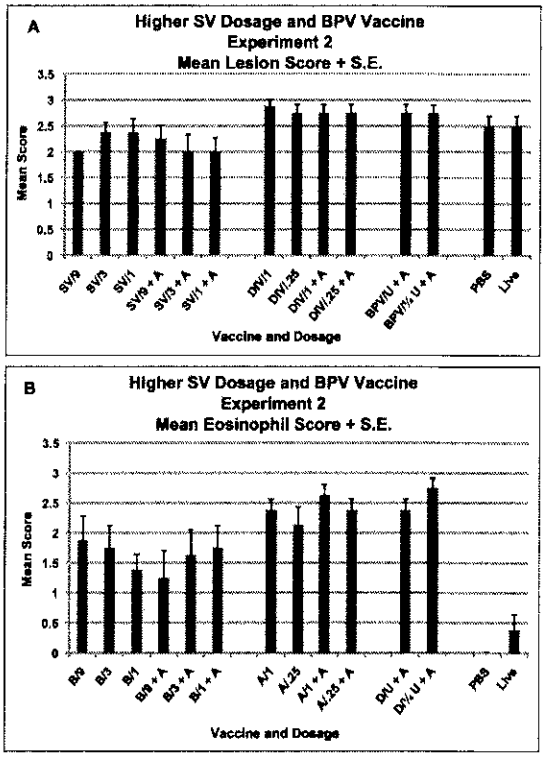

Figure 4. Higher Dosages of SV Vaccine plus DIV and BPV Vaccine Comparisons, Experiment 2. Mean lung cellular infiltration/lesion pathology and mean percent eosinophils in infiltrates for each vaccine dosage group two days after challenge with SARS-CoV. A. Mean lesion score and standard error of the mean (S.E.) for each vaccine dosage group. Scores are mean of scores for seven to eight mice per group. Scoring - 0 - no definite pathology, 1 - mild peribronchiole and perivascular cellular infiltration, 2 - moderate peribronchiole and perivascular cellular infiltration, 3 - severe peribronchiolar and perivascular cellular infiltration with thickening of alveolar walls, alveolar infiltration and bronchiole epithelial cell necrosis and debris in the lumen. Ten to 20 microscopy fields were scored for each mouse lung. B. Mean score and standard error of the mean (S.E.) for eosinophils as percent of infiltrating cells for each vaccine dosage group. Scores are mean of scores for seven to eight mice per group. Scoring: 0 - <5% of cells, 1 - 5–10% of cells, 2 - 10–20% of cells, 3 - >20% of cells. Ten to 20 microscopy fields were scored for each mouse lung. Analyses: A. Mean lesion scores were different $p<.001$. Mean scores were lower for SV than DIV $p<.001$ and less than BPV $p=.006$. B. Mean eosinophil scores were lower for SV than DIV $p<.001$ and less than BPV $p<.001$. Eosinophil scores greater for SV than PBS or live virus $p<.001$.
doi:10.1371/journal.pone.0035421.g004

Balb/c mice [37–39]. PBS and live virus controls were again included and trivalent 2010-11 formulation influenza vaccine at a dosage of 12 μg per component was given to assess vaccine specificity.

Neutralizing antibody titers are shown in figure 7A. Geometric mean titers for the highest dose of the DI vaccine were higher for those vaccine groups in the Balb/c mice than the C57BL/6 mice but only the nonadjuvanted DI vaccine group was significantly higher ($p=0.008$, Mann Whitney U). The serum antibody responses after BPV and live virus administration were similar for the two mouse strains. After challenge, mean lung virus titers

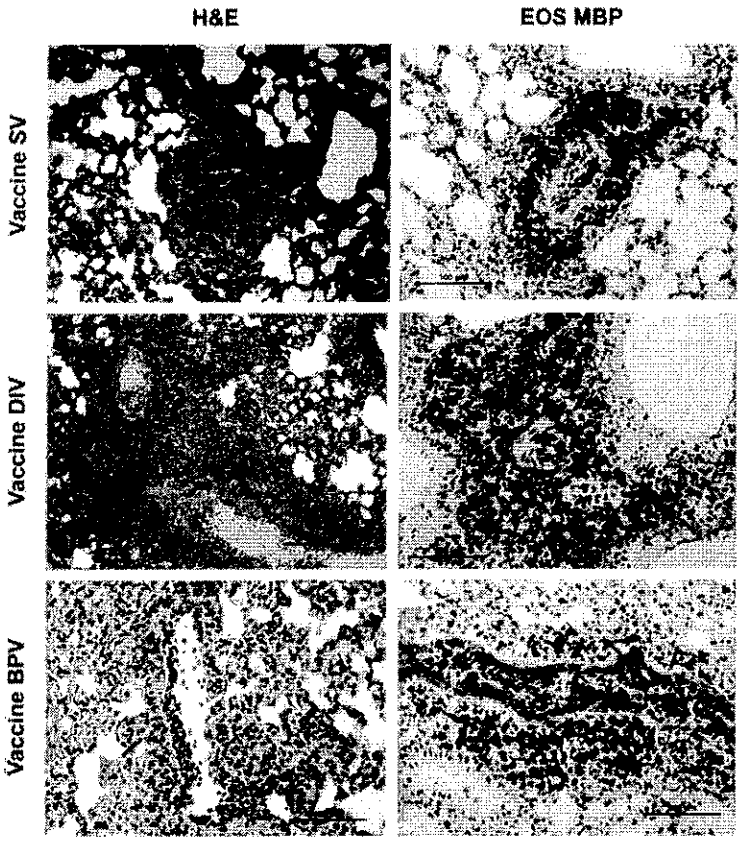

Figure 5. Photographs of Lung Tissue. Representative photomicrographs of lung tissue two days after challenge of Balb/c mice with SARS-CoV that had previously been given a SARS-CoV vaccine. Lung sections were separately stained with hematoxylin and eosin (H&E) and an immunohistochemical protocol using an eosinophil-specific staining procedure with a monoclonal antibody to a major basic protein of eosinophils. DAB chromogen provided the brown eosinophil identity stain. The procedure and antibody were kindly provided by the Lee Laboratory, Mayo Clinic, Arizona. The H&E stain column is on the left and eosinophil-specific major basic protein (EOS MBP) stain column is on the right. Vaccines: double inactivated whole virus (DIV), β propiolactone inactivated whole virus vaccine (BPV). As shown in the images, eosinophils are prominent (brown DAB staining) in all sections examined. Exposure to SARS-CoV is associated with prominent inflammatory infiltrates characterized by a predominant eosinophilic component.
doi:10.1371/journal.pone.0035421.g005

were similar for the PBS control challenged mice of both mouse strains ($10^{6.7\ 7.3}$ $TCID_{50}$/g lung) (figure 7B). None of the Balb/c mouse groups given either vaccine or live virus earlier yielded virus after challenge but some virus was detected in C57BL/6 mice given the DIV without alum and the BPV with alum (C57BL/6 versus Balb/c, $p = 0.004$, Mann Whitney U).

Mean lung lesion scores two days after challenge were similar for all groups and indicated a moderate to severe degree of cellular infiltration ($p>0.05$ for each, Anova) (figure 8A). However, eosinophil scores were significantly different between groups ($p<0.001$, Anova) with significantly lower scores for nonvaccine groups than for vaccine groups of both mouse strains ($p<0.001$ for all comparable group comparisons, Tukey's HSD). Eosinophil scores for the vaccine groups were not different between the two mouse strains ($p>0.05$, t test) (figure 8B). Photomicrographs of the different vaccine and mouse strain groups are shown in figure 9. Both vaccines in both mouse strains exhibited significant cellular infiltrations that included numerous eosinophils as shown in the MBP stained sections, a finding consistent with a hypersensitivity component of the pathology. Prior influenza vaccine did not lead to an eosinophil infiltration in the lung lesions after challenge.

Discussion

The emergence of the disease SARS and the rapid identification of its severity and high risk for death prompted a rapid mobilization for control at the major sites of occurrence and at the international level. Part of this response was for development of vaccines for potential use in control, a potential facilitated by the rapid identification of the causative agent, a new coronavirus [8–9]. Applying the principles of infection control brought the epidemic under control but a concern for reemergence naturally or a deliberate release supported continuation of a vaccine development effort so as to have the knowledge and capability necessary for preparing and using an effective vaccine should a need arise. For this purpose, the National Institute of Allergy and Infectious Diseases supported preparation of vaccines for evaluation for potential use in humans. This effort was hampered by the

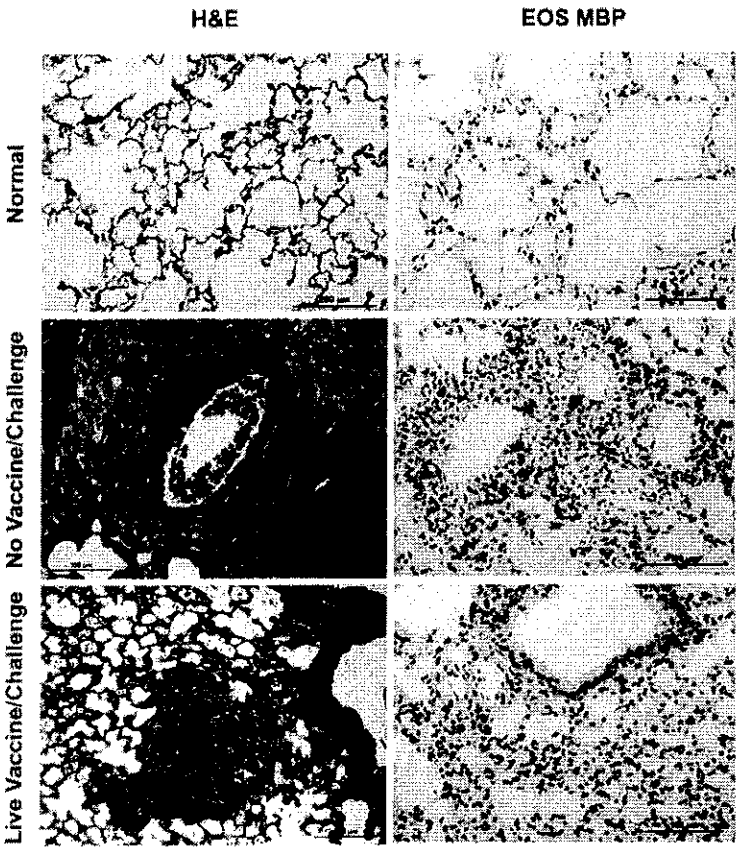

Figure 6. Photomicrographs of Lung Tissue. Representative photomicrographs of lung tissue from unvaccinated unchallenged mice (normal) and from Balb/c mice two days after challenge with SARS-CoV that had previously been given PBS only (no vaccine) or live virus. H&E and immunohistochemical stains for eosinophil major basic protein were performed as described for figure 5. The H&E column is on the left and the Eos MBP column is on the right. Shown are sections from normal mice (no vaccine or live virus) and mice given PBS (no vaccine) or live SARS-CoV and then challenged with SARS-CoV. As shown in the middle and bottom row images, although exposure to SARS-CoV elicits inflammatory infiltrates and accumulation of debris in the bronchial lumen, eosinophils in all groups remain within normal limits.
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occurrence in the initial preclinical trial of an immunopathogenic-type lung disease among ferrets and Cynomolgus monkeys given a whole virus vaccine adjuvanted with alum and challenged with infectious SARS-CoV [14]. That lung disease exhibited the characteristics of a Th2-type immunopathology with eosinophils in the lung sections suggesting hypersensitivity that was reminiscent of the descriptions of the Th2-type immunopathologic reaction in young children given an inactivated RSV vaccine and subsequently infected with naturally-occurring RSV [32–33]. Most of these children experienced severe disease with infection that led to a high frequency of hospitalizations; two children died from the infection [33,40,41]. The conclusion from that experience was clear; RSV lung disease was enhanced by the prior vaccination. Subsequent studies in animal models that are thought to mimic the human experience indicate RSV inactivated vaccine induces an increased $CD4^+$ T lymphocyte response, primarily of Th2 cells and the occurrence of immune complex depositions in lung tissues [32,42,43]. This type of tissue response is associated with an increase in type 2 cytokines including IL4, IL5, and IL13 and an influx of eosinophils into the infected lung; [32,33,42,44]. Histologic sections of tissues exhibiting this type of response have a notable eosinophilic component in the cellular infiltrates. Recent studies indicate that the Th2-type immune response has both innate and adaptive immune response components [33,43].

In addition to the RSV experience, concern for an inappropriate response among persons vaccinated with a SARS-CoV vaccine emanated from experiences with coronavirus infections and disease in animals that included enhanced disease among infected animals vaccinated earlier with a coronavirus vaccine [31]. Feline infectious peritonitis coronavirus (FIPV) is a well-known example of antibody-mediated enhanced uptake of virus in macrophages that disseminate and increase virus quantities that lead to enhanced disease [31,45]. Antigen-antibody complex formation with complement activation can also occur in that infection and some other coronavirus infections in animals. Thus, concern for safety of administering SARS-CoV vaccines to humans became an early concern in vaccine development.

As a site proposed for testing vaccines in humans, we requested and were given approval for evaluating different vaccine candidates for safety and effectiveness. Two whole coronavirus

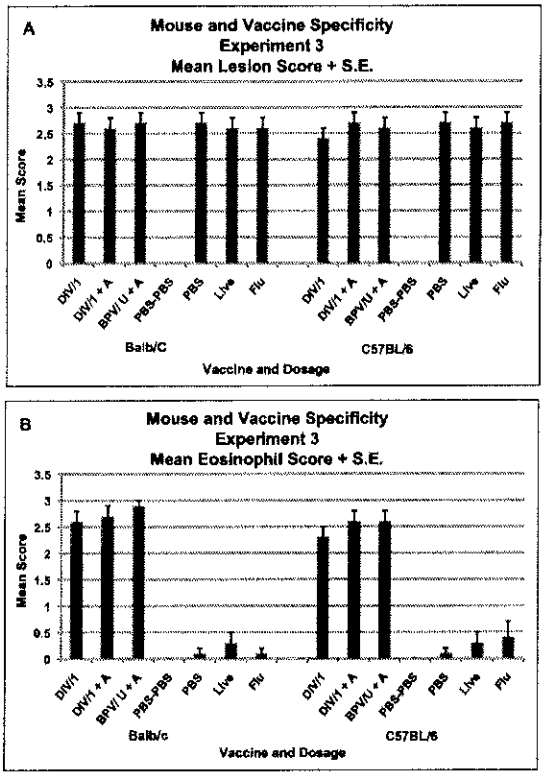

Figure 7. Mouse and Vaccine Specificity, Experiment 3. Serum neutralizing (neut) antibody and lung virus titers for each vaccine dosage group. A. Geometric mean serum antibody titer and standard error of the mean (S.E.) on day 56 for each vaccine dosage group for each mouse strain (Balb/c or C57BL/6). Five mice per group given 0.1 ml of vaccine intramuscularly on days 0 and 28. B. Geometric mean virus titer (log_{10} $TCID_{50}$/g) and standard error of the mean (S.E.) in lungs on day 58 (two days after SARS-CoV challenge for each vaccine dosage group for each mouse strain. Seven to eight mice per group. Vaccines: Double inactivated whole virus, (DIV), β propiolactone inactivated whole virus (BPV), with alum (+A). Analyses: A. GMT for highest DIV dosage without alum greater for Balb/c than C57BL/6 $p = .008$ but not for alum $p > .05$. GMT for the BPV vaccine and live virus were not different for the two strains $p > .05$. B. GMT for PBS control mice were not different $p > .05$. GMT for DIV without alum and BPV with alum greater for C57BL/6 than Balb/c $p = .004$.
doi:10.1371/journal.pone.0035421.g007

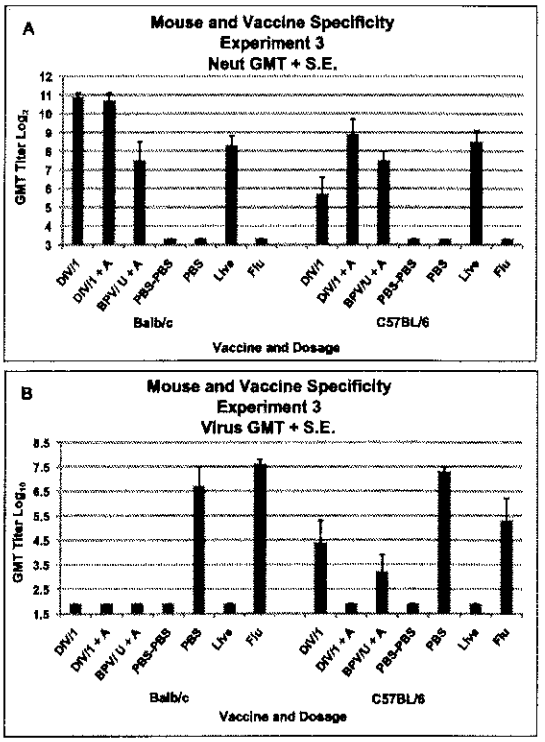

Figure 8. Mouse and Vaccine Specificity, Experiment 3. Mean lung cellular infiltration/lesion pathology and percent eosinophils in infiltrates for each vaccine dosage group for each mouse strain (Balb/c or C57BL/6) two days after challenge with SARS-CoV. A. Mean lesion score and standard error of the mean (S.E.) for each vaccine dosage group. Scores are mean of scores for seven to eight mice per group. Scoring 0 - no definite pathology, 1 - mild peribronchiole and perivascular cellular infiltration, 2 - moderate peribronchiole and perivascular cellular infiltration, 3 - severe peribronchiole and perivascular cellular infiltration with thickening of alveolar walls, alveolar infiltration and bronchiole epithelial cell necrosis and debris in the lumen. Ten to 20 microscopy fields were scored for each mouse lung. B. Mean score and standard error of the mean (S.E.) for eosinophils as percent of infiltrating cells for each vaccine dosage group. Scores are mean of scores for seven to eight mice per group. Scoring: 0 - <5% of cells, 1 - 5–10% of cells, 2 - 10–20% of cells, 3 - >20% of cells. Ten to 20 microscopy fields were scored for each mouse lung. Analyses: A. Mean lesion scores were not different $p > .05$. B. Mean eosinophil scores were different $p < .001$. Mean scores for vaccine groups greater than non-vaccine groups for Balb/c and C57BL/6 $p < .001$ for all comparisons. Mean eosinophil scores for the same groups not different for Balb/c and C57BL/6 $p > .05$.
doi:10.1371/journal.pone.0035421.g008

vaccines, one rDNA-expressed S protein vaccine and a VLP vaccine prepared by us were evaluated in a Balb/c mouse model, initially described by others, of SARS-CoV [46,47]. The concern for an occurrence of lung immunopathology on challenge of mice vaccinated with an inactivated virus vaccine, as reported by Haagmans, et al. for ferrets and nonhuman primates, was seen by us after challenge of mice vaccinated with a SARS VLP vaccine [20]. This finding was duplicated in an experiment reported here and was also seen in mice vaccinated with a range of dosages of a double-inactivated whole virus vaccine (DIV) and an rDNA S protein vaccine (SV) although the immunopathologic reaction appeared reduced among animals given the S protein vaccine when compared to those given the whole virus vaccine. In later experiments, these findings were confirmed and the vaccine utilized by Haagmans, et al. was also shown to induce the immunopathology in mice. Thus, all four vaccines evaluated induced the immunopathology; however, all four also induced neutralizing antibody and protection against infection when compared to control challenged animals.

The immunopathology in all experiments in the present study occurred in the absence of detectable virus in lungs of mice two days after challenge with infectious virus. In two experiments, a live virus group subsequently challenged with live virus was included. These challenged animals also exhibited similar histopathologic changes after challenge although no infectious virus was detected in lungs on day two; however, in the latter case, the infiltrates were nearly 100%

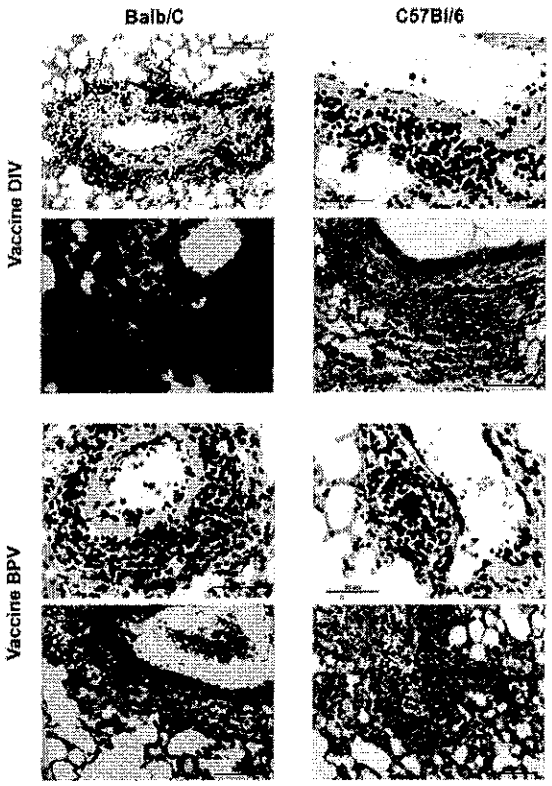

Figure 9. Photomicrographs of Lung Tissue. Representative photomicrographs of lung tissue two days after challenge of Balb/c and C57BL/6 mice that had previously been given a SARS-CoV vaccine. Lung sections were separately stained with H&E (pink and blue micrographs) or the immunohistochemical stain for eosinophil major basic protein (blue and brown micrographs). Balb/c mice lung sections are in the left column and C57BL/6 are in the right column; doubly inactivated whole virus vaccine is in the upper four panels and those from mice given the β propiolactone inactivated whole virus vaccine are in the lower four panels. Pathologic changes observed (inflammatory infiltrates) are similar in Balb/c and C57BL/6 and eosinophils are prominent in both groups.
doi:10.1371/journal.pone.0035421.g009

monocytes and lymphocytes without the eosinophil component seen in the vaccinated challenged animals. In a separate test to assess the effects of the challenge inoculum, mice were given an IN challenge with 10^{6}TCID$_{50}$ of inactivated whole SARS-CoV. Lungs of these animals revealed minimal or no histopathologic damage (data not shown). These findings suggest that virus replication probably occurred early after challenge, including in animals given live CoV earlier, and is required for development of pathology, including for the immunopathology. Infection would have been transient, below the limit of detection two days after challenge, or neutralized in lung homogenates before testing for virus.. Nevertheless, the Th2-type immunopathology pattern was seen only in animals given an inactivated vaccine earlier.

During the course of these experiments, a report appeared describing a similar immunopathologic-type reaction with prominent eosinophils in SARS-CoV challenged Balb/c mice that had been given Venezuelan equine encephalitis (VEE) vector containing the SARS nucleocapsid protein gene [18]. Those challenged animals exhibited infection similar to unvaccinated animals as well as Th2-type immunopathology. A similar experiment with a VEE vector containing only the S gene exhibited protection against infection and no immunopathology. More recently, this group has reported immunopathology with prominent eosinophil infiltration after SARS-CoV challenge in Balb/c mice vaccinated with the same double-inactivated whole virus vaccine used in our experiments [28]. They attribute the immunopathologic reaction following these SARS-CoV vaccinations to presence of the nucleocapsid protein (N) in the vaccine.

In another report, vaccinia was used as a vector vaccine for immunizing Balb/c mice with each of the SARS-CoV structural proteins (N, S, membrane, and envelope) and then challenged with SARS-CoV [21]. Virus infection was present in all groups after challenge but reduced in the S vector vaccine group. Histopathology scores were high for the N containing vector group and low for the S containing group and for the vehicle control group. Eosinophilic infiltrates and IL-5 were increased in the N vaccine group but only IL-5 was increased in the S vaccine group.

To be certain the Th2 type immunopathology was elicited by the S protein vaccine in our studies and in hopes a greater immune response would result from higher dosages of the vaccine and induce greater protection against infection as well as reduce or prevent the immunopathology, our experiment 2 used up to 9 μg of the S protein for immunization. While increased titers of serum antibody were induced and no virus was detected day two after challenge in most animals, the Th2-type immunopathology occurred after challenge, and the immunopathology seen earlier after vaccination with the DI whole virus vaccine was seen again. This experiment also included the whole virus vaccine tested earlier in ferrets and nonhuman primates where the Th2-type immunopathology was initially seen. That vaccine, the BPV in this report, exhibited a pattern of antibody response, protection against infection and occurrence of immunopathology after challenge similar to the DI whole virus vaccine (DIV).

A final experiment was conducted to evaluate specificity. The Balb/c mouse was compared to C57BL/6 mice which do not exhibit the Th2 response bias known to occur in Balb/c mice. C57BL/6 mice in that same experiment exhibited results on challenge similar to those seen in Balb/c mice. Challenge of animals given prior influenza vaccine were infected and exhibited histopathologic damage similar to animals given PBS earlier; neither group exhibited the eosinophil infiltrations seen in animals given a SARS-CoV vaccine.

In these various experiments alum was used as an adjuvant and this adjuvant is known to promote a Th2 type bias to immune responses [48]. However, the immunopathology seen in vaccinated-challenged animals also occurred in animals given vaccine without alum. In an effort to determine whether an adjuvant that induced a bias for a Th1-type response would protect and prevent the immunopathology, we initiated an experiment where the DI PBS suspended vaccine was adjuvanted with Freund's complete adjuvant, a Th1-type adjuvant. However, this experiment was aborted by the September, 2008, Hurricane Ike induced flood of Galveston, Texas. An experiment with a SARS-CoV whole virus vaccine with and without GlaxoSmithKline (GSK) adjuvant ASO1 in hamsters has been reported [25]. This adjuvant is thought to induce Th1-type immune responses [49]. The authors indicate no lung immunopathology was seen among animals after challenge, including the group given vaccine without adjuvant; however, whether the hamster model could develop a Th2-type immunopathology is uncertain. Finally, a number of other studies of vaccines in animal model systems have been reported but presence or absence of immunopathology after challenge was not reported.

Table 2. Summary of Reported Protection and Immunopathology In Animal Model Studies with SARS Coronavirus Vaccines.

Animal Model	Vaccine[1]	Protection[2]	Immunopathology[3]
Mice	Whole virus[tr]		
	w alum	Yes	Yes
	Whole virus[25,tr]		
	w alum	Yes	Yes
	wo alum	Yes	Yes
	VLP[17,tr]		
	w alum	Yes	Yes
	wo alum	Yes	Yes
	S Protein[tr]		
	w alum	Yes	Yes
	wo alum	Yes	Yes
	VEE Vector[15]		
	for N protein	No	Yes
	for S protein	Yes	No
	Vaccinia vector[18]		
	for N protein	No	Yes
	for S protein	Yes	?No
Ferrets	Whole virus[11]		
	w alum	Yes	Yes
Nonhuman Primate[4]	Whole virus[11]		
	w alum	Yes	Yes
Hamsters	Whole virus[22]		
	w ASO1	Yes	No

[1]Reference for each indicated; tr = this report; w = with, wo = without.
[2]Protection against infection (reduced lung virus after challenge).
[3]Th2-type immunopathology as indicated by cellular infiltrates with prominence of eosinophils.
[4]Cynomolgus monkeys.
doi:10.1371/journal.pone.0035421.t002

A summary of the SARS-CoV vaccine evaluations in animal models (including the current report) that indicated an evaluation for immunopathology after challenge is presented in Table 2. As noted all vaccines containing S protein induced protection against infection while the studies with VEE and vaccinia vector containing the N protein gene only did not. Also shown is that a Th2-type immunopathology was seen after challenge of all vaccinated animals when evaluation for immunopathology was reported except the study in hamsters with a GSK whole virus vaccine. Thus, inactivated whole virus vaccines whether inactivated with formalin or beta propiolactone and whether given with our without alum adjuvant exhibited a Th2-type immunopathologic in lungs after challenge. As indicated, two reports attributed the immunopathology to presence of the N protein in the vaccine; however, we found the same immunopathologic reaction in animals given S protein vaccine only, although it appeared to be of lesser intensity. Thus, a Th2-type immunopathologic reaction on challenge of vaccinated animals has occurred in three of four animal models (not in hamsters) including two different inbred mouse strains with four different types of SARS-CoV vaccines with and without alum adjuvant. An inactivated vaccine preparation that does not induce this result in mice, ferrets and nonhuman primates has not been reported.

This combined experience provides concern for trials with SARS-CoV vaccines in humans. Clinical trials with SARS coronavirus vaccines have been conducted and reported to induce antibody responses and to be "safe" [29,30]. However, the evidence for safety is for a short period of observation. The concern arising from the present report is for an immunopathologic reaction occurring among vaccinated individuals on exposure to infectious SARS-CoV, the basis for developing a vaccine for SARS. Additional safety concerns relate to effectiveness and safety against antigenic variants of SARS-CoV and for safety of vaccinated persons exposed to other coronaviruses, particularly those of the type 2 group. Our study with a VLP SARS vaccine contained the N protein of mouse hepatitis virus and Bolles, et al., reported the immunopathology in mice occurs for heterologous Gp2b CoV vaccines after challenge [25]. This concern emanates from the proposal that the N protein may be the dominant antigen provoking the immunopathologic reaction.

Because of well documented severity of the respiratory disease among infants given an inactivated RSV vaccine and subsequently infected with RSV that is considered to be attributable to a Th2-type immunopathologic reaction and a large number of studies in the Balb/c mouse model that have described and elucidated many components of the immunopathologic reaction to RSV vaccines, the similarity to the SARS-CoV vaccine evaluations in Balb/c mice supports caution for clinical vaccine trials with SARS-CoV vaccines in humans. Of interest are the similar occurrences in C57BL/6 mice and in ferrets and nonhuman primates that provide alternative models for elucidating vaccine-induced mechanisms for occurrences of Th2 immunopathologic reactions after infection. As indicated, strong animal model evidence indicates expression of the N protein by SARS-CoV vector vaccines can induce sensitization leading to a Th2 -type immunopathology with infection. In contrast to our results, those studies did not find clear evidence of the Th2 type immunopathology on challenge of mice given a vector vaccine for the S protein. The finding of a Th-2-type pathology in our studies in animals immunized with an rDNA-produced S protein is unequivocal. In this regard, animal model studies with FIPV in cats and RSV in mice have indicated that viral surface proteins may be the sensitizing protein of inactivated vaccines for immunopathology with infection [32,45]. This suggests that presentation of the S protein in a vector format may direct immune responses in a different way so that sensitization does not occur.

Limitations of the present studies include their performance in mice only and uncertainty of the relevance of rodent models to SARS-CoV vaccines in humans. Additionally, a more intense study for virus replication including quantitative RT-PCR assays might have confirmed the probability that virus replication is required for induction of the immunopathology after vaccination. Evaluations of mechanisms for the immunopathology, including immunoglobulin and cytokine responses to vaccines and tests for antigen-antibody complexes in tissues exhibiting the reaction, could have strengthened the Th2-type immunopathology finding. Finally, a successful study with a Th1-type adjuvant that did not exhibit the Th2 pathology after challenge would have confirmed a Th2 bias to immune responses as well as provide a potential safe vaccination approach for SARS.

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Author Contributions

Conceived and designed the experiments: RBC CJP C-TT. Performed the experiments: C-TT ES NI-Y PGN TG. Analyzed the data: RLA RBC C-TT. Contributed reagents/materials/analysis tools: RBC C-TT RLA ES. Wrote the paper: RBC C-TT ES.

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The failed negotiations mean Argentinian citizens, unlike those in neighbouring countries, do not have access to Pfizer's vaccine, leaving them with Russia's Sputnik V vaccine, AstraZeneca's vaccine and those delivered through Covax. The government is also negotiating to acquire vaccines from Moderna, Sinopharm and CanSino.

"Pfizer misbehaved with Argentina," said Ginés González Garcia, Argentina's minister of health. "Its intolerance with us was tremendous."

The same demands were made of Brazil's Ministry of Health. Pfizer asked to be indemnified and asked the ministry to put up sovereign assets as collateral, as well as create a guarantee fund with money deposited in a foreign bank account. In January, the ministry refused these terms, describing the clauses as "abusive".

An official from another Latin American country, which cannot be named, described talks unfolding similarly. They said the government began negotiating with Pfizer in July, before the vaccine was approved. There was a perception that Pfizer's negotiators had a "good cop, bad cop" routine, with the "bad cop" pressing the government to buy more doses.

"[At that time] there was not a single drug or vaccine in the world with this kind of technology that had been shown to be safe and effective ... You had this lady putting pressure saying: 'Buy more, you're going to kill people, people are going to die because of you,'" the official said.

Negotiations became fraught when the company asked for additional indemnity. The government had never awarded any kind of indemnity before and did not want to waive liability, but Pfizer said this was non-negotiable. Negotiations continued and eventually a deal was signed, but after a delay of three months.

As Pfizer has only 2 billion doses to sell across the world this year – apparently on a first come, first served basis – the official is angry about a delay that likely pushed the country further back in the queue.

One of the reasons the government wanted Pfizer's vaccines was because the company said they could be delivered quickly. Yet in the contract, Pfizer wanted to reserve the right to modify the schedule. There was no room for negotiation. "It was take it or leave it," said the official.

The official said: "Five years in the future when these confidentiality agreements are over you will learn what really happened in these negotiations."

Pfizer told the *Bureau*: "Pfizer and BioNTech are firmly committed to working with governments and other relevant stakeholders to ensure equitable and affordable access to our COVID-19 vaccine for people around the world."

This article was first published (https://www.thebureauinvestigates.com/stories/2021-02-23/held-to-ransom-pfizer-demands-governments-gamble-with-state-assets-to-secure-vaccine-deal) by The Bureau of Investigative Journalism *and has been republished here under the terms of a Creative Commons BY NC ND 3.0 license (https://creativecommons.org/licenses/by-nc-nd/3.0/).*

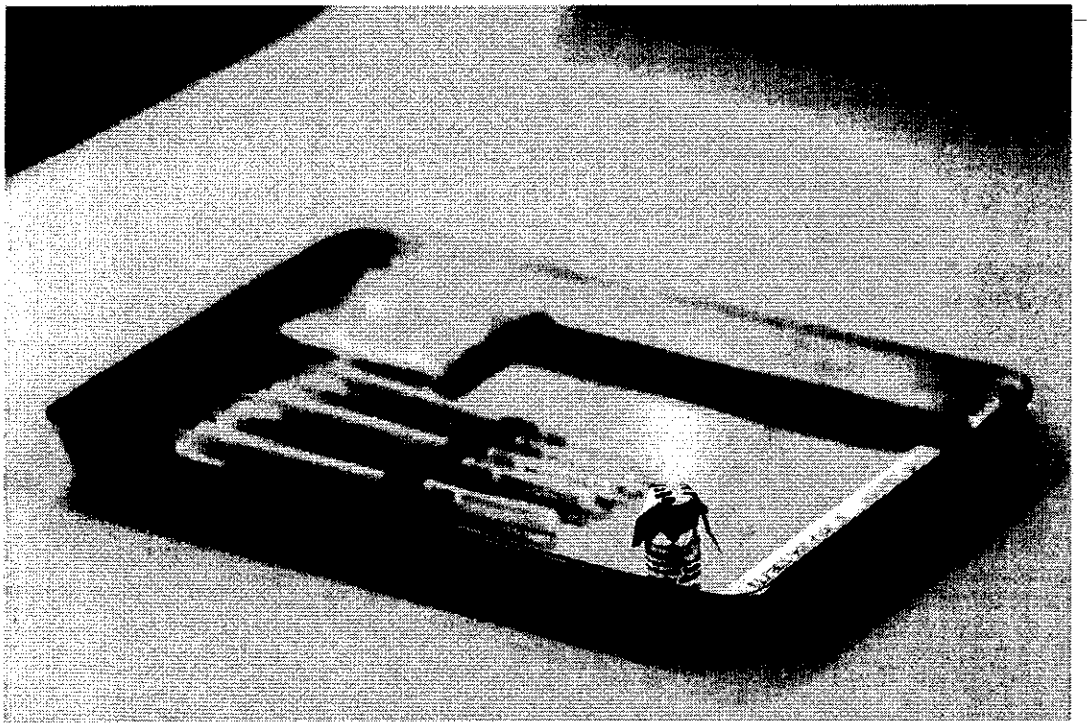
The official said talks soon became tense and complicated: "Instead of giving in on some points, Pfizer demanded more and more." In addition to the changes in the new law, it asked Argentina to take out international insurance to pay for potential future cases against the company (countries were also asked to do this during the H1N1 outbreak).

In late December, Pfizer made another unexpected request: that the government put up sovereign assets - which might include federal bank reserves, embassy buildings or military bases – as collateral.

"We offered to pay for millions of doses in advance, we accepted this international insurance, but the last request was unusual: Pfizer demanded that the sovereign assets of Argentina also be part of the legal support," the official said. "It was an extreme demand that I had only heard when the foreign debt had to be negotiated, but both in that case and in this one, we rejected it immediately."

Also read: A Brief History of Pharmaceutical Profiteering (https://science.thewire.in/health/covid-19-big-pharma-taxpayer-funded-development-profiteering/)

'Good cop, bad cop'



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A vial of the Pfizer-BioNTech COVID-19 vaccine lies on a tray at Tokyo Medical Centre, February 17, 2021. Photo: Behrouz Mehri/Pool via Reuters

"Some liability protection is warranted, but certainly not for fraud, gross negligence, mismanagement, failure to follow good manufacturing practices," said Gostin. "Companies have no right to ask for indemnity for these things."

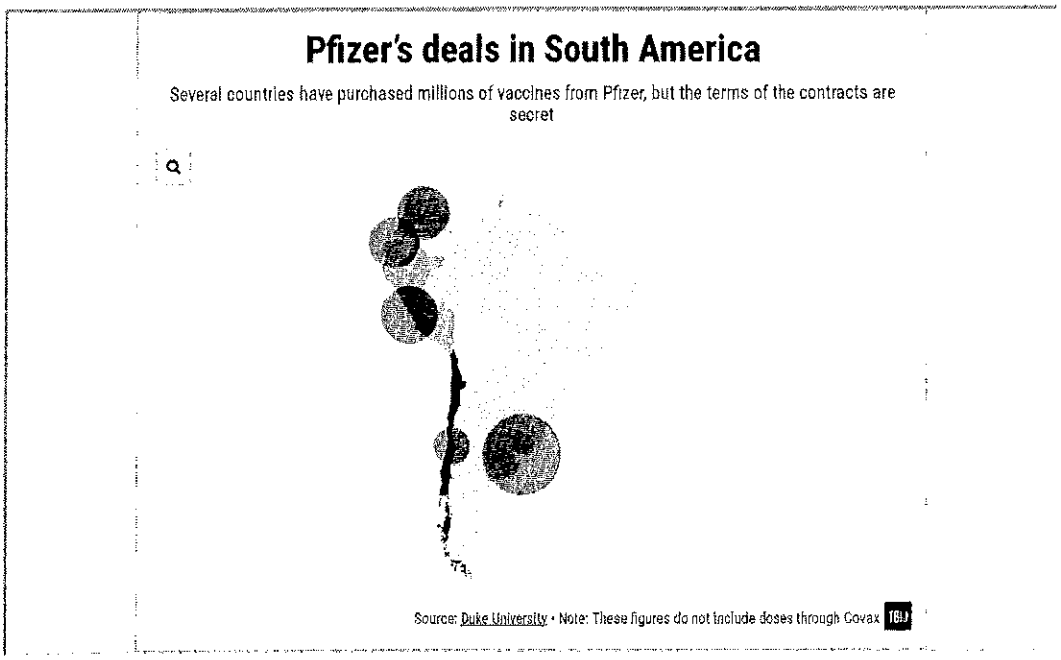
Dr Mark Eccleston-Turner, a lecturer in global health law at Keele University, said Pfizer and other manufacturers have received government funding to research and develop the vaccines and are now pushing the potential costs of adverse effects back on to governments, including those in low- and middle-income countries. (Pfizer's partner BioNTech was given $445 million by the German government to develop a vaccine and the US government agreed a deal in July to pre-order 100m doses for nearly $2 billion, before the vaccine had even entered phase three trials. Pfizer expects to make sales of $15 billion worth of vaccines in 2021.)

In Eccleston-Turner's opinion, it looks like Pfizer "is trying to eke out as much profit and minimise its risk at every juncture with this vaccine development then this vaccine rollout. Now, the vaccine development has been heavily subsidised already. So there's very minimal risk for the manufacturer involved there."

The Bureau spoke to officials from two countries, who all described how meetings with Pfizer began promisingly but quickly turned sour, and reviewed a report by the Brazilian Ministry of Health.

The Argentinian Ministry of Health began negotiating with the company in June and President Alberto Fernández held a meeting with Pfizer Argentina's CEO the following month. During subsequent meetings Pfizer asked to be indemnified against the cost of any future civil claims. Although this had never been done before, Congress passed a new law in October allowing for it. However, Pfizer was not happy with the phrasing of the legislation, according to an official from the president's office. The government believed Pfizer should be liable for any acts of negligence or malice. Pfizer, said the official, disagreed.

The government did offer to amend the existing law to make it clear "negligence" meant problems in the distribution and delivery of the vaccines. But Pfizer was still not satisfied. It asked the government to amend the legislation through a new decree; Fernández refused.



"Argentina could compensate for the vaccine's adverse effects, but not if Pfizer makes a mistake," said the official, who has detailed knowledge of the negotiations. "For example, what would happen if Pfizer unintentionally interrupted the vaccine's cold chain [of -70° C transport and storage] ... and a citizen wants to sue them? It would not be fair for Argentina to pay for a Pfizer error."

However, the government officials from Argentina and the unnamed country who spoke to the Bureau felt Pfizer's demands went beyond those of other vaccine companies, and beyond those of Covax, an organisation created to ensure low-income countries can access vaccines, which is also requiring its members to indemnify manufacturers. This presents an additional burden for some countries because it means having to hire specialist lawyers and sometimes pass complex new legislation, so manufacturers' liabilities can be waived.

Also read: COVID-19 Is Boosting Demand for Universal Healthcare – but Won't Get Us There (https://science.thewire.in/health/covid-19-india-universal-health-care-universalism/)

'An extreme demand'

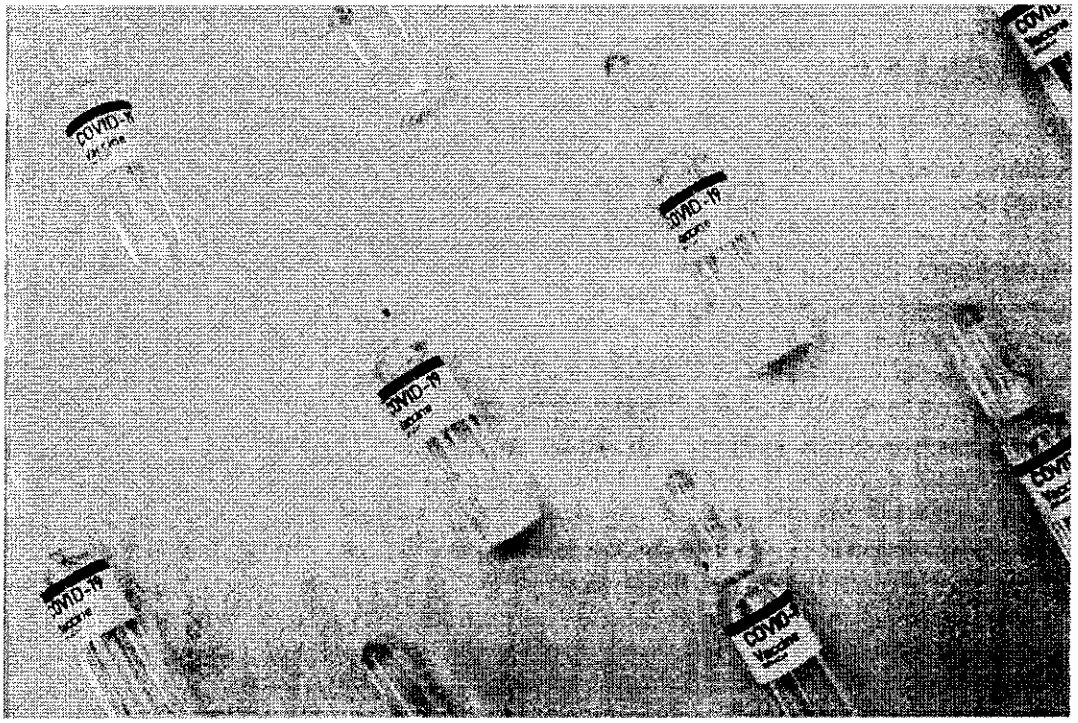


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Photo: Alena Shekhovtcova/Pexels

Pfizer asked for additional indemnity from civil cases, meaning that the company would not be held liable for rare adverse effects or for its own acts of negligence, fraud or malice. This includes those linked to company practices – say, if Pfizer sent the wrong vaccine or made errors during manufacturing.

Officials from Argentina and the other Latin American country, which cannot be named as it has signed a confidentiality agreement with Pfizer, said the company's negotiators demanded additional indemnity against any civil claims citizens might file if they experienced adverse effects after being inoculated. In Argentina and Brazil, Pfizer asked for sovereign assets to be put up as collateral for any future legal costs.

One official who was present in the unnamed country's negotiations described Pfizer's demands as "high-level bullying" and said the government felt like it was being "held to ransom" in order to access life-saving vaccines.

Campaigners are already warning of a "vaccine apartheid" in which rich Western countries may be inoculated years before poorer regions. Now, legal experts have raised concerns that Pfizer's demands amount to an abuse of power.

"Pharmaceutical companies shouldn't be using their power to limit life-saving vaccines in low- and middle-income countries," said Professor Lawrence Gostin, director of the World Health Organisation's Collaborating Centre on National and Global Health Law. "[This] seems to be exactly what they're doing."

Protection against liability shouldn't be used as "the sword of Damocles hanging over the heads of desperate countries with a desperate population," he added.

Pfizer has been in talks with more than 100 countries and supranational organisations, and has supply agreements with nine countries in Latin America and the Caribbean: Chile, Colombia, Costa Rica, Dominican Republic, Ecuador, Mexico, Panama, Peru, and Uruguay. The terms of those deals are unknown.

Pfizer told the *Bureau*: "Globally, we have also allocated doses to low- and lower-middle-income countries at a not-for-profit price, including an advance purchase agreement with Covax to provide up to 40 million doses in 2021. We are committed to supporting efforts aimed at providing developing countries with the same access to vaccines as the rest of the world." It declined to comment on ongoing private negotiations.

Most governments are offering indemnity – exemption from legal liability – to the vaccine manufacturers they are buying from. This means that a citizen who suffers an adverse effect after being vaccinated can file a claim against the manufacturer and, if successful, the government would pay the compensation. In some countries people can also apply for compensation through specific structures without going to court.

This is fairly typical for vaccines administered in a pandemic. In many cases adverse effects are so rare that they do not show up in clinical trials and only become apparent once hundreds of thousands of people have received the vaccine (a 2009 H1N1 flu vaccine, for example, was eventually linked to narcolepsy). Because manufacturers have developed vaccines quickly and because they protect everyone in society, governments often agree to cover the cost of compensation.

HEALTH (HTTPS://SCIENCE.THEWIRE.IN/CATEGORY/HEALTH/), WORLD (HTTPS://SCIENCE.THEWIRE.IN/CATEGORY/EXTERNAL-AFFAIRS/WORLD/)

Pfizer Demands Governments Gamble With State Assets To Secure Vaccine Deal

24/02/2021

A medical worker fills a syringe with a dose of the Pfizer-BioNTech COVID-19 vaccine, Tokyo Medical Centre, February 17, 2021. Photo: Behrouz Mehri/Pool via Reuters/File Photo.

Pfizer has been accused of "bullying" Latin American governments in COVID-19 vaccine negotiations and has asked some countries to put up sovereign assets, such as embassy buildings and military bases, as a guarantee against the cost of any future legal cases, the *Bureau of Investigative Journalism* can reveal.

In the case of one country, demands made by the pharmaceutical giant led to a three-month delay in a vaccine deal being agreed. For Argentina and Brazil, no national deals were agreed at all. Any hold-up in countries receiving vaccines means more people contracting COVID-19 and potentially dying.

"Around 17 million people in the E.U. and U.K. have now received our vaccine, and the number of cases of blood clots reported in this group is lower than the hundreds of cases that would be expected among the general population", Ann Taylor, the company's chief medical officer, said in a statement. Experts have agreed, stating that instances of blood clots and rarer thrombocytopenia cases are no higher among those who received the jab than the general population. On Friday, the International Society on Thrombosis and Haemostasis said that "the small number of reported thrombotic events relative to the millions of administered Covid-19 vaccinations does not suggest a direct link".

Both the World Health Organization and the European Medicines Agency have insisted that the shot is safe and that countries continue using it. In a statement, the latter said that "many thousands of people develop blood clots annually in the E.U. for different reasons and that "the number of thromboembolic events overall in vaccinated people seems not to be higher than that seen in the general population". Notably, it added that "it currently remains of the view that the benefits of the AstraZeneca vaccine in preventing COVID-19, with its associated risk of hospitalization and death, outweigh the risks of side effects".

**Click below to enlarge (charted by Statista)*

Mar 16, 2021, 05:59am EDT | 9,373 views

Which Countries Have Stopped Using The AstraZeneca Vaccine? [Infographic]

Niall McCarthy Contributor ⓘ
Business
Data journalist covering technological, societal and media topics

A growing list of countries, mainly in Europe, have suspended use of the AstraZeneca vaccine as the continent faces a third wave of Covid-19. The European Union's vaccination drive was already painfully slow and the move to suspend shots of its cheapest and most flexible jab at the worst possible time have thrown it into further disarray. Austria was the first European country to sound the alarm regarding potential blood clots caused by the AstraZeneca vaccine while Denmark became the first one to suspend its use outright last Thursday.

It was swiftly followed by others such as Norway and Ireland before some of Europe's largest economies also announced their own suspensions. Germany, France and Italy said they were halting use of the vaccine yesterday while several countries outside of Europe have also announced their own suspensions or halted rollout plans including Thailand, Indonesia, the Democratic Republic of the Congo and Venezuela. British-Swedish pharmaceutical company AstraZeneca has strongly defended its vaccine, stating that there is no link to increased risk of fatal brain hemorrhages and blood clots.

NEWS · News

Southern California suicides down during coronavirus pandemic — but not among young people

In three of four Los Angeles-area counties, suicides among minors rose in 2020, causing concern about isolation during distance learning