Evidence for Residual Immunity to Smallpox After Vaccination and Implications for Re-emergence

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ABSTRACT Introduction: Smallpox has been eradicated but advances in synthetic biology have increased the risk of its re-emergence. Residual immunity in individuals who were previously vaccinated may mitigate the impact of an outbreak, but there is a high degree of uncertainty about the duration and degree of residual immunity. Both cell-mediated and humoral immunity are thought to be important but the exact mechanisms of protection are unclear. Guidelines usually suggest vaccine-induced immunity wanes to zero after 3–10 years post vaccination, whereas other estimates show long term immunity over decades. Materials and Methods: A systematic review of the literature was conducted to quantify the duration and extent of residual immunity to smallpox after vaccination. Results: Twenty-nine papers related to quantifying residual immunity to smallpox after vaccination were identified: neutralizing antibody levels were used as immune correlates of protection in 11/16 retrospective cross-sectional studies, 2/3 epidemiological studies, 6/7 prospective vaccine trials and 0/3 modeling studies. Duration of protection of >20 years was consistently shown in the 16 retrospective cross-sectional studies, while the lowest estimated duration of protection was 11.7 years among the modeling studies. Childhood vaccination conferred longer duration of protection than vaccination in adulthood, and multiple vaccinations did not appear to improve immunity. Conclusions: Most studies suggest a longer duration of residual immunity (at least 20 years) than assumed in smallpox guidelines. Estimates from modeling studies were less but still greater than the 3–10 years suggested by the WHO Committee on International Quarantine or US CDC guidelines. These recommendations were probably based on observations and studies conducted while smallpox was endemic. The cut-off values for pre-existing antibody levels of >1:20 and >1:32 reported during the period of endemic smallpox circulation may not be relevant to the contemporary population, but have been used as a threshold for identifying people with residual immunity in post-eradication era studies. Of the total antibodies produced in response to smallpox vaccination, neutralizing antibodies have shown to contribute significantly to immunological memory. Although the mechanism of immunological memory and boosting is unclear, revaccination is likely to result in a more robust response. There is a need to improve the evidence base for estimates on residual immunity to better inform planning and preparedness for re-emergent smallpox.

INTRODUCTION

Smallpox was declared eradicated in 1980. Smallpox was a suitable disease to select for eradication as it met the criteria for eradicability; no animal host, a moderate reproductive number and an effective vaccine.[1] However, re-emergent smallpox remains a risk.[2,3] Advances in synthetic biology have altered the risk landscape, with Canadian researchers synthesizing an extinct poxvirus in a laboratory using mail-order DNA for less than $100,000 in 2017.[4] The methods for this process were published in an open access journal in 2018.[5] Vaccination is a key component of prevention and preparedness planning. As residual immunity in previously vaccinated individuals may mitigate the impact of an outbreak, it is important to estimate the duration of protection following vaccination to understand the contemporary population's immunological susceptibility.

Edward Jenner proposed that immunity to smallpox was life-long once infected or vaccinated.[6–8] Assumptions that are made in modeling studies and planning documents for smallpox are widely divergent reflecting a high level of uncertainty and range from long-lasting to non-existent immunity.[9–11] Mass vaccination was discontinued after the disease's eradication in 1980.[12] More than 90% of Americans born before 1971 have been vaccinated against smallpox.[13] But in other countries such as Australia universal smallpox vaccination was never used,[14] and a lower proportion of the population have residual immunity.[15]

Both cell-mediated immunity (CMI) and humoral immunity are important in smallpox infection and vaccination response.[13] Field and trial observations indicate that those who have neutralizing antibody (Nab) levels below the threshold appear to be protected.[16,17] Furthermore, subclinical (asymptomatic) infections, which could occur after vaccination, may have boosted the immune response and have provided an inaccurate view of the duration of protection.[13] CMI is associated more with recovery from smallpox, while humoral immunity is thought to be more important in

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protection from infection. CD4+ and CD8+ T cell activity reach peak levels in a shorter time after vaccination or during primary acute viral infection compared to antibody responses.[18] Depletion of CD4+ and CD8+ cells in Rhesus macaques 6 months after DryVax vaccination did not reduce protection from severe disease suggesting that anti-vaccinia antibodies alone can protect against death from infection.[19] Nab confer protection mainly by binding to proteins at the surface of the virus and blocking the infection via neutralization.[20]

Immunity wanes with time since vaccination[21–23] but there is no consensus as to the residual duration and degree of vaccine-induced immunity. With the eradication of smallpox it may be impossible to definitively establish immunological correlates of protection against the disease in humans. This has resulted in a diverse range of estimates for duration of protection from smallpox infection and vaccination.[13]

Since Bernoulli's smallpox model in 1766, mathematical modeling has been used to assess the risk of transmission and the potential impact of smallpox.[24,25] Models provided a means of threat assessment which could be used in preparedness planning.[26] Models generally consider vaccine-induced immunity, but there is much variation in the modeling approaches to population immunity such as immunosenescence,[27] age-related waning immunity and medical immunosuppression.[9,28]

It is critical to quantify residual immunity correctly to assess the potential impact of a smallpox outbreak. Vaccine-derived immunity, level of population immunosuppression along with other factors contribute to population susceptibility such as immunosenescence linked with an ageing population.

In comparing two cities with and without past population smallpox vaccination, we estimated that 22% of New York's population (with past population vaccination in the United States) and 11% of Sydney's population (in the absence of population vaccination in Australia) are vaccinated.[15] In Australia, a significant proportion of the population that are vaccinated is largely the result of immigration. This may play an important role in mitigating the impact of an epidemic. Historical data suggests that death occurs in up to 30-55% of unvaccinated individuals, which decreases to 2% in people vaccinated between 0 and 10 years previously.[6,13] Vaccinated individuals may have some immunity against severe forms of smallpox and death.[29] Assumptions about residual vaccine-induced immunity are important, as most model outputs are highly sensitive to changes in immune status.[9,28] In the model comparing New York and Sydney populations, we showed that with optimistic assumptions of residual immunity related to host immunosuppression, modeled infection rates are 31% lower in New York and 17% lower in Sydney.[15] The proportion of the population at increased risk of serious adverse events from smallpox vaccines is also increasing due to the increase in the number of people living with immune-compromising conditions.[15,30–37] In modeling re-emergent smallpox, failing to consider population-level immunosuppression, incorrect assumptions about residual immunity and considerations on age-related waning immunity may substantially influence model outputs.

In the context of this background, we aimed to conduct a systematic review of the literature to quantify residual immunity to smallpox vaccination to inform preparedness planning for the possibility of re-emergent smallpox.

METHODS

Search Strategy and Selection Criteria

We systematically searched for and reviewed clinical, epidemiological and modeling studies that presented information on the duration of protection to smallpox from prior vaccination and we set no restrictions on population demographics.

To retrieve information, electronic databases: PubMED (1930 to present), Scopus (1823 to present), and EMBASE (1947 to present) were searched for relevant articles according to the selection criteria specified below.[37] We used the PRISMA criteria for systematic review.[38] The date of the last search was October 17, 2017. Within the articles, the reference lists were also screened and checked for completeness. The search terms "smallpox" and "immunity" were used in combination as medical subject headings (MeSH) for PubMED and Emtree subheadings for EMBASE. In Scopus, where there is no equivalent MesH terms, search terms were used as keywords. Only peer-reviewed articles published in English were retrieved. We identified eligible studies by scanning titles and abstracts resulting from the search described. Eligibility and risk of bias assessment were separately done by two authors (MK and XC) and validated by AC. Assessment of bias was done using the ROBINS-I tool[39] to assess observational studies and non- randomized studies of intervention, the RoB 2 tool for randomized trials[40] and relevant portions of the "Checklist for critical appraisal and data extraction for systematic reviews of prediction modeling studies" (CHARMS)[41] for modeling studies.

Studies that were based on models not specifically developed for smallpox were not included. Immunological and phylogenetic modeling studies and theoretical models for preparedness for smallpox outbreak were excluded. Studies on variola minor or vaccinia virus, clinical trials where the number of years since last vaccination for all participants was less than 5 years, and review articles and editorials were excluded.

Data Extraction

Data from all included studies were extracted with respect to type of immune response measured or reported, age of

participants, information on vaccination history such as type of vaccine and distribution of participants by number of vaccinations and number of years since vaccination. Information was classified by type: retrospective or prospective, cross sectional analysis, clinical trial, modeling studies, or epidemiological studies based on outbreaks. Reciprocal geometric mean tire values were used for comparison for the Nab level pre and post-vaccination for the prospective clinical studies. Objective measures of protective immunity such as duration of protection, vaccine take, proportion of participants that sero-converted and reciprocal mean titers for neutralizing antibodies were extracted.

Evaluation of the full text including appendices was completed for the 109 studies obtained for articles that incorporated assumptions or methods for calculating residual immunity (Fig. 1). Three reviewers (VC, XC and MK) were involved in reviewing full text articles and in case of discrepancies, a fourth reviewer (AC) was involved.

RESULTS

Twenty-nine papers related to quantifying residual immunity to smallpox after vaccination were included for analysis: 16 retrospective cross-sectional studies (Table I, Supplemental Table 1), three prospective cohort studies (Table II, Supplemental Table 2), seven prospective vaccine trials (Table III) and three modeling studies (Supplemental Table 4).

Retrospective Cross-sectional Studies

The results of retrospective cross sectional studies are shown in Table I. Eight retrospective cross-sectional studies assessed residual immunity by measuring neutralizing antibody levels,[41–48] four studies looked specifically at cell-mediated responses such as CD4+ T cells and CD8+ T cells[49–52] and three studies looked at both neutralizing antibody levels and T cell responses.[13,53–55] One study looked at measured complement-fixing antibodies and other components of CMI[55] whilst the other studies looked at vaccinia virus-specific IGg levels[43,46,54] and total circulating antibody levels.[52] In these studies, the duration of protection was inferred from the duration the immune response that was stable since primary vaccination. These protection correlates were used to assess the duration of protective efficacy of the vaccine against smallpox. The duration of protection in studies assessing both types of responses were the widest-ranging from 1 to 75 year, followed by 20 to 88 years in studies looking specifically at cell mediated responses and the narrowest range of 20 to about 55 years in studies measuring humoral responses.

Nine studies addressed the efficacy of vaccine against smallpox in terms of immunogenicity. Seroconversion rates were >90% in three studies.[13,44,46] Hatakeyama et al. reported the highest rates of seropositive individuals in those born in 1969 and 1970.[42] In a study conducted in 1971 with 143 contacts of smallpox cases, 4% were vaccinated within the last 3 years, 96.6% were vaccinated within the last 10 years of the study period, and 58.7% were sero-positive.[55]

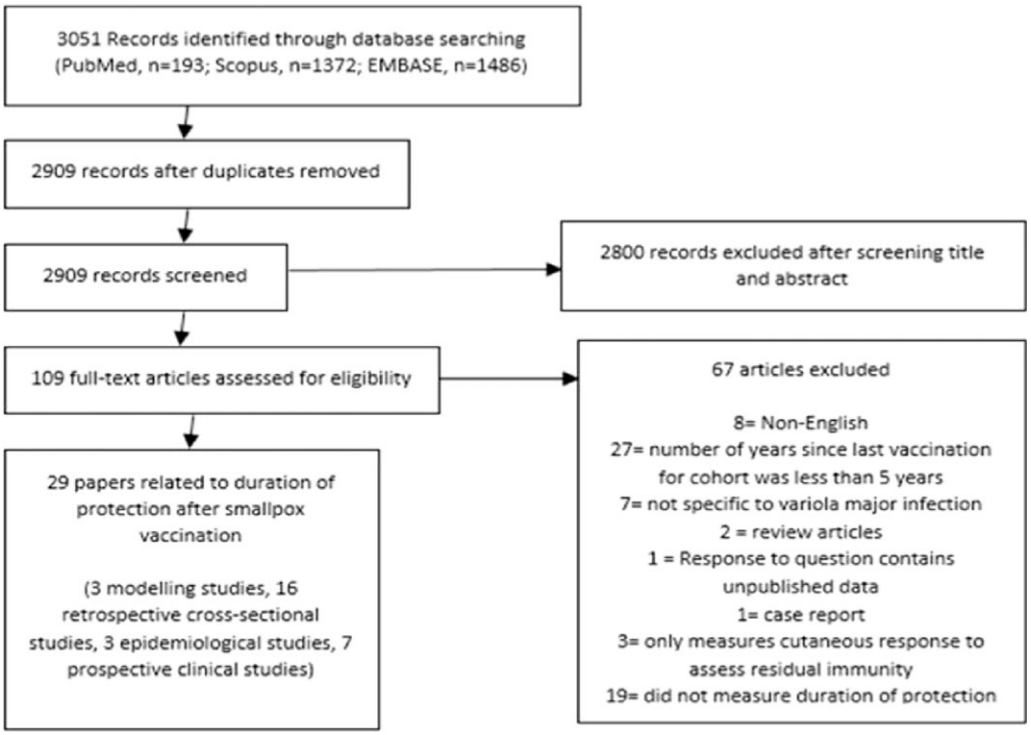

FIGURE 1. Process of selection and reviewing articles

TABLE I. Summary of Retrospective Cross-sectional Analysis

Author, Year	Type of Immune Response Measured		Sample Population	Residual Immunity	
	Humoral	Cell Mediated		Duration of Protection	Population Group (% Sero-positive)
McCarthy, Downie & Bradley, 1958 (42)	✓		86 unvaccinated young adult students, laboratory staff & recent recruits of H.M. Forces. 66 revaccinated staff of the Ministry of Health	20–40 years	
Heiner et al., 1971 (56)	✓	✓	143 contacts of smallpox cases in 20 rural villages in Lahore and Bahawalnagar Districts of West Pakistan and 62 controls (Group 2: Pakistani staff employees best vaccinated, Group 1: intermediate level, Group 3: American volunteers least well vaccinated) from 5 of those villages in which recent smallpox outbreak has occurred but no vaccination campaign held		Contacts (58.7), Group1 (11.3), Group 2 (40.5), Group 3 (15.0)*
El-ad et al., 1990 (43)	✓		140 volunteers from 2 sources, new 18-year old recruits born in Israel for the Israel Defense force and stored sera of reservists	20–30 years	
Demkowicz et al., 1996 (50)		✓	30 Healthy HIV-1 seronegative donors and 22 asymptomatic HIV-1-seropositive donors	50 years	
Crotty et al., 2003 (55)	✓	✓	27 healthy volunteers	>50 years	
Gallwitz et al., 2003 (44)	✓		204 adults about half from laboratory in California and Texas, USA		Born 1920 or earlier (1.05), 1921–1930 (7.4), 1931–1940 (8.8), 1941–1950 (15.2), 1951–1960 (25.5), 1961–1970 (11.8),1971–1980 (0.5)*
Hammarlund et al., 2003 (13)	✓	✓	26 controls, 241 volunteers vaccinated in the USA, 65 volunteers vaccinated in 34 foreign countries	1–75 years	90-95
Amara et al., 2004 (51)		✓	15 native American and 4 Southeast Asian employees from Emory University, USA	>55 years	
Hsieh et al., 2004 (53)		✓	220 healthy subjects from Taiwanese general population of certain ages	20–30 years	
Combadiere et al., 2004 (52)		✓	79 vaccinated volunteers, 10 controls	>45 years	72.5
Hatakeyama et al., 2005 (45)	✓		876 Japanese individuals	27 to 53 years	Born in 1969 (90), born in 1970 (93.3%), born in 1972 (88.6%), born in 1973 (79.3), born in 1974 (45.8), born in 1975 (6.5)
Putz et al., 2005 (46)	✓		642 anonymous serum samples collected at the Central Laboratory in the General Hospital of Siena, Italy	>25 years	Italian population (46)
Kim et al., 2007 (54)	✓	✓	83 healthy volunteers from Korean population	25–50 years	
Taub et al., 2008 (47)	✓		from 246 participants of the Baltimore Longitudinal Study of Aging inclusive of 8 who had documented childhood smallpox infections	88 years in 97% of	participants
98.6					
Liu et al., 2012 (48)	✓		278 health donors from Beijing and Anhui province, China with known smallpox vaccination history, 222 unvaccinated people born after 1980	>40 years	Born before 1980 (7.6), 31–40 age group (5.5), 41–56 age group (10.3)
Slike et al., 2017 (49)	✓		475 serum samples from the Department of Defence Serum Repository established since 1989 obtained from healthy individuals	>30 years	*Vaccinated >2 y ACAM (32.0), 5 y Dryvax (30.7),10 y–20 y DryVax (2.0), Negative controls (12.0)

For additional information regarding study populations refer to Supplemental Table 1.

Slike et al. found a 31.0% seroconversion rate amongst those vaccinated within at least two years of revaccination with the ACAM2000 smallpox vaccine and those vaccinated between 1997 and 2007 had a 28.7% lower sero-conversion rate than

TABLE II. Summary of Prospective Clinical Studies

Author, Year	Study Period	Study Design	Residual Immunity: Neutralizing Antibody GMT Level Mean (95% CI) or (p-Value) Pre-vaccination	Residual Immunity: Neutralizing Antibody GMT Level Mean (95% CI) or (p-Value) Post-vaccination	Population Sub Group (% Sero-positive)	Vaccine Take
Stienlauf et al., 1999 (61)	Not specified	Non-randomized study	19 (14–26)	80 (68–93)	87/99 (87.8%) of those vaccinated once at birth	
Orr et al., 2004 (62)	2002–2003	Non-randomized, open-label study		Time since last vaccination <10 y, 572 (233–1404); ≥10 y–<20 y, 673 (260–1740); ≥20 y, 420 (130–1351)	Time since last vaccination <10 y (39.4%); ≥10 y–<20 y (40%); ≥20 y, (79.3%), $p < 0.0001$	Time since last vaccination <10 y, (39.4%); ≥10 y–<20 y, (54.0%); ≥20 y, (74.1%), $p = 0.001$
Cummings et al., 2008 (59)	Jan 2003	Non-randomized trial			Primary vaccinees were significantly more likely to have a positive result at days 14 ($p = 0.007$) and 21 ($p = 0.018$) than were revaccinees	
Saito et al., 2009 (60)	2002–2005	Non-randomized vaccine trial	Higher in revaccinated group ($p < 0.001$) and those in group D > group B ($p = 0.003$), group D > group C ($p = 0.04$)	Primary vaccinees ≠ revaccinees ($p = 0.40$). among revaccinees, group B> group D ($p = 0.05$)	Revaccinees (B = 60.0%, C = 52.3%, D = 67.7%) <primary vaccines (B = 90.2%, C = 81.2%, D = 99.3%), ($p < 0.01$). Revaccinees highest in group B > group D ($p = 0.002$)	Primary vacinees (B = 94.4, C % = 93.2%, D = 95.9%); Revaccinees (B = 86.6%, C = 85.0%, D = 88.2%)
Haselow et al., 2015 (63)	During 2003 smallpox	vaccination campaign	Non-randomized post vaccination descriptive surveillance study	Highest in ≥60 age group 293 (347), followed by 40–49 age group 284 (463); highest for those ≥3 vaccinations, 8290 (1160) and highest in those vaccinated in 1970s, 293 (491) followed by those vaccinated in 1940s, 273 (188)	Highest observed in those vaccinated in 1940s, 2530 (2160); highest percentage difference between pre and post vaccination levels in this group as well, 871%; highest percentage difference between pre and post vaccination levels in those vaccinated ≥3 times, 226%.	
155/160 (96.9%) included in the		serosurvey had vaccine take				
Nishiyama et al., 2015 (58)	2005–2010	Post-marketing surveillance study		Primary vaccinees ≠ revaccinees (not statistically significant difference at 1,4 and 7 months). Primary and re-vaccinees at 7months < revaccinees at baseline (statistically significant)	Primary vaccines > revaccinees at 1,4 and 7 months (statistically significant). Younger vaccinees tended to show a higher seroconversion rate than older vaccines.	Primary vaccines (94.4%) > Revaccinees (81.7%). Odds ratio at 95% CI = 0.265; 0.113–0.624

(continued)

TABLE II. Continued

Author, Year	Study Period	Study Design	Residual Immunity: Neutralizing Antibody GMT Level Mean (95% CI) or (*p*-Value) Pre-vaccination	Residual Immunity: Neutralizing Antibody GMT Level Mean (95% CI) or (*p*-Value) Post-vaccination	Population Sub Group (% Sero-positive)	Vaccine Take
Greenberg et al., 2016 (57)	2009–2010	Randomized double blind placebo-controlled phase II trial group MM – 2 doses of MVA, group PM- 1 dose of placebo and 1 dose of MVA		GMT levels declined during the 6-month follow up period. ELISA and PRNT titers were still consistently above baseline titers.	98.5% of subjects in group MM and 94.8% from group PM using Elisa; 72.1% and 69.0% using PRNT. At week 32: 59.3% (45.7,71.9) in group MM and 58.6 (44.9,71.4% in group PM for ELISA, 55.9% (42.4,68.8) and 41.4 (28.6,55.1) for group MM and group PM for PRNT	

For additional information regarding the study populations, please refer to Supplemental Table 2.

TABLE III. Summary of Epidemiological Studies

Author, Year	Study Period	Sample Population	Age-Related Cohorts (Number of Participants, Number of Vaccinated Participants) or Age Range	Residual immunity: Group (Case-Fatality Rate)	Residual immunity: Group (Secondary Attack Rate,%)	Residual immunity: Mean Neutralizing Antibody Titers Level (% Participants That Were Sero-positive That Contracted Smallpox)
Heiner et al., 1971 (66)	1968–1970	464 family contacts of the first cases in villages of 6 rural districts in Punjab Province of West Pakistan where 47 smallpox outbreaks had occurred.	<1 y (21,2), 1 y–4 y (65,22), 5 y–9 y (82,57), 10 y–14 y (56,49), 15 y–19 y (38,30), ≥20 y (202,171)	Unvaccinated (20.5%), vaccinated (0.0%)	Unvaccinated (70.8%), vaccinated (4.8%). Vaccinated < unvaccinated ($p = 0.034$).	-
Mack et al., 1972 (64)	35 days	184 compound contacts with no previous history of smallpox, of these 146 contacts of smallpox cases identified in Lahore City, Punjab in West Pakistan	Children aged =<14 y (73)	-	-	>1:32, among susceptible contacts (3/15, 20%), >1:32, among contacts with pre-existing antibodies (0/127, <1%)
Sarkar et al., 1975 (65)	3 weeks	57 volunteer contacts of 30 smallpox index cases in Calcutta area	4 y to 70 y	-	-	≥1:20 (0/13, 0.0%). <1:20 (6/43, 14%)

those vaccinated within five years of revaccination.[48] Gallwitz et al. found a mean seroconversion rate of 25.5% for 76 participants born between 1951 to 1960, compared to 8.8% average seroconversion rate amongst 20 participants born between 1931 and 1940 or 11.8% amongst the 41 participants born between 1961 and 1970.[43] Hammarland et al. reported a 90-95% seroconversion rate in a population where 44.6% were vaccinated between 1953 and 1972.[13] A 72.5% seroconversion in participants vaccinated between 1943 and 1989 was observed by Combadiere et al. where 40.5% of those were vaccinated between 1959 and 1968.[51] Putz et al. observed a 46% seroconversion rate in a population-based study of 642 samples in Italy from participants aged 11 to 102 years old.[45] Taub et al. measured a mean 98.6% seroconversion rate amongst 246 participants where the number of years since last smallpox vaccination ranged from 13 to 88 (median=61years).[46] Liu et al. found the lowest mean seroconversion rate of 5.5% amongst those born 1981 to 1972, followed by those born before 1980 (7.6%), with the highest seroconversion rates among those born between 1971 and 1956 (10.3%).[47] For Nab, seroconversion rates measured by neutralization assays varied amongst the studies but the highest rates were observed among those born between 1950 and 1989.

The maximum number of vaccinations per person recorded was 14 for one individual and 13 for another individual in two different studies.[13,50] Hammarlund et al.[13] reported that additional vaccinations, which ranged from 3–5 to as many as 6–14 immunizations did not result in any further increase in long term antibody production.

All studies focused on healthy subjects, except for one study that included an immunocompromised population of asymptomatic HIV-1 seropositive people.[49]

There was potential for confounding to be present as there could be other factors that predict the immune response, limited information on other factors such as age, gender were collected and reported. Volunteer bias could be present in the studies included as though sampling frame was described, details on recruitment of volunteers not specified for the studies. There is the possibility of recall bias as six studies ascertained vaccination history through self-reporting and not by the absence or presence of vaccination scar[52,53,55] and official records from military or medical records[42,46] (refer to Supplementary Table 3).

Prospective Vaccine Trials

The results of prospective vaccine trials are shown in Table II. All trials used different vaccine strains which were administered by scarification or multi-puncture techniques except in Greenberg et al. where the vaccine was administered subcutaneously via injection.[55] The number of insertions for the pressure pricking was different in vaccinia-naïve and previously-vaccinated individuals in two studies.[58,59] The follow-up period for the trials ranged from 21 days[57] to the longest 26–30 weeks.[56] The average duration of the follow-up period was 2.8 months (standard deviation = 2.6). First-generation vaccines were used in four trials[57,59–61] while third-generation vaccines were used in three trials.[57–59]

Four studies obtained participants from the military with two studies from the Japan Self Defense Force,[57,59] two studies from the Israel Defense Force[60,61] while one study obtained participants from the healthcare setting.[62] Two studies[56,58] did not provide details of the occupation of participants. All participants were healthy individuals except in one study where 10 individuals had allergies.[57] The highest pre-vaccination neutralizing antibody levels were seen in those born between 1953 and 1963,[59] those above 60 years old in 2015 and those who had at least three previous vaccinations.[62] The highest post-vaccination Nab levels were seen in those <10 years old.[61]

Primary vaccinees were defined as those who were not vaccinated prior to the commencement of study and revaccinees were defined as those who had been previously-vaccinated before the trials. We identified two studies that used first-generation vaccines and one study that used third-generation vaccine that suggested Nab titers in revaccinees do not differ significantly from those found in primary vaccinees. Steinlauf et al. found that Nab titers level before revaccination at 18 years old, was detected only in 87 out of 99 soldiers who had been first vaccinated at birth and their GMT two months after revaccination (80, 95% CI 68-93) was significantly higher than at baseline (19, 95% CI 14–26).[58] They also found that, although not statistically significant, those previously vaccinated at birth and at age eight years old had higher titers compared to those vaccinated at birth.[58] Haselow reported that there was no statistically significant increase in pre- and post- vaccination titers with higher number of previous vaccinations, increasing age, and longer intervals since time of last vaccination.[62] Saito et al. found that before vaccination, among those who were previously-vaccinated, Nab titers levels were significantly higher in those who were born between 1953 and 1963 compared to after 1969.[59] After vaccination, there were no significant difference in Nab titers levels between primary vaccinees and revaccinees in the same study.[59] Among the revaccinees, post-vaccination Nab titers was marginally higher in those born between 1953 and 1963 compared to the other revaccinees ($p = 0.05$).

Orr et al. reported that the time since last vaccination significantly correlated with seroconversion rates, and that pre-existing antibody titers level had a positive association with seroconversion rate.[61] Of those previously-vaccinated within ten years of revaccination, 40% seroconverted, while 74–79% of those previously-vaccinated more than 20 years before revaccination seroconverted. Greenberg et al. observed that revaccinees who had their first vaccination more than 40 years ago and were 56 to 80 years of age at time of study, had detectable baseline Nab titers levels. Nab titers levels declined in the eight months (344.6, 95% CI 288.9–411.1) after these subjects completed a two-dose schedule for revaccination but were still significantly higher than baseline levels (129.0, 95% CI 100.3–165.8).[56]

Two studies using third-generation vaccine[58,60] and one study using first-generation vaccine[59] found that primary vaccinees were significantly more likely than revaccinees to have seroconverted. Cummings et al. found that primary vaccines had a significantly higher seroconversion rate (61.5%) than revaccinees (42.9%) within 2–3 weeks of vaccination.[58] Primary vaccinees had a statistically higher seroconversion rate at one, four and seven months following vaccination compared in revaccinees in one study.[57] Saito et al. found 94.4% of primary vaccinees and 86.6% of revaccinees were seropositive.[59] The same study reported that 90.2% of primary vaccinees seroconverted compared to 60.0% of revaccinees.

Two studies, one using first-generation vaccine[61] and another using third-generation vaccine[58] found that those with lower pre-existing antibody levels had a higher rate of seroconversion.[59] Orr et al. reported that 26.0% of those with pre-existing antibody titers of at least 1600 seroconverted, compared to 86.0% of those with a lower pre-existing antibody levels of less than 200.[61] No significant difference was found in Nab titers levels between primary

and revaccinees at one, four and seven months after LC16-Kaketsuken vaccination, although both groups had higher antibody titers levels compared to baseline. One study found that found that GMT Nab titers were significantly higher in those who did not seroconvert (36.6, 95% CI 28.0–47.8) compared to those who seroconverted (10.7, 95% CI 8.8–13.1).[59] In this study, those who received primary vaccination at ages less than 20 years old had a higher seroconversion rate (100.0%) compared to those who received primary vaccination at older age, 33.3% for those more than 50 years old at seven months postrevaccination.

Orr et al. and Haselow found no statistically significant difference in antibody titers between males and females.[61,62]

Of the seven prospective vaccine trials, six studies were non-randomized studies of intervention while only one study was a double-blinded placebo controlled randomized trial. Greenberg et al. used randomization by electronic system to select participants into trial groups.[56] Serological analysis for 100 samples was done randomly in Nishiyama et al. without further details on the how randomization was carried out.[57] Some missing data reported in four studies for reasons reported ranged from participant no-show or lost sample and it is uncertain if there are any significant differences between those whose data is missing or those who are included in analyses except in Haselow, 2015 where differences are reported (Refer to Supplementary Table 3).

Epidemiological Studies

The results of epidemiologic studies are shown in Table III. Three prospective cohort studies followed contacts of smallpox index cases for almost a month to one year in endemic countries during periods of outbreak.[63–65] All three studies included children aged less than 14 while Sarkar et al. did not include any children less than four.[63] In Heiner et al. the case-fatality rate in the vaccinated cohort was zero compared to 20.5% in the unvaccinated cohort and the secondary attack rate in the vaccinated cohort was 66.0% lower than in the unvaccinated cohort ($p = 0.034$).[65] One study showed that smallpox patients' contacts who had neutralizing titers <1:32 against vaccinia virus were more susceptible to smallpox infection (20% of contacts infected) than contacts with pre-existing antibody titers≥1:32 (0 contacts infected).[64] A smaller study showed similar results after 6/43, or 14% of contacts, with neutralizing titers <1.20 contracted smallpox, whereas 0/13 contacts with titers ≥1:20 contracted the disease.[63]

There was potential for confounding to be present as there could be other factors that predict the immune response, information on other factors such as age, gender, location, schooling experience were collected but not all the information was reported. Possibility of misclassification bias in confirming smallpox cases either by visual inspection using lesion density system to count lesions on areas of body could be present. During endemic settings presence of subclinical infections of smallpox could have resulted in measurement and misclassification bias among controls and contacts (refer to Supplementary Table 3).

Modelling Studies

Modelling studies are shown in Supplementary Table 4. All three modeling studies which used data from outbreaks in the Europe and Australia were stochastic compartmental models. Nishiura et al.[66] estimated that the median duration of protection from disease was 11.7–28.4 years.[66] They also reported that vaccinated individuals have more than 50% probability of protection from severe disease even 50 years after primary vaccination and that within 20 years of vaccination severe cases will be extremely rare. Nishiura & Eichner[67] estimated that the median duration of protection against severe and fatal disease was 31.7 and 53.9 years and protection from disease was observed in 52.6% after 50 years since vaccination. Loss of immunity against disease was estimated as 2.19%/year[67] and against fatal disease as 1.41%/year.[67]

Sensitivity analyses for parameters were carried out for worst-case and best-case scenarios with rationale provided for the modeling studies. When interpreting the results, comparison was made with other studies and discussion of generalizability, strengths and limitations were included for all three modeling studies. Results of further assessment of bias included in Supplementary Table 3.

DISCUSSION

Protection greater than 20 years was seen in 16 retrospective cross-sectional studies, while modeling studies' lowest estimate of 11.7 years was closer to the number of years (between three to ten) after which CDC recommends revaccination depending on the setting.[66] The changing risk of re-emergent smallpox necessitates better understanding of the impact of a contemporary epidemic and the role of residual vaccine-induced immunity.[68] In countries where universal smallpox vaccination was practised, over 20% of the population may have residual vaccine-induced immunity, but its degree and duration is uncertain.[15] Overall we found evidence of long-lasting immunity from the persistence of Nab after smallpox vaccination especially amongst the retrospective cross sectional studies.[13,42–55] Before revaccination, Nab titers levels were higher in revaccinees than in primary vacineees in most studies. Similarly, those who received primary vaccination at a younger age demonstrated higher Nab titers levels in adulthood. This suggests that Nab does persist in vaccinated individuals, especially if vaccinated in childhood. If so, this would support a recommendation that the interval to revaccinate individuals can be longer than the current three to five years. However, other factors such as risk of occupational exposure, number of previous vaccinations, age

of primary vaccination and status of immune system should also be considered and studied further.

The highest rates of seroconversion were observed among those born in the period coinciding with initiation on the WHO smallpox eradication campaign in 1959, or who received their first vaccination during the period overlapping the duration of the eradication campaign.[42,43] Those who received primary vaccination in childhood were more likely to have higher levels of protection than those vaccinated in adulthood.[57–59] This suggests that the protective levels required are likely varied by age, previous exposure to virus or vaccine strains and age at which this exposure has occurred. This effect is seen in research on other highly infectious viruses.[69]

Despite the different methodologies used to identify the Nab, the findings regarding seroconversion rates and Nab titers levels were similar. This suggests that the methodologies do not significantly bias the results. The studies which exclusively focused on Nab may underestimate the duration of residual immunity conferred by smallpox vaccines as other components of the immune system have been shown to increase post vaccination and have been shown to contribute to immunity in animal models. Four studies found an increase of CD4+ and CD8+ T cells after targeted cohort was vaccinated but before Nab were produced.[51–53] This means that vaccinees without detectable Nab level could also be protected by cellular immunity.

The studies which used mathematical modeling were based on vaccination history, rates of infection and the data from actual outbreaks. The immune responses in the retrospective studies were also measured using various advanced biological techniques which were not available in the pre-eradication era of smallpox. Therefore, immunological correlates of protection are uncertain, and it is unknown if the Nab tire threshold levels as a measure of immunity are sufficient to protect against actual disease. In this respect, current CDC recommendations appear to reflect a conservative estimate of immunity. The estimates of modeling studies incorporate the realistic biological effects of ageing and waning immunity by assuming lower attack rates and less severe infection in those vaccinated in older age groups produce more robust estimates of duration of immunity.[15] However, our most recent study, which examined the influence of residual immunity from past vaccination and immunosuppression, found the highest death rates in older people, despite past vaccination.[15] With increasing time since vaccination, we found that host immunosuppression (from a range of iatrogenic and medical factors) is a stronger predictor of severity of modeled smallpox than past vaccination, due to substantial waning of immunity over time and almost four decades since eradication.

The wide range in estimates for residual immunity could be due factors other than the number of vaccinations or time since last vaccination. In most of the reviewed studies, the vaccine history is elicited by physical examination for the presence of scar, or through self-reporting. Studies reporting the variation in Nab titer as a function of time since last vaccination could be used as a guide in determining the schedule of boosters. Booster doses of vaccination could increase antibody responses but are unlikely to produce prolonged levels of Nab above the critical threshold.[13] Recently, other factors have been shown to affect residual immunity to smallpox such as gender, ethnicity, gene polymorphisms and type of smallpox vaccine received.[18,69–74] Clinical proof of efficacy in the post-eradication era is not possible, although animal studies can be indicative. For example, since the LC16m8 vaccine has never been tested against smallpox in the field, protective efficacy was evaluated in various animal studies using mouse, rabbit and monkeypox models.[73–78] The clinical trials included in this review used first-generation[59,61,62,75] and third-generation vaccines.[55,58,60] Although second-generation vaccines were developed based on first-generation vaccines to comply with modern manufacturing guidelines, there were no studies of these that fit the inclusion criteria. Due to the higher risk of adverse events and limited use, it is possible that more recent studies focused on third-generation vaccines instead.[79] The only available titers cut-off values for pre-existing antibody levels in humans were[63] reported during the period of endemic smallpox circulation and may not be relevant to the contemporary population. Yet these cut-off values have been used to classify people with residual immunity in post-eradication era studies.

Due to the inclusion of 28 studies with generally lower level quality of evidence associated with them and 1 double blinded placebo controlled randomized clinical trial, the evidence for the duration of protection with smallpox vaccines is limited by the presence of bias of mainly volunteer bias, recall bias and misclassification bias and lack of detail regarding the populations included in the observational studies limits the applicability of the result.

This review highlights the scarcity of high-quality evidence regarding duration of protection with smallpox vaccines and the corresponding uncertainty about duration. An important aspect for further research is the cellular immune response after vaccination against smallpox. The reason that Nab titers between primary and revaccinees were not significantly different in some of the clinical studies needs to be explored further. The protective level may be different with age as well as other factors such as pre-existing conditions that could affect the immune response. In the event of a smallpox epidemic, health workers who have had previous vaccination could be prioritized for revaccination, to enable rapid and robust protection for front line workers. There is a need to improve the evidence base using high quality randomized controlled trials and more well-designed studies for estimates on duration of residual immunity against smallpox in the contemporary population for pandemic planning and to avoid risk of bias in methodology.

SUPPLEMENTARY MATERIAL

Supplementary material is available at *Military Medicine* online.

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