Rickettsial vaccines with emphasis on epidemic typhus

THEODORE E. WOODWARD

The invitation to contribute a brief review of an old subject — the rickettsial diseases — was accepted with great pleasure. Any recognition given to James Gear is little, indeed, when compared with his long and remarkably significant contributions made in the noble cause of preventive medicine and tropical medical science. Humble and unassuming, James Gear has dedicated his life to fulfilment of the highest standards of medical practice, to the conduct of relevant research and to educational pursuits. He is one of South Africa's proudest sons. All that is needed to admire him is to know him.

One of the earliest protective preventive measures for classic typhus was the eating of human lice. The value of this procedure is undocumented. Among the first inactivated vaccines for rickettsial infections was the type made from infected tick tissues by Spencer and Parker.¹ Favourable results were reported for protection against Rocky Mountain spotted fever based on the decline of infection in vaccinated persons. Control trials were not conducted. Severe local reactions resulted from the injection of the tick tissue vaccine. When tick-derived vaccines were studied later in volunteers in a small controlled vaccine trial after challenge injection using a viable pathogenic strain of Rickettsia rickettsii little if any protective value was demonstrated.² Conceivably the vaccine had lost some of its protective potency after long storage in ampoules.

The Weigl louse-intestine vaccine used from 1920 to 1930 was difficult to produce because the technique of preparation necessitated the inoculation of lice intrarectally with viable R. prowazekii.³ After inoculation, lice needed to be fed on the skin of convalescent typhus patients twice daily for a week or more which permitted rickettsiae to multiply in the intestinal cells of the arthropod. Louse intestines were then individually harvested, and the rickettsiae were harvested with formalin which resulted in a protective vaccine.

It was observed this technique first as a young medical officer soon after arrival in North Africa in 1942. Guinea-pigs infected with R. mooseri (typhi) were placed in deep porcelain-lined sawdust pits, each of which contained 30000 - 40 000 starved fleas; the infected animal soon died of exsanguination. Several additional infected guinea-pigs were placed in the tubs until the fleas were well infected with the murine typhus rickettsiae. To collect the rickettsial-laden flea faeces, healthy cotton rats were placed in the pit. In only a few hours, they died of blood loss and then appeared brown in colour because of a coat of flea faeces. In such tubs, fleas could feed on many rats. The dead animal was removed and technicians, without any real protection, picked up the brownish rat hair and rubbed it over a fine mesh which permitted the flea faeces to drop into a glass Petri dish. Such heaps of flea faeces were then measured in milligram amounts and sealed in glass ampoules. These ampoules were then crated along with a diluent of saline water and a small amount of ox bile. These cartons were dispatched throughout Morocco and Algeria to various district health stations.

Another interesting finding was that the simple taking of oral temperature in enough antigen for 20 doses. Another interesting finding was the development of fever in other vaccine recipients who were not clinically ill and who did not develop Proteus OX19 or complement-fixing (CF) antibodies to rickettsial antigens. Indeed, I learned that the simple taking of oral temperature in the search for a larger and richer rickettsial production line. A significant hazard of the animal aerosolization technique was the sneezing of mice with the resultant spraying of the room with virulent rickettsiae. Often the inoculator became infected.

An attenuated strain of epidemic typhus vaccine known as strain E has been tested in volunteers and under field conditions. This viable vaccine stimulates the production of humoral antibodies and prevents the classic form of typhus, particularly when booster doses are given. One of the limiting features, which has precluded further development, is a moderate to severe reaction rate and the possible threat of reversion of the attenuated strain to one of virulence.⁵

Perhaps the most unique antityphus vaccine was made by Blanc and Baltazard⁶ of the Pasteur Institute in Casablanca. I observed this technique first as a young medical officer soon after arrival in North Africa in 1942. Guinea-pigs infected with R. mooseri (typhi) were placed in deep porcelain-lined sawdust pits, each of which contained 30000 - 40 000 starved fleas; the infected animal soon died of exsanguination. Several additional infected guinea-pigs were placed in the tubs until the fleas were well infected with the murine typhus rickettsiae. To collect the rickettsial-laden flea faeces, healthy cotton rats were placed in the pit. In only a few hours, they died of blood loss and then appeared brown in colour because of a coat of flea faeces. In such tubs, fleas could feed on many rats. The dead animal was removed and technicians, without any real protection, picked up the brownish rat hair and rubbed it over a fine mesh which permitted the flea faeces to drop into a glass Petri dish. Such heaps of flea faeces were then measured in milligram amounts and sealed in glass ampoules.

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For I vaccine dose 100 louse intestines were necessary. Based on epidemiological data, the vaccine appeared to be protective against classic typhus.

Laigret,⁴ the French rickettsiologist, developed an attenuated vaccine made from murine brain tissues. Such vaccines were reactive because of the extraneous protein-laden tissues present in the product.

Later, Castaneda,⁵ a contemporary of Zinsser, developed an inactivated epidemic typhus vaccine by forcing mice to inhale droplets of rickettsial-laden suspensions; on being placed in a cold room, mice developed a pneumonia rich with rickettsiae. Lungs were harvested and the rickettsiae were inactivated with formalin, which resulted in a protective vaccine.

Giroud and Panthier,⁶ and Giroud⁷ extended this technique to include rabbits, dogs, sheep and even considered camels in the search for a larger and richer rickettsial production line. A significant hazard of the animal aerosolization technique was the sneezing of mice with the resultant spraying of the room with virulent rickettsiae. Often the inoculator became infected.

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Huge numbers of Muslim citizens, who came for a regular sugar ration, were then injected with the vaccine which was reconstituted just before inoculation, i.e. dried flea faeces placed in a small amount of ox bile/saline diluent. This so-called 'attenuated' vaccine produced an unknown number of cases of murine typhus fever said to be milder than usual because of the attenuating effect of the ox bile. Incidentally, Zinsser always argued with Blanc during international meetings that rickettsiae were not made less virulent in this manner. It was known from previous studies in animals and observations in humans that murine typhus fever provided resistance to the more virulent epidemic louse-borne typhus fever.

Careful assessment of the Blanc vaccine was conducted by testing volunteers for pre- and post-vaccine antibodies and clinical evaluation of vaccinees. In two separate series, it appeared that approximately 10% of the vaccinees developed murine typhus fever, suggesting that they were the beneficiaries of most of the particles of flea faeces from a particular vial of dried material. One vial of dried flea faeces was said to contain enough antigen for 20 doses. Another interesting finding was the development of fever in other vaccine recipients who were not clinically ill and who did not develop Proteus OX19 or complement-fixing (CF) antibodies to rickettsial antigens. Indeed, I learned that the simple taking of oral temperature in...
a large number of persons will show a small percentage of elevations without an obvious cause!

A later study of this vaccine in volunteers showed that protection against epidemic typhus occurred only when the flea faeces in the vaccine was viable and caused a mild murine typhus infection with serological conversion after vaccination. Most vaccinees received no antigen and developed no immunity.

Cox\textsuperscript{10} adapted the Goodpasture egg-embryo technique for the cultivation of rickettsia. Rickettsiae multiplied avidly in the yolk sac of the viable embryo. The method of vaccine production coupled with ether extraction and freeze-thawing of the infected chorio-allantoic membranes resulted in clean rickettsial suspensions. The resulting vaccines either for \textit{R. prowazekii} or \textit{R. rickettsii} were effective, particularly when care was taken to produce a product with high titres of antigen.

From 1942 to 1944 epidemic typhus was very prevalent in the indigenous population in North Africa, and the incidence of human louse infestation was high. British military forces experienced epidemic typhus with fatal cases during their march across North Africa. American military forces landed in Morocco and Algeria in 1942 having been previously inoculated with an inactivated vaccine which contained suspensions of whole \textit{R. prowazekii}. Because of the potential threat of epidemic typhus to Allied forces, a trial of the newly-available epidemic typhus vaccine was conducted in collaboration with French health authorities. This trial was considered of even more importance because many doses of the viable flea-faeces vaccine were being used throughout Morocco and Algeria. There was always the threat as put forward by Zinsser\textsuperscript{11} and Zinsser and Castaneda\textsuperscript{12} that the human body louse might acquire the murine typhus rickettsia and transform its characteristics to that of the epidemic type.

Studies on human infection with \textit{R. prowazekii}

Prophylactic efficacy of non-viable typhus vaccine (Cox and Durand type)

The ability of vaccines made from purified suspensions of inactivated \textit{R. prowazekii} to prevent epidemic typhus fever is accepted. The contributions of Weigl, Castaneda, Durand and Giraud, and Cox\textsuperscript{10} and others represent a milestone of considerable magnitude, since the investigations of these scientists helped lead to effective control of one of mankind's major afflictions.

It is now 40 years since the following studies of the efficacy of two types of epidemic typhus vaccine were performed. The work was conducted in collaboration with Georges Blanc, Director of l'Institut Pasteur, Morocco, and Marcel Baltazard, Deputy Director. Drs Blanc and Baltazard ranked with the world's top rickettsiologists. It was my privilege to have worked with and learned from them while assigned as an army officer to the Pasteur Institute for special clinical and laboratory work on typhus fever. Records of this work were made available, in official reports, to investigators in the field and are now published in full.

These studies, performed in man, showed that vaccines made from inactivated strains of \textit{R. prowazekii} were effective in preventing infection or serious sequelae of typhus fever in the small number of volunteers evaluated. Salient features pertaining to methods of immunization, infection, serological response and clinical reaction to infections are briefly described.

Patients and methods

Twelve long-term prisoners, aged 20 - 40 years and with no history of typhus fever, volunteered for this study under guidance of French health authorities. None of the volunteers showed antibodies to rickettsial antigens before immunization with killed vaccines. Each subject was healthy and without physical impairment. Subsequently, each was given a certificate of acknowledgment for his contribution to the advancement of medical knowledge.

Three volunteers received Cox-type vaccine (Mulford M 400-4) in doses of 1.0, 1.5 and 1.5 ml at weekly intervals. Six received the same vaccine in one dose of 4.0 ml. There were no significant reactions in either group. Three volunteers received the Durand-type vaccine (Pasteur Institute, Algiers, FAL 36). Two subjects received an initial dose of 1.0 ml and subsequently two doses of 1.5 ml at weekly intervals. One volunteer received 4.0 ml in one dose. There were no serious reactions.

Source of rickettsiae and method of infection

A guinea-pig-adapted strain of \textit{R. prowazekii} was utilized for the infection. The strain was originally isolated from a typhus patient with classic signs of this disease. Then 1 ml of a 20% suspension of guinea-pig brain material, obtained on the third febrile day, was administered subcutaneously to the previously immunized subjects. The infecting inoculum for each volunteer represented approximately 10\textsuperscript{7} infectious guinea-pig doses of viable \textit{R. prowazekii}.

Control observations

Two macaques were each injected subcutaneously with 1.0 ml of the challenge material used for each group of volunteers. The 12 volunteers were distributed in four separate tests, hence 8 monkeys were employed as controls. Six of these primates died and the other 2 recovered after a severe experimental infection. Rickettsiae were identified regularly in the smears of tissues of dead guinea-pigs and monkeys.

Criteria used for appraising the clinical reactions to typhus infection

Class 0 — no reaction; class I — sporadic temperature elevation without headache, rash or other clinical manifestations; class II — continuous temperature for 4 days or more without rash, slight to moderate headache and slight toxaemia; class III — continuous temperature, typical typhus rash, moderate toxaemia; patient regarded as moderately ill; and class IV — classic typhus fever, i.e. typical rash, delirium, toxaemia, circulatory weakness, azotaemia, etc.

Results

Cox vaccine (egg-embryo type)

All 3 volunteers given Cox-type vaccine in three doses developed CF antibodies ranging from titres of 1:10 — 1:160 2 - 3 weeks after the first dose. Two of these volunteers showed no antibodies just before infection. The inoculum of viable typhus rickettsiae was given from 50 to 75 days after immunization. The serological data and clinical reaction to infection are presented in Table 1. One subject (case 1), with a CF titre of 1:160 at the time of challenge, developed a mild reaction (class I) without headache or rash. A rise in CF titre after the infection did not occur. Two subjects (cases 2 and 3), without detectable antibody at time of infection, developed mild febrile courses of 6 and 8 days, respectively. Neither developed a rash or manifested toxaemia. Slight headache was present. Increases in titre of CF antibodies were noted after infection.
TABLE I. EPIDEMIC TYPHUS VACCINE STUDIES IN VOLUNTEERS (COX AND DURAND VACCINES). VIABLE CHALLENGE: R. PROWAZEKI

<table>
<thead>
<tr>
<th>Case</th>
<th>Vaccine type</th>
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<th>Highest after infection</th>
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<td>Durand</td>
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*CF titres to R. prowazeki antigen. Controls: Macaca sylvanus — 8 monkeys: 6 deaths, 2 severe infections.
*Clinical key: 0 = no reaction; I = sporadic temperature rise only; II < 4 days temperature, no rash; III = continuous temperature, rash, moderate illness; IV = classic severe typhus.

Of the 6 volunteers given Cox-type vaccine in one dose, all but 1 (case 4) developed CF antibodies (Table I). Three volunteers (cases 5, 6 and 8) showed antibody titres ranging from 1:80 to 1:640 at the time of infection. There was no clinical reaction to infection and moreover, the titres of antibody declined after the injection of this seemingly large inoculum of viable rickettsiae. Two members of this series (cases 7 and 9) developed low titres after vaccination, 1:40 and 1:20, respectively, but showed no antibody at the time of infection. Their clinical reaction following challenge was class II, with slight headache and mild toxic signs but no rash. The febrile course was 7 and 9 days for cases 7 and 9, respectively. These volunteers developed high titres after the infection. Patient 4 did not have demonstrable CF antibodies at any time after vaccination. He, too, developed a class II reaction as a result of the challenge and had a febrile course of approximately 10 days followed by a rise in CF antibodies.

In no patient in this series was the clinical reaction to infection regarded as severe. The patients were all ambulatory throughout the active stages.

Durand (inactivated rat lung-type) vaccine

Three volunteers received the rat lung-type of non-viable vaccine. Of 2 subjects given three graded doses, 1 did not develop demonstrable antibody (case 10). In another (case 11), a titre of 1:80 appeared, which subsequently declined to zero before infection. Case 10 developed a mild clinical reaction classified between I and II with a febrile course of approximately 6 days. No rash occurred. An antibody rise occurred in convalescence. The second subject (case 11) developed clinical signs compatible with mild typhus fever, i.e. headache, sparse rash and continuous fever for 11 days. This patient was regarded as class III. A significant antibody rise was demonstrated during convalescence. The final volunteer (case 12), who received 4.0 ml vaccine in one dose, developed a mild reaction to infection without a rash (class I - II). An antibody response was demonstrated.

Summary of results

Killed typhus vaccines, when administered subcutaneously, provoked little local or systemic reaction when given in graded doses of 1.0 ml or in a larger dose of 4.0 ml. Typhus CF antibodies developed after immunization. There was a tendency to develop higher titres after a large single dose.

Four immunized volunteers who showed significant CF antibody titres of 1:80 - 1:640 at the time of infection with viable rickettsiae developed either no clinical disease or a very mild reaction. Moreover, in such persons, the titre of CF antibody did not rise after the injection of viable Rickettsiae.

Six volunteers who had no demonstrable antibody at the time of infection, although they had an initial low titre, did develop a mild to moderate illness. None of this group was regarded as seriously ill. Significant increases in antibody occurred in all members of this group with overt evidence of a mild clinical infection. In only one instance (volunteer 11) was the classic picture of mild typhus fever demonstrated. Two volunteers who had no detectable antibodies following vaccination (cases 4 and 10) had a mild febrile illness following infections.

Conclusions

The state of the art of rickettsial vaccines is unsettled. The remarkable beneficial efficacy of the broader spectrum antibiotics for therapy of patients with Rocky Mountain spotted fever, typhus and other rickettsioses has dampened enthusiasm for this type of research. Another factor is the relatively low incidence of these infections and a limited market for preventive vaccines.

Inactivated R. prowazekii vaccines do reduce the severity, duration and mortality in those immunized persons who contract epidemic (classic) typhus fever. No controlled data have assessed reduction in incidence of typhus following vaccination. Typhus vaccine is available commercially. Strain E, the attenuated-vaccine strain of R. prowazekii, does provide substantial protection against illness; this vaccine may produce a modified type of typhus infection. It is not available at present.

In human vaccination-challenge studies, the Rocky Mountain spotted fever vaccines failed to protect against clinical illness although the incubation periods in vaccinees was lengthened and the severity of illness appeared to be diminished when compared with controls. There does appear to be a limited beneficial effect. Vaccines for Rocky Mountain spotted fever are not commercially available in the USA.

There are no commercially available vaccines for murine typhus, scrub typhus, rickettsial pox or tick typhus of the eastern hemisphere.

Inactivated vaccines are effective for prevention of Q fever (Coxella burnetii infection). Q-fever vaccines are under experimental study and available for laboratory investigators and those persons exposed to heavily-infected aerosols.

Grateful thanks and affection, for help and guidance, are expressed to Dr Georges Blanc and Dr Marcel Baltazard, now deceased. Special appreciation is given to health authorities of...
Morocco and other administrative officials for their assistance in helping make these old vaccine studies possible. The technical assistance of various staff members of the Pasteur Institute is gratefully acknowledged. The writer is grateful to his dear friend and associate Dr Joseph E. Smadel, who gave helpful suggestions in the preparation of this report.

REFERENCES

10. Cox HH. Use of yolk sac of developing chick embryo as medium for growing rickettsiae of Rocky Mountain spotted fever and typhus groups. Publ Health Rep 1938; 53: 2241-2247.

Chlamydial infections of the eye and genital tract in southern Africa

R. C. BALLARD, H. G. FEHLER

In the light of recent developments in chlamydial research, there appears little doubt that chlamydial infections are among the most common communicable diseases afflicting man. These organisms, previously thought to be 'large viruses' and now recognized as obligate intracellular bacteria, were originally thought to be responsible for the relatively rare diseases psittacosis and lymphogranuloma venereum (LGV) and also for the blinding eye disease trachoma in endemic areas. During the past 15 years interest in the chlamydiae has increased considerably as it has gradually become apparent that Chlamydia trachomatis (serovars D-K) were responsible for a large number of sexually transmitted infections previously classified as 'idiopathic'. Until recently, little was known of the relative importance of genital tract infections and their sequelae in developing countries, especially in regions where the 'traditional' chlamydial diseases have remained a significant cause of morbidity.

Southern Africa is a typical example of such a region, which incidentally has a long history of chlamydial research. Indeed, Professor J.H.S. Gear and his colleagues at the South African Institute for Medical Research were one of the first groups in the world to isolate the causative organism of trachoma in the yolk-sacs of fertile hen's eggs. This historical association with chlamydial organisms and the subsequent development and application of tissue culture and serological techniques has resulted in many aetiological and epidemiological studies of chlamydial infections of the eye and genital tract being undertaken on the subcontinent. These studies are relevant not only to the local situation, but indicate that a considerable amount of morbidity caused by these infections may be found in other comparable developing regions.

Trachoma

Despite overwhelming clinical evidence to suggest that trachoma was endemic in the northern Transvaal, many doubts were expressed whether the disease described was, in fact, trachoma.2 These doubts arose from the belief, at the time, that the negroid races were less susceptible to the disease than other races. However, incontrovertible evidence that the disease was indeed trachoma was presented in 1952 by Amies et al., when classic intracytoplasmic inclusion bodies were demonstrated in 25 of 109 (23%) conjunctival scrapings obtained from children with presumed trachoma in Sekukuniland.

Between 1955 and 1959 many attempts were made to isolate the causative agent in tissue cultures and the yolk sacs of chick embryos. These efforts finally resulted in the isolation of an agent from the eye of a 7-year-old girl seen at Jane Furse Hospital, Sekukuniland.4 Once isolated, this agent was used to inoculate the eyes of a volunteer who developed an acute conjunctivitis 3 days later. Swabs and scrapings taken at weekly intervals before starting therapy resulted in demonstration of typical inclusion bodies in conjunctival epithelial cells and re-isolation of the organism in the yolk sacs of chick embryos.5 Subsequently the infection was successfully transmitted to baboons (Papio ursinus) and persistent, though low-grade infection was demonstrated for many months.6 These and later pioneering studies on trachoma undertaken by Professor Gear and his ophthalmologist colleague Dr Graham Scott indicated the widespread nature of the disease and identified it as a typical intracytoplasmic inclusion body infection.