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The assessment in sheep of an inactivated vaccine of parainfluenza 3 virus incorporating double stranded RNA (BRL 5907*) as adjuvant

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SUMMARY

The serological responses of conventionally reared sheep were compared after vaccination with inactivated parainfluenza 3 (PI3) virus incorporated in three different adjuvants. Inactivated PI3 virus with the double-stranded RNA, BRL 5907 in an oil emulsion was shown to stimulate higher serum antibody titres over the first 5 weeks after vaccination than virus with and without BCG emulsified in oil. The ability of this vaccine to protect specific pathogen-free lambs against challenge with PI3 virus was examined in a second experiment. In this experiment the vaccine stimulated virus neutralizing and haemagglutination inhibiting antibodies in the serum. After intranasal and intratracheal inoculation with PI3 virus at challenge, vaccinated lambs showed no clinical illness and virus isolation was confined, except in one lamb, to the first two days. In contrast, unvaccinated lambs developed respiratory disease and virus was isolated daily for 7 days after challenge.

INTRODUCTION

Experiments in specific pathogen-free (SPF) lambs have shown that an oil-emulsion inactivated vaccine of parainfluenza 3 (PI3) virus protects against challenge infection (Smith, 1975). However the associated tissue reaction and hypersensitization due to the mycobacterial component in this vaccine may preclude its acceptance for use in the field. Double-stranded polyribonucleic acids have been incorporated in oil-emulsion vaccines and have been shown to have adjuvant effects. The naturally occurring double-stranded RNA, BRL 5907* enhances the humoral immune response in mice (Cunnington & Naysmith, 1975) and incorporated in an oil-emulsion vaccine of inactivated Newcastle disease virus, stimulated high antibody titres which were maintained for up to 8 weeks (Gough, Allan, Knight & Leiper, 1974). In this study the effect of replacing the mycobacterial component in the oil-emulsion inactivated P13 virus vaccine by BRL 5907 was examined. Initially an experiment to assess the serological response to vaccination with a vaccine incorporating BRL 5907 was carried out in conventionally reared sheep. A further trial was conducted in specific pathogen-free lambs which were challenged with PI3 virus.

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MATERIALS AND METHODS

Sheep

In Experiment I, twenty-four 6-month old Cheviot sheep were reared under conventional conditions and on the basis of their prevaccination serum antibody titres to PI3 virus were allocated into four groups of six. Two sheep from each group were housed together in each of three isolation rooms. In Experiment II 8 hysterectomy-derived, colostrum deprived Dorset lambs were reared under specific pathogen-free conditions. These lambs were allocated to 2 groups of 4 lambs and each group was housed in an isolation room.

Virus

The ovine G2 isolate of PI3 (Hore, 1966) at the sixth pass was produced on secondary fetal lamb kidney (FLK) monolayers using medium 199* with 2% inactivated fetal bovine serum. In Experiments I and II virus used for vaccination had a titre of $10^{6\cdot0}$ TCID 50/ml. and $10^{8\cdot4}$ TCID 50/ml. respectively. Virus used to challenge lambs in Experiment II was produced on FLK cells overlaid with Eagle's medium with 1% fetal bovine serum and at the time of challenge had a titre of $10^{7\cdot5}$ TCID 50/ml.

Vaccines

Virus used for the preparation of vaccines in both experiments was inactivated by treatment with 0·5% formaldehyde for 72 hr. at room temperature. In Experiment I three vaccines were prepared from a pool of inactivated PI3 virus. These vaccines which all contained the same concentration of virus per unit volume were:

Vaccine A: virus emulsified with an equal volume of incomplete Freund's adjuvant (IFA) consisting of 20% Falba in Bayol F.

Vaccine B: virus with freeze dried BCG (250 μg./ml. tissue culture fluid) emulsified with an equal volume of IFA.

Vaccine C: virus with BRL 5907 (0·5 mg./ml. tissue culture fluid) emulsified with an equal volume of IFA.

In Experiment II only one vaccine, prepared as vaccine C was used.

Challenge

Sheep in Experiment I were not challenged but lambs in Experiment II were challenged by administering intratracheally 8·0 ml. and intranasally 2·0 ml. of infective culture fluid. Titration of a sample of the challenge inoculum showed that each lamb received $10^{8\cdot5}$ TCID 50 of infective PI3.

Virus isolation

Nasopharyngeal swabs were taken via the right nostril of lambs in Experiment I, placed immediately into 3·0 ml. of transport medium and held on solid CO_2. Samples collected in this way were either thawed and inoculated into FLK cul-

* Wellcome Reagents Ltd, Beckenham, Kent.

tures in tubes within 2 hr. or stored at −70° C. until inoculation. After 6 days incubation at 37° C., guinea-pig red blood cells were added to the medium and cultures were examined for haemadsorption.

Virus neutralization and haemagglutination inhibition tests

The microneutralization test described previously by Smith (1975) was used to titrate neutralizing antibody to PI3 in serum samples obtained in Experiment II. Haemagglutination inhibiting (HAI) activity in serum samples was estimated as described previously (Smith, Dawson, Wells & Burrells, 1976) and differences in serum antibody titres between vaccination groups in Experiment I were analysed using the Mann–Whitney test (Snedecor & Cochran, 1967).

Blood samples were collected from the jugular vein and the harvested sera were stored at −20° C. until used.

Experiment I

In this experiment there were 4 groups consisting of 6 sheep in each group. Each sheep in groups 1, 2 and 3 received an intramuscular injection of 2 ml. of vaccine A, B, or C respectively. Group 4 was not vaccinated and represented an in-contact control group. Blood samples were collected before vaccination and thereafter at weekly intervals for 6 weeks, with a final sample collected 8 weeks after vaccination.

Experiment II

In this experiment there were 2 groups (groups 5 and 6) each consisting of 4 hysterectomy-derived lambs. Lambs in Group 5 were vaccinated at 10 days of age with 2 ml. of vaccine C given intramuscularly. Twenty-five days after vaccination, lambs in both groups were challenged with $10^{8\cdot5}$ TCID 50 of infective PI3 given intratracheally and intranasally as described. Serum samples were collected before vaccination and on days 8, 15, 25 and 36 after vaccination. Nasopharyngeal swabs were taken before challenge and then daily for 10 days.

Pathology

All lambs in Experiment II were killed and examined at necropsy 10 days after challenge. Portions of lung tissue were fixed in formal saline and sectioned for histopathological examination.

RESULTS

Experiment I

Response to vaccination

The mean serum HAI antibody responses of each group to vaccination with the inactivated PI3 vaccines are shown in Fig. 1. All vaccines resulted in mean serum antibody responses which were significantly different ($P < 0\cdot05$) from that of the lambs in the in-contact control group at 7 days after vaccination and the antibody titres continued to be significantly different ($P < 0\cdot01$) for the duration of the experiment. The geometric mean titres of the group vaccinated with vaccine *C* containing BRL 5907 were higher than those of the other two vaccinated groups

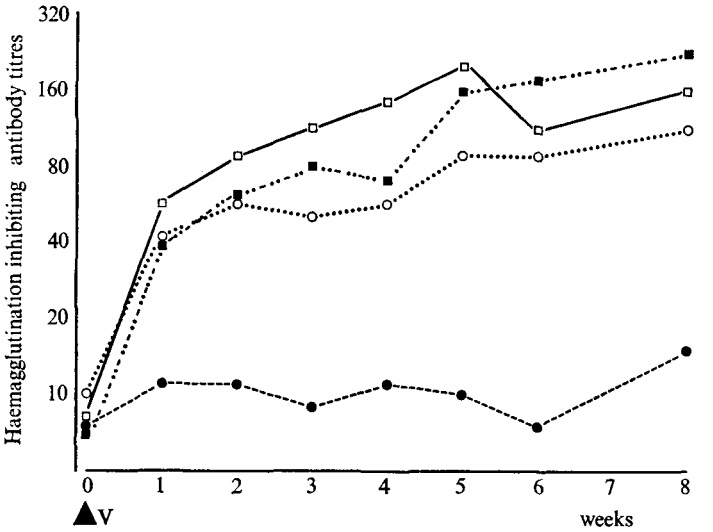

Fig. 1. Experiment I. Group geometric mean serum haemagglutination inhibiting antibody titres. ●------●, unvaccinated in-contact control group; ○.....○, vaccine A treated group; ■·····■, vaccine B treated group; and ¦□——□, vaccine C treated group.

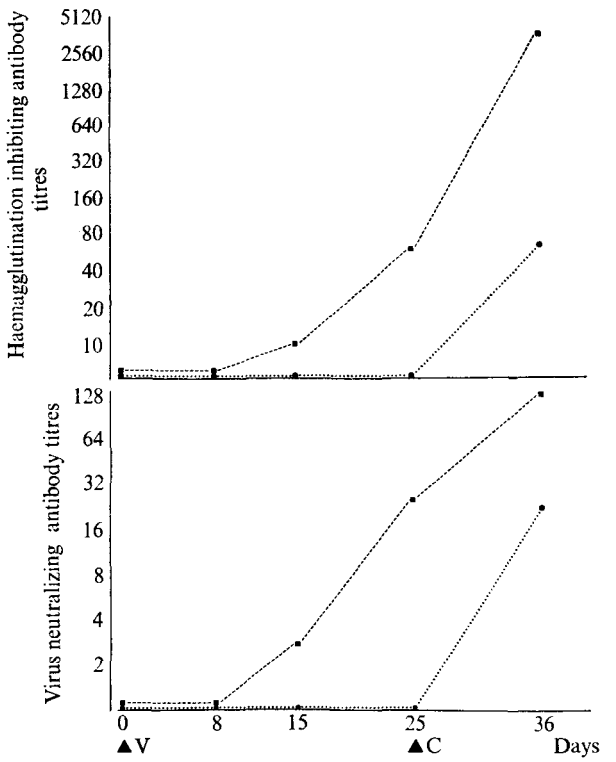

Fig. 2. Experiment II. Group geometric mean serum haemagglutination inhibiting and virus neutralizing antibody titres ●.....● unvaccinated incontact control group and ■——■ vaccine C treated group. Vaccine (V) was administered at day 0 and challenge (C) was after 25 days.

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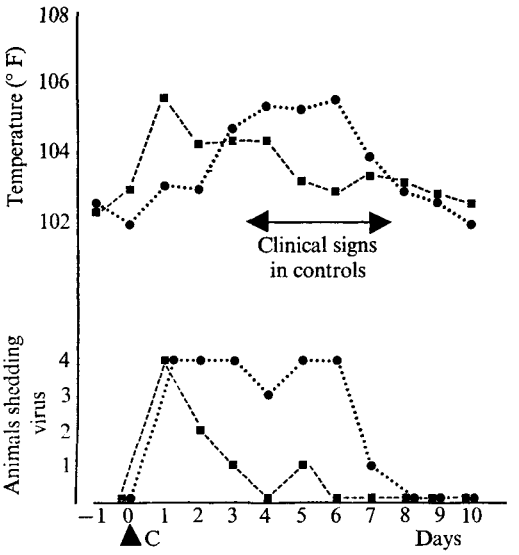

Fig. 3. Experiment II. Occurrence of clinical signs, group body temperature means and virus isolation from in-contact controls (●.....●) and vaccine C treated vaccinates (■——■) after challenge (C).

for the first 5 weeks after vaccination and at weeks 4 and 5 were significantly different ($P < 0{\cdot}05$) from those of the sheep vaccinated with vaccine A. No statistically significant differences between vaccinated groups were observed at other times.

On the basis of these results vaccine C was selected for assessment in the challenge experiment (Experiment II) in SPF lambs.

Experiment II

Response to vaccination

The group mean serological responses in the lambs injected with vaccine C are shown in Fig. 2. On day 25 when the lambs were challenged, geometric mean virus neutralization and haemagglutination inhibiting antibody titres were 25·0 and 47·6 respectively. These values were similar to those achieved in the control group at 10 days after challenge with live virus at which time, however, a further rise in serum antibody titre had occurred in vaccinated animals.

Response to challenge

The clinical response to challenge and relationship to virus shedding is summarized in Fig. 3. Lambs in the vaccinated group all showed a febrile response on the day after challenge but were otherwise clinically normal and were not anorexic. The febrile response seen in lambs in the control group coincided with obvious clinical signs of tachypnoea, dyspnoea, anorexia and general dullness. Virus was isolated from lambs in the control group for up to 7 days after challenge. Virus was also isolated from all the vaccinated lambs 24 hr. after challenge but from only one lamb on days 3 and 5 after challenge.

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Pathology

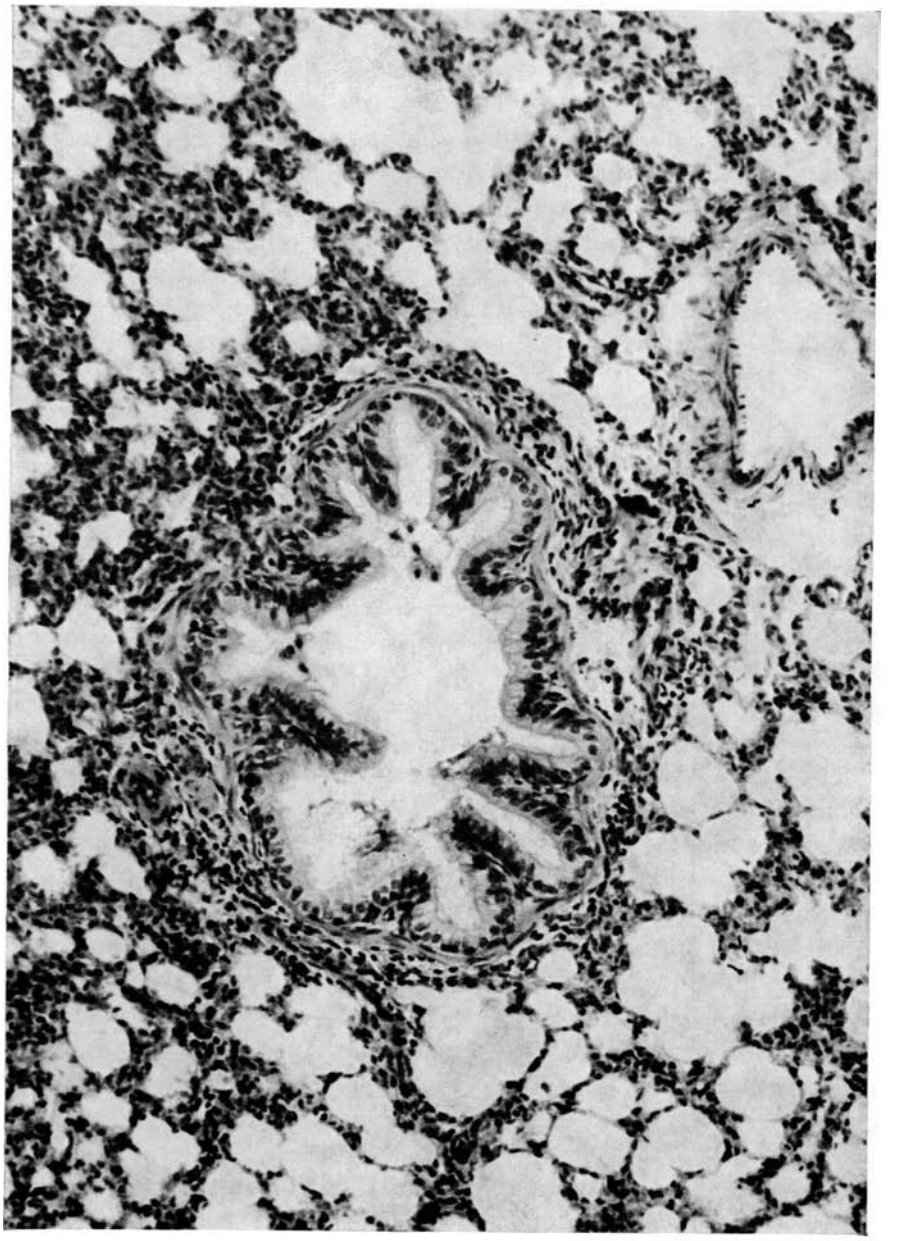
At necropsy there was no difference between the macroscopic appearance of lungs from vaccinated animals and controls in Experiment II. Histopathological examination of lungs from lambs in both groups revealed a mild bronchiolitis as characterized by epithelial hyperplasia with hypersecretion of mucin and infiltration of the peribronchiolar connective tissue by lymphoid cells (Plate 1). In lungs from vaccinated lambs, more lymphoid cells were present which were frequently arranged into small lymphoreticular nodules. There was also infiltration of interalveolar septa by lymphoid cells and hyperplasia of free and fixed macrophages, in the lungs of the vaccinated animals.

DISCUSSION

The results obtained in Experiment I confirm that inactivated PI3 virus incorporated in IFA is capable of stimulating a serum antibody response (Gilmour *et al.* 1968). Inclusion of both BRL 5907 or BCG in this vaccine appeared to increase the serological response but only vaccine C containing BRL 5907 stimulated serum antibody titres which were significantly different from those produced by the IFA vaccine. Thus vaccine C was selected for assessment in the challenge experiment using SPF lambs (Experiment II) in order to determine whether this vaccine was capable of stimulating a protective immune response.

In a previous experiment when unvaccinated SPF lambs were challenged with PI3 administered by aerosol (Smith, 1975) no clinical illness was observed although virus replicated in the upper respiratory tract. However, in the present experiment clinical signs were obvious in unvaccinated lambs when PI3 was administered by combined intratracheal injection and intranasal instillation in a manner similar to that described by Hore & Stevenson (1967). The clinical response was also greater than that observed by these workers and this may have been due to the greater concentration of virus or the larger volume of infected tissue culture fluid inoculated. It is significant, however, that the clinical signs were lessened by prior vaccination with vaccine C. The febrile response in the vaccinated group during the day after challenge was attributed to an immediate-type of hypersensitive response similar to that observed previously (Smith, 1975). Apart from this mild response the vaccinated group remained clinically normal and clearly a considerable degree of protection was conferred by vaccination with BRL 5907 adjuvanted vaccine. This contention is further supported by the results of attempts at virus isolation which showed a lower incidence of recovery of PI3 from the vaccinated group after challenge.

Despite the clinical response in the unvaccinated control lambs after challenge no microscopic lesions were observed at necropsy. This observation is consistent with findings in a further experiment in which SPF lambs were challenged with PI3 in a similar manner (unpublished results). It is suggested that the clinical signs observed in unvaccinated lambs may have been associated with a bronchiolar and alveolar exudate similar to that described by Stevenson (1968) which had



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largely resolved by the time of necropsy. The severity of the hyperplasia of the bronchiolar epithelium attributable to PI3 (Hore & Stevenson, 1967) was not reduced by vaccination. However, the increased numbers of lymphoid cells and free macrophages present in the lungs of vaccinated lambs may be a reflexion of the protective immune response induced by challenge in these animals.

It is clear from the results of these experiments that the inactivated vaccine of PI3 incorporating BRL 5907 in IFA produced a significant serological response and successfully protected SPF lambs against clinical illness produced by challenge with live virus. Further work is necessary to determine whether a vaccine of this type can reduce the incidence of clinical disease in the field.

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EXPLANATION OF PLATE

Experiment II. Epithelial hyperplasia with infiltration of peribronchiolar connective tissue by lymphoid cells in a section of bronchiole from the lung of an unvaccinated control lamb.