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Development of methods to measure humoral immune responses against selected antigens in the common marmoset (*Callithrix jacchus*) and the effect of pyridostigmine bromide administration

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Abstract

This methodological study was carried out in preparation for a major long term study, also reported in this volume, which was designed to investigate whether the combination of vaccines and pyridostigmine bromide (PB) could have been responsible for adverse signs and symptoms reported by a number of veterans of the 1990/1991 Gulf conflict. In this context, the marmoset has been used to model aspects of the human immune system.

The purposes of this methodological study were to select appropriate immunochemical reagents to measure humoral responses induced in marmosets in response to selected health and hygiene and biological warfare vaccines and to initially assess the effects of PB on the responses recorded. Vaccines were administered at 1/5th of a human dose, and also investigated in combination with the nerve agent pretreatment compound PB. PB dosing was selected to induce an inhibition of erythrocyte acetylcholinesterase by 30%. In order to assess the functionality of the immune system, antibody responses to a neo-antigen (keyhole limpet haemocyanin—KLH), administered some 2 months following the completion of the vaccination schedule, were measured.

The present study identified appropriate isotyping reporter reagents which cross-reacted with equivalent marmoset immunoglobulins. Robust antibody responses were identified against anthrax protective antigen (PA), whole cell pertussis vaccine and KLH, while weaker responses were measured against cholera and typhoid vaccines. The killed whole cell plague vaccine induced a response which was at the limit of detection of the assay. Coadministered PB had no discernable effect on immunological responses in this study.

Keywords: Vaccines; Non-human primate; Marmoset; KLH; Pyridostigmine bromide; Isotyping reagents; Humoral responses

1. Introduction

During the build up to the 1990/1991 Persian Gulf conflict, many UK service personnel were administered a range of vaccines over a relatively short time period, including those against endemic health and hygiene diseases and anti-biological warfare agents. They were

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also issued PB as a pretreatment against the potential threat of nerve agent poisoning.

A series of studies has been conducted in order to assess whether combined administration of the pretreatments (vaccines with or without PB) resulted in adverse health consequences. Non-human primates were considered to be essential because of their close phylogeny to man, which enabled extrapolation of data to be optimized. Marmosets were selected because of the practical and welfare considerations associated with their relatively small size, and their growing acceptance in fields of pharmacology [1–3], toxicology [4,5], behaviour [6,7] and sleep assessment [8], all key aspects which were incorporated into a fully controlled long-term study, *ibid*.

In the first of this series of studies, a number of dose levels of vaccines were assessed in a non-primate species, the guinea pig, in order to identify a dose level sufficient to induce immune responses but without producing any adverse health consequences [9]. A vaccine dose level of 1/5th of a human dose was found to be acceptable. This study allowed the quantification in the marmoset of antibody responses to a group of relevant vaccine antigens.

The marmoset, however, had not previously been characterised as an immunological model for man and consequently suitable immunoreagents, such as immunoglobulin isotyping reagents for the determination of specific antibody response, were not available for this species. The study reported here focused on the development of suitable assays based on commercially available reagents that were identified to be cross-reactive with marmoset immunoglobulins. Antibody responses to selected vaccine antigens and KLH were measured by Enzyme Linked Immunosorbent Assay (ELISA). Isotyping reagents, suitable for marmoset immunoglobulins, were screened using Western blotting techniques.

This part of the study also provided an opportunity to undertake a preliminary investigation of the effects of PB administered at the same time as some of the vaccines. It has been suggested that modulation of immune responses might possibly occur via acetylcholine-based signalling, partly because T cells appear to have the full complement of molecular machinery necessary to undertake acetylcholine homeostasis and to use this neurotransmitter to modulate their activation [10]. Since PB binds to (carbamoylates) acetylcholinesterase it is possible that this compound might exert some effect on the function of the T cell.

Functional integrity of immune responses, 18 months after administration of treatments, was tested by measuring antibody responses against the neo-antigen, KLH, a T cell dependent antigen [11].

2. Materials and methods

2.1. Animals

Twelve marmosets (six males, six females, weighing 300–500 g) were used, which had been bred at Dstl, Porton Down, UK. The work was conducted with due regard to the social and environmental needs of this non-human primate species and under the aegis of a project license granted within the legislation of the UK Animals (Scientific Procedures) Act 1986 along with an internal ethical review process. Animals were assigned to either of two groups of $n=6$ animals (3 male, 3 female). One group received vaccines and PB and the other group received vaccines and saline.

They were housed at a mean temperature of 25 °C and a relative humidity of 40%, in two cage units per animal, linked by a vertical cage extension fitted with a tray at the base containing forage mix. Animals were kept on a 12 h light/dark cycle with 30 min dawn and dusk effects to simulate conditions in the wild. Water was available ad libitum and the animals were fed once a day with primate pellets and fruit.

2.2. Dosing schedules

Vaccines were administered according to a schedule which reflected, as closely as possible according to information available, that given to service personnel prior to the 1990/1991 conflict. Vaccines were administered at 1/5th human dose (based on volume) and are detailed in Table 1. All vaccines used in the study were in contemporary clinical use except for whole cell pertussis vaccine, which had subsequently been replaced with an acellular version. This vaccine was administered to a number of military personnel engaged in the first Persian Gulf conflict and it was necessary to manufacture a preparation which matched as closely as possible the original product specification. This was undertaken by The Centre for Applied Microbiological Research (now HPA, Porton Down).

PB or saline was administered by mini-osmotic pump between days 15 and 44 during the vaccine dosing schedule.

In order to minimize local trauma from multiple vaccinations on a single day, several intramuscular sites were used. Anthrax and pertussis vaccine injections were administered at adjacent sites but in the opposite limb to that of the plague vaccine.

2.3. PB administration

PB, at a dose of 500 μg/kg/day, the dose level which was intended to result in an inhibition of erythrocyte acetylcholine esterase (as assessed using a standard colorimetric assay; [12]) of approximately 30%, was administered over 28 days (days 15–44) by means of subcutaneously implanted mini-osmotic pumps (Alzet Corp., USA). The mini-osmotic pumps were implanted subcutaneously below the right scapula under general anaesthesia (alphaxalone and alphadalone acetate; Saffan, Glaxo) i.m. 5 min following premedication with diazepam (Valium, Roche) i.m. Pumps contained either 0.9%