

Pollinex® Quattro Ragweed: safety evaluation of a new allergy vaccine adjuvanted with monophosphoryl lipid A (MPL®) for the treatment of ragweed pollen allergy

Paul Baldrick,¹ Derek Richardson,² Stefan R. Woroniecki^{2,*} and Beverley Lees²

¹ Scientific and Regulatory Consulting, Covance Laboratories Ltd, Otley Road, Harrogate HG3 1PY, UK

² Allergy Therapeutics (UK) Ltd, Dominion Way, Worthing BN14 8SA, UK

Received 4 December 2006; Revised 13 December 2006; Accepted 18 December 2006

ABSTRACT: A novel allergy vaccine (Pollinex® Quattro Ragweed) has been developed for the prevention or relief of allergic symptoms caused by pollen from *Ambrosia* spp. (ragweed). An extract from the pollen (chemically modified by glutaraldehyde) is adsorbed onto L-tyrosine with addition of the immunostimulatory adjuvant, monophosphoryl lipid A (MPL®). A specific preclinical safety testing strategy was developed to support clinical use and comprised reference to preclinical data available for the marketed non-MPL® adjuvanted form of the ragweed vaccine (Pollinex® R) and a new repeat dose toxicity study in the rat. Studies with Pollinex® R comprised single dose subcutaneous toxicity studies in mice and rats, repeat dose (10 injections over 20 days) parenteral toxicity studies in rats and dogs, an *in vitro* gene mutation assay along with single and multiple injection local tolerance studies in rats and dogs. The repeat dose subcutaneous toxicity study with Pollinex® Quattro Ragweed involved seven injections over 3 weeks (which was more aggressive than the four weekly doses used in the clinic) with dose levels of up to 0.5 ml per animal used. Overall, the product showed no toxicological findings of significance at levels greatly in excess of those proposed for clinical use. As is a feature with vaccination, some dose site irritation was seen. Copyright © 2007 John Wiley & Sons, Ltd.

KEY WORDS: ragweed pollen; allergy vaccines; Pollinex® Quattro Ragweed; MPL® adjuvant; toxicity studies

Introduction

Allergic asthma and allergy-related disorders such as rhinitis are becoming increasingly prevalent in the developed world. A particularly important allergenic weed is ragweed (*Ambrosia* spp.) which has been reported as the major source of airborne allergenic protein in the United States with at least 10% of the overall population sensitised to short ragweed and a prevalence of about 50% in atopic individuals (Taramarcaz *et al.*, 2005; Wopfner *et al.*, 2005). Indeed, a recent survey of over 10 000 individuals showed a positive skin test response of 26% to ragweed for the period 1988–1994 which was over 2-fold higher than the prevalence rate seen in an earlier survey covering the period 1976–1980 (Arbes *et al.*, 2005). Ragweed allergy is also rapidly increasing in Europe (notably in France, Italy, Austria, Hungary, Croatia and Bulgaria) where up to 12% of the population in some of these countries are hypersensitive to this type

of pollen (Taramarcaz *et al.*, 2005). Over the years, allergy vaccination, also known as specific immunotherapy (SIT) has been recognised as a safe and efficacious treatment strategy for patients suffering from ragweed and other pollen allergy; gradually increasing quantities of specific allergen are given in order to reduce symptoms to that allergen upon subsequent exposure (e.g. Bousquet *et al.*, 1998; Ebner, 1999; Malling, 2000; Rolland *et al.*, 2000). Such vaccination is believed to exert its beneficial effects on the immune system, at least in part, by modifying the T-helper 2 (Th2) lymphocyte response to subsequent natural allergen exposure. Studies of successful allergy vaccination have shown that the Th2 cellular response influencing the expression of interleukins IL-4, IL-5 and IgE antibody is depressed in favour of a more Th1 like response, which is associated with increased IFN- γ and IL-10 or IL-12 (Bellinghausen *et al.*, 1997; Durham and Till, 1998; Jung *et al.*, 1995). Continuing development in this field has improved safety and efficacy by addressing manufacturing quality (better characterised and standardised allergen extracts), optimal dosing, allergen modification (to reduce allergenicity whilst maintaining immunogenicity), adjuvant adsorption (to control release) and adjuvant activity (to assist immunomodulatory action). A new range of vaccines

* Correspondence to: Stefan Woroniecki, Allergy Therapeutics Ltd, Dominion Way, Worthing, West Sussex, BN14 8SA, UK.
E-mail: Stefan.woroniecki@allergytherapeutics.com

(Pollinex® Quattro) embraces these properties resulting in the alleviation of symptoms caused by many pollens. Indeed, current formulations are designed to provide a vaccine that is efficacious with only four doses in contrast to longer administration schedules in use for other allergy vaccines. Pollinex® Quattro Ragweed contains a short ragweed pollen extract which is chemically modified by glutaraldehyde and adsorbed onto L-tyrosine with addition of the immunostimulatory adjuvant, monophosphoryl lipid A (MPL®). Short ragweed is the most widespread species, and it is not necessary to employ mixtures of ragweed pollens since there is significant cross-reactivity between species (White and Bernstein, 2003).

Vaccines employing allergen modification and improved adsorption are not new and include the Modified pollen Allergens Tyrosine Adsorbed (MATA) vaccines derived from extracts of one or more pollens which are chemically modified by glutaraldehyde (to produce allergoids) and adsorbed onto L-tyrosine (e.g. Wheeler *et al.*, 1982). Variants of the MATA range are currently marketed in various countries under various brand names (e.g. Pollinex®) and have been in use for at least 25 years; indeed, Pollinex® R, a modified ragweed tyrosine adsorbate, has been commercially available in Canada since the 1980s (www.hc-sc.ca). An array of data are available to show the safe and efficacious use of MATAs using grass pollen (including ragweed) extracts (e.g. Bonifazi *et al.*, 1991; Drachenberg *et al.*, 2001; 2003; Ko *et al.*, 1987; Muehlethaler *et al.*, 1990; Palmer and Balacescu, 1977). Indeed, a recent study with Pollinex® Quattro Ragweed, using a four-injection course, has demonstrated the vaccine to be well tolerated and to significantly reduce allergy symptoms (www.allergytherapeutics.com). Other publications show that L-tyrosine is a safe adjuvant for human use and has both an adsorptive role (as a short-term depot) and a favourable immunological effect (Baldrick *et al.*, 2002a; Newland *et al.*, 1999; Wheeler *et al.*, 1982; Wheeler and Woroniecki, 2001). The addition of MPL®, a detoxified form of lipid A derived from the lipopolysaccharide of *Salmonella minnesota* R595, as an immunostimulatory adjuvant to a range of vaccine materials is the subject of ongoing clinical trials for many companies. Indeed, the adjuvant has been licensed for use in the melanoma vaccine, Melacine® in Canada and in the hepatitis vaccine, Fendrix® in Europe (www.emea.eu.int-a; www.pslgroup.com). Published non-clinical and clinical data indicate that MPL® is safe and effective with excellent immunological adjuvant activity through its ability to induce a skew from a Th2-like to a Th1-like situation (Alving, 1993; Baldrick *et al.*, 2000b; Lodmell *et al.*, 2000; Moore *et al.*, 1999; Puggioni *et al.*, 2005; Thoelen *et al.*, 1998; Ulrich and Myers, 1995). Mechanistic studies indicate that it works as a Toll-Like 4 Receptor (TLR4) agonist, directly activating innate immune mech-

anisms such as induction of pro-inflammatory cytokines with resulting favourable T and B cell responses (e.g. De Becker *et al.*, 2000; Ismaili *et al.*, 2002; Martin *et al.*, 2003). *In vitro* and *in vivo* studies show that non-toxic doses of MPL® have ideal adjuvant properties at dose levels at, or in the region of, 50 µg MPL®, a level which is used in human allergy vaccine formulations (Baldrick *et al.*, 2002a). Immunogenicity studies in rodents have shown that the presence of L-tyrosine/MPL® with a model antigen provides a Th1 skewed effect inducing IgG2a and IgG2b responses but minimal IgE response (Wheeler *et al.*, 2001).

Clinical trials with Pollinex® Quattro vaccines are progressing and this paper reports preclinical studies that were performed to show that the materials, in this case Pollinex® Quattro Ragweed, have given no cause for toxicological safety concerns. Relatively little global 'regulatory' guidance for safety testing of these types of product exists. The World Health Organisation (WHO) has designated standardised allergen extracts as 'vaccines' (Bousquet *et al.*, 1998). In Europe, guidance exists for preclinical evaluation of vaccines (www.emea.eu.int-b) and the WHO has recently issued guidance on the same topic (www.who.int). The principles mentioned in these documents have been followed but due to the nature of Pollinex® Quattro Ragweed, a specific safety testing strategy was adopted with a new repeat dose toxicity investigation in the rat.

Materials and Methods

The repeat dose toxicity study in the rat with Pollinex® Quattro Ragweed was performed at Charles River Laboratories, BioLabs Europe, Ballina, Co. Mayo, Eire in 2004 and conformed to Good Laboratory Practice (GLP). Other preclinical studies with glutaraldehyde-modified ragweed pollen extract (adsorbed onto L-tyrosine) to which Allergy Therapeutics Ltd has access, performed in the 1970s, were carried out in various testing establishments before the introduction of GLP but were considered to be scientifically robust.

Test Material and Formulation

The Pollinex® Quattro Ragweed vaccine employs a modified ragweed pollen (short or common ragweed, *Ambrosia elatior*, *Ambrosia artimisiifolia*) extract which is adsorbed by co-precipitation to L-tyrosine and then to MPL® adjuvant. The product is analogous to the ragweed-containing Pollinex® R vaccine and similar to some forms of the grass pollen vaccine marketed as Pollinex®, with the exception that MPL® is not present. An aqueous extract of ragweed pollen is chemically modified with glutaraldehyde which covalently binds to primary amino

Table 1. Composition of test material^a

Ingredient	Function	Quantity per ml	Reference to Standards
Active substance			
Allergen extract (short ragweed allergoid)	Active ingredient	12 000 SU	HSE ^b
Other ingredients			
L-Tyrosine	Adsorbant base	20 mg (2%)	Ph.Eur.
Monophosphoryl lipid A/AF (MPL [®])	Adjuvant	0.1 mg	HSE ^c
Phenol	Preservative	5 mg (0.5%)	Ph.Eur.
Sodium chloride	Tonicity	9 mg	Ph.Eur.
Disodium hydrogen phosphate dodecahydrate and sodium dihydrogen phosphate dihydrate	Buffering and tonicity	Not exceeding 4 mg	Ph.Eur.
Water for injection	Diluent	to 1 ml	Ph.Eur.

^a The formulation control material was identical except that active ingredient and MPL[®] were not present.

^b HSE — in-house specifications, vaccines are quantified in SU — Standardised Units ml⁻¹ (12 000 SU contains approximately 20 µg of major ragweed pollen allergen Amb a 1).

^c HSE — methodology described in Hopkins *et al.* (2001).

groups on the extracted protein. This procedure gives a modified allergen (also known as an allergoid) which retains the ability to reproduce the immunological response to the native allergen, but with the safety benefit of reduced allergenicity or IgE reactivity (Moran and Wheeler, 1976; Moran *et al.*, 1977; Wheeler *et al.*, 1976). A measured antigenic value is used for the starting pollen material which forms the basis for the calculation of immunological potency in Standardised Units ml⁻¹ (SU ml⁻¹) for the final product. The composition of test material is given in Table 1.

The test material for the repeat dose toxicity study with Pollinex[®] Quattro Ragweed vaccine was formulated into vials containing 12 000 SU ml⁻¹ of ragweed pollen allergoid, 100 µg ml⁻¹ MPL[®] adjuvant and 20 mg ml⁻¹ tyrosine and was administered as supplied at volumes of 0.05 and 0.5 ml. The latter volume resulted in a level of 6000 SU ml⁻¹ allergoid which is the highest dose of Pollinex Quattro Ragweed vaccine.

Single Dose Toxicity Studies

Groups of male and female mice or rats were given a single subcutaneous injection of modified ragweed pollen tyrosine adsorbate vaccine (Pollinex[®] R) in four separate studies as detailed in Table 2. Although these studies were performed in the 1970s, they followed a standard single dose study design and it is felt that they are useful in supporting an assessment of toxicity following acute subcutaneous dosing with a high ragweed pollen allergoid concentration.

Repeat Dose Toxicity Studies

Groups of male and female rats or dogs were given a single intramuscular (rat) or subcutaneous (dog) injection

of modified ragweed pollen tyrosine adsorbate vaccine every second day over 20 consecutive days as detailed in Table 2. These studies, which were performed in the 1970s, are probably deficient by modern standards in that only one vaccine-treated group was tested and low numbers of non-rodents were used in the study design, although standard repeat dose parameters were assessed as part of the study design. Thus, it is felt that they are useful in supporting an evaluation of general toxicity following repeated parenteral dosing with a high ragweed pollen allergoid concentration.

The repeat dose toxicity study with Pollinex[®] Quattro Ragweed vaccine took the form of a 3-week intermittent dose subcutaneous toxicity study in the rat followed by a 2-week treatment-free period. Groups of 10 male and 10 female Han Wistar rats (CrI:WI (GlX/BRL/Han) BR from Charles River Labs, Margate Kent, UK) were given a single subcutaneous dose of vaccine at dose volumes of 0.05 and 0.5 ml per rat twice weekly for 3 weeks (a total of 7 doses) — groups 3 and 4, respectively. This dose regimen is much more aggressive than that used in the clinic (four weekly doses). Two control groups (one — group 1 — receiving 0.9% sodium chloride for intravenous infusion, Baxter Healthcare Ltd, Norfolk, UK and the other — group 2 — receiving the vehicle formulation without the allergoid or MPL[®] — see Table 1) of 10 males and 10 females were also dosed on the same seven occasions over 3 weeks at a dose volume of 0.5 ml per rat. Rats were approximately 8 weeks old and had body weights of 209–262 g (males) and 144–187 g (females) on study initiation. They were allocated to the various study groups by a randomisation procedure based on body weight and, individually identified by ear punch and animal number recorded on the dorsal surface of the tail. The animals were generally maintained under conditions of 21–23 °C, 44–56% relative humidity and a 12 h light/dark cycle. Standard rodent diet (a minimum of 500 g/cage) and water (available *ad libitum*) were

Table 2. Summary of toxicology studies performed with modified ragweed pollen tyrosine adsorbate vaccine

Study type	Study design	Results	Reference
Single dose toxicity in mouse or rat	Groups of 6 male and 6 female mice or rats were given one subcutaneous dose at 77 000–280 000 Noon units ^a kg ⁻¹ at a dose volume of up to 20 ml kg ⁻¹ and observed for 14 days	No deaths or signs of systemic toxicity. Local dose site reaction seen	Unpublished data, 1974
Single dose toxicity in mouse or rat	Groups of 6 male and 6 female mice or rats were given one subcutaneous dose at 10 000–40 000 protein nitrogen units ^b kg ⁻¹ at a dose volume of up to 4.2 ml kg ⁻¹ and observed for 14 days	No deaths, signs of systemic toxicity or dose site reactions seen	Unpublished data, 1978
Repeat dose toxicity in rat	Groups of 12 male and 12 female Sprague-Dawley rats received an intramuscular injection of either saline or test material at 14 000 Noon units ml ⁻¹ at a dose volume of 0.2 ml per animal twice weekly over 20 days (a total of 10 doses). Animals were assessed for clinical signs, body weight and food consumption, clinical pathology and urinalysis, organ weights plus macroscopic and microscopic changes	Notable study findings comprised an initial loss or arrest of body weight (both sexes) plus slightly reduced food intake (females), raised neutrophil values (both sexes) and spleen weight (males). Dose site findings of a few small foci of white material within the musculature and moderate reactive change (focal necrosis in the intramuscular connective tissue with oedema and occasionally, fibrocytic proliferation) among test material-treated animals; controls only showed slight muscle reddening at the injection site	Unpublished data, 1974
Repeat dose toxicity in dog	Groups of 2 male and 2 female Beagle dogs received a subcutaneous injection of either saline or test material at 14 000 Noon units ml ⁻¹ at a dose volume of 0.5 ml per animal twice weekly over 20 days (a total of 10 doses). Animals were assessed for clinical signs, body weight and food consumption, clinical pathology and urinalysis, organ weights plus macroscopic and microscopic changes	Study findings were restricted to dose site effects. Among test material-treated animals, post-dose injection site swelling was seen with macroscopic findings of localised thickening/lamination of the subcutaneous tissue with diffuse haemorrhage for up to 5 days after dosing; a focus of whitish material was also seen within the thickened tissue. Microscopically, moderate oedema, inflammation and foci of necrosis occurred within the subcutaneous connective tissue for up to 3 days after injection followed by rapid tissue regeneration. Saline controls only showed minimal post-injection swelling and occasional subcutaneous haemorrhage with minimal inflammatory foci	Unpublished data, 1974

Local tolerance in rat	Groups of 3 male and 3 female Sprague-Dawley rats were given a single or multiple intramuscular injections of test material into the right thigh muscle at 14 000 Noon units ml ⁻¹ and a dose volume of 0.2 ml as follows: single injection with 1, 3, 5 and 7 days recovery, 4, 7 or 10 injections on separate days with one day recovery and 10 injections on separate days with 7 or 14 days recovery before killing. A similar dose regimen was used for the left thigh muscle using saline. Examinations comprised clinical signs plus macroscopic and microscopic examination of the injection site	Findings were restricted to minimal or moderate swelling of the injection site along with macroscopic findings of slight firmness of the muscle and/or reddening through to the presence of pockets of pale yellow/white material and/or minimal haemorrhage and mild oedema. Following single injection, these findings had generally resolved by 7 days post-dose. A gradual recovery was seen following multiple injections, with minimal reactions at 7 days and no reactions at 14 days of recovery. Microscopic examination after single injection revealed oedema and chronic inflammation of the intermuscular connective tissue with occasional necrosis at one or 3 days post-post; minimal or no findings occurred with the longer recovery period. Following multiple injections necrosis, inflammation and/or fibrocytic proliferation and haemorrhage were seen; a gradual recovery was noted with recovery complete 14 days post-dose. Minimal findings were seen at saline-treated sites	Unpublished data, 1974
Local tolerance in dog	Groups of one male and one female Beagle dogs were given single or multiple subcutaneous injections of test material at 14 000 Noon units ml ⁻¹ and a dose volume of 0.5 ml per site as follows: one group received single injections at 4 different left dorsal sites on day 1, 3, 5 and 7 and, another group received up to 4 injections at 4 different left dorsal sites on days 1, 3, 5 and 7 or 7, 9, 11 and 13 or 13, 15, 17 and 19; both groups were killed on the day following the final injection (day 20). Control animals were injected on the right dorsal side with vehicle (saline) under the same experimental conditions and at an equivalent dose volume. Animals were observed for clinical signs, injection site reaction, body weights and food and water intake. Macroscopic and microscopic examination concentrated on the injection site	There was no effect on clinical signs, body weight, food and water intake. Small subcutaneous swellings following injection were seen along with macroscopic findings of localised thickening and lamination of the subcutaneous tissue with haemorrhage and/or white material at the injection sites 1 and 3 days following the last injection; minimal haemorrhage at 5 days and no reaction at greater than 5 days were seen. Injection site histopathology showed marked oedema and necrotic debris and/or widespread polymorph infiltration and, with multiple injections, mixed inflammatory cells and early fibroblastic proliferation. A gradual recovery was seen such that minimal findings or no abnormalities were seen at 7 and 14 days, respectively, after the last injection. Minimal swelling, occasional haemorrhage and minimal inflammatory foci were seen at saline injected sites	Unpublished data, 1974

^a Noon unit = quantity of soluble allergen extracted from 1 µg allergen source (i.e. ragweed pollen).

^b Protein nitrogen unit = 0.01 µg of phosphotungstic acid-precipitable protein nitrogen, quantified by the Kjeldahl method.

provided throughout the study. Rats were housed five to a cage.

All rats dosed with 0.05 ml and 5 animals/sex/group from the two control and 0.5 ml groups were killed after 3 weeks; the remaining rats were retained for a 2-week recovery period. The following study parameters were measured: mortality and clinical observations, body weights, food consumption, ophthalmoscopy, clinical pathology and urinalysis, organ weights plus macroscopic and microscopic examination. Clinical observations were made daily. Body weights were recorded during acclimatisation, on day 1, twice weekly and prior to necropsy. Food consumption was measured on a weekly basis and was expressed as g rat⁻¹ week⁻¹. An ophthalmoscopic examination was performed predose (all animals) and at the end of week 3 (controls and high dose); examination was carried out using an ophthalmoscope after induction of mydriasis. On the day before necropsy, the rats were moved to metabolism cages overnight to allow collection of urine samples. The following parameters were measured: volume, specific gravity, pH, glucose, blood, ketones, protein, bilirubin, urobilinogen, reducing substances and microscopy of the sediments. Prior to killing at the end of week 3 or after the 2 week non-dose recovery period, blood samples for haematology and clinical chemistry assessment were collected by cardiac puncture. Samples were collected in EDTA and lithium heparin tubes, respectively. Haematology parameters measured comprised: haemoglobin, red blood cell count, packed cell volume, mean cell volume, mean cell haemoglobin, mean cell haemoglobin concentration and white blood cell count. Clinical chemistry parameters measured comprised: aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, sodium, potassium, chloride, calcium, inorganic phosphate, urea, total bilirubin, creatinine, total protein, albumin, globulin, albumin/globulin ratio, total cholesterol and glucose.

Rats were killed after 3 weeks of treatment or, after the 2 week recovery period, by sodium pentobarbitone overdose prior to exsanguination and then examined grossly for pathological changes. Selected organs were weighed (paired organs together) and comprised: adrenals, kidneys, spleen, liver, heart, brain, pituitary, testes + epididymides and ovaries. Following processing for light microscopy, the following organs/tissues were examined histopathologically for all animals of the control and high dose groups (as well as injection sites from the low dose group): adrenals, axillary lymph nodes, brain, caecum, colon, duodenum, femur with bone marrow and articular surface, gross lesions, heart and aorta, ileum, injection sites, jejunum, kidneys, larynx and trachea, liver, lungs with mainstem bronchi, mammary gland (female), mandibular and mesenteric lymph nodes, oesophagus, ovaries, pancreas, Peyer's patches, pituitary, prostate, rectum, salivary glands, sciatic nerves, seminal vesicles, skin, spinal cord (at three levels), spleen, sternum with bone marrow,

stomach, testes + epididymides, thymus, thyroids + parathyroids, tongue, trachea, urinary bladder, uterus and vagina.

Statistical analysis involved comparison of Group 1 with Groups 2, 3 and 4. Body weight and clinical pathology parameters were analysed using mixed models analysis of variance (ANOVA) and/or covariance (ANCOVA). Probability values of $P < 0.05$ were considered statistically significant.

Genotoxicity

The two components of modified ragweed L-tyrosine adsorbate (Pollinex R), i.e. modified ragweed extract 3% w/v and L-tyrosine suspension were tested separately for mutagenic potential in three histidine-requiring strains (TA98, TA100 and TA1537) of *Salmonella typhimurium* both in the absence and presence of metabolic activation by an Aroclor 1254-induced rat liver post mitochondrial fraction (S9). Levels of up to 3000 µg of modified ragweed extract/plate (Unpublished data, 1978) and 1000 µg of L-tyrosine/plate (Baldrick *et al.*, 2002b) were tested, using distilled water as solvent; positive and negative control treatments were also included as part of the study design. Although this study was deficient by modern standards, it is felt that it is useful in confirming a lack of genotoxic potential from modified ragweed extract and tyrosine suspension. L-Tyrosine was shown to be non-genotoxic elsewhere (Baldrick *et al.*, 2002b).

Local Tolerance Studies

A comprehensive study has been carried out in rats and dogs to examine dose site effects following parenteral administration of modified ragweed pollen tyrosine adsorbate vaccine as detailed in Table 2. Although these studies were performed in the 1970s, they followed a study design of injection followed by macroscopic and microscopic examination and it is felt that they are useful in supporting an assessment of local tolerance for a ragweed pollen vaccine.

Results

Single Dose Toxicity Studies

Not unexpectedly, no evidence of toxicity was seen following high acute subcutaneous doses of modified ragweed pollen tyrosine adsorbate vaccine to mice or rats (see Table 2). The doses used were in the order of up to 2-fold the ragweed allergoid concentration used in the new Pollinex® Quattro Ragweed.

Repeat Dose Toxicity Studies

No evidence of toxicity was seen following 10 parenteral doses of modified ragweed pollen tyrosine adsorbate vaccine, given over 20 days, to rats or dogs (see Table 2). The doses used were approximately the same as the ragweed allergoid concentration used in the new Pollinex® Quattro Ragweed. In the rat, slight increases

in neutrophils and spleen weight were seen. For both species, evidence of a local dose site reaction was seen.

Single subcutaneous doses of Pollinex® Quattro Ragweed vaccine at 0.05 and 0.5 ml per rat twice weekly for 3 weeks were well tolerated with no obvious signs of systemic toxicity (see Table 3). The only notable statistically significant changes compared with the saline control in clinical pathology parameters were a slight

Table 3. Key effect of repeat subcutaneous administration of Pollinex® Quattro Ragweed on various toxicity study parameters after a 3 week treatment and 2 week recovery period (week 5)

Parameter		Dose level (ml per animal) and findings $\pm$ standard deviation			
		0.9% saline	Vehicle	0.05	0.5
Haemoglobin (g dl ⁻¹) week 3	M	14.0 $\pm$ 0.7	13.8 $\pm$ 0.4	14.1 $\pm$ 0.6	13.5 $\pm$ 0.2*
	F	14.4 $\pm$ 0.5	13.7 $\pm$ 0.3	13.9 $\pm$ 0.3	12.5 $\pm$ 1.1*
Haemoglobin (g dl ⁻¹) week 5	M	14.1 $\pm$ 0.5	14.1 $\pm$ 0.5	—	13.9 $\pm$ 0.5
	F	13.8 $\pm$ 1.0	14.0 $\pm$ 0.5	—	13.3 $\pm$ 0.9
Packed cell volume (%) week 3	M	42.3 $\pm$ 2.4	41.3 $\pm$ 1.0	42.2 $\pm$ 1.9	40.8 $\pm$ 0.9*
	F	43.0 $\pm$ 1.4	41.0 $\pm$ 0.9	41.6 $\pm$ 1.2	37.6 $\pm$ 3.1*
Packed cell volume (%) week 5	M	41.5 $\pm$ 1.9	41.8 $\pm$ 1.7	—	41.0 $\pm$ 1.7
	F	41.0 $\pm$ 3.0	41.4 $\pm$ 1.3	—	39.5 $\pm$ 2.3
Mean cell volume (fl) week 3	M	56.9 $\pm$ 1.1	54.8 $\pm$ 1.1	54.9 $\pm$ 1.3	53.1 $\pm$ 1.4*
	F	55.4 $\pm$ 1.7	55.3 $\pm$ 0.5	55.3 $\pm$ 1.6	55.0 $\pm$ 1.6*
Mean cell volume (fl) week 5	M	52.4 $\pm$ 1.0	53.7 $\pm$ 1.3	—	52.1 $\pm$ 1.2
	F	54.0 $\pm$ 1.1	53.6 $\pm$ 0.9	—	53.2 $\pm$ 1.6
Mean cell haemoglobin week 3	M	18.8 $\pm$ 0.2	18.4 $\pm$ 0.5	18.4 $\pm$ 0.5	17.5 $\pm$ 0.5*
	F	18.5 $\pm$ 0.6	18.5 $\pm$ 0.2	18.4 $\pm$ 0.6	18.3 $\pm$ 0.6*
Mean cell haemoglobin week 5	M	17.9 $\pm$ 0.6	18.2 $\pm$ 0.6	—	17.6 $\pm$ 0.5
	F	18.2 $\pm$ 0.4	18.1 $\pm$ 0.6	—	17.9 $\pm$ 0.6
Albumin (g l ⁻¹) week 3	M	35.5 $\pm$ 2.4	34.3 $\pm$ 1.3	33.5 $\pm$ 1.0	33.4 $\pm$ 1.5*
	F	36.9 $\pm$ 1.8	36.9 $\pm$ 1.5	36.4 $\pm$ 1.7	33.3 $\pm$ 1.8*
Albumin (g l ⁻¹) week 5	M	33.9 $\pm$ 1.2	35.6 $\pm$ 1.2	—	32.7 $\pm$ 1.2
	F	37.7 $\pm$ 2.9	37.4 $\pm$ 1.5	—	38.8 $\pm$ 1.8
Globulin (g l ⁻¹) week 3	M	24.7 $\pm$ 1.7	23.7 $\pm$ 0.8	22.6 $\pm$ 2.3	27.1 $\pm$ 1.0*
	F	20.5 $\pm$ 1.1	19.9 $\pm$ 1.6	26.0 $\pm$ 1.8*	24.6 $\pm$ 3.3*
Globulin (g l ⁻¹) week 5	M	23.8 $\pm$ 1.5	22.9 $\pm$ 1.6	—	25.4 $\pm$ 2.4
	F	26.1 $\pm$ 2.2	23.9 $\pm$ 1.6	—	25.6 $\pm$ 2.1
Spleen weight (g) ^a week 3	M	0.675 $\pm$ 0.09	0.682 $\pm$ 0.03	0.6811 $\pm$ 0.11	0.768 $\pm$ 0.06
	F	0.455 $\pm$ 0.02	0.442 $\pm$ 0.06	0.466 $\pm$ 0.07	0.505 $\pm$ 0.03
Spleen weight (g) ^a week 5	M	0.730 $\pm$ 0.14	0.759 $\pm$ 0.11	—	0.736 $\pm$ 0.06
	F	0.549 $\pm$ 0.05	0.492 $\pm$ 0.05	—	0.532 $\pm$ 0.13
Liver weight (g) ^a week 3	M	9.92 $\pm$ 0.51	9.44 $\pm$ 0.58	10.46 $\pm$ 1.09	11.27 $\pm$ 1.20
	F	6.81 $\pm$ 0.86	6.91 $\pm$ 0.53	6.87 $\pm$ 0.07	6.46 $\pm$ 0.54
Liver weight (g) ^a week 5	M	11.29 $\pm$ 1.29	10.98 $\pm$ 0.92	—	10.30 $\pm$ 0.48
	F	7.54 $\pm$ 0.75	7.89 $\pm$ 0.37	—	7.43 $\pm$ 1.21
Macroscopic findings after 3 weeks ^b		Yes ^c	Yes ^c	Yes ^c	Yes ^c
Macroscopic findings after recovery ^b		No ^c	Yes ^c	—	Yes ^c
Microscopic findings after 3 weeks ^b	M	F	M	M	M ^d
Granulomatous inflammation	0/5	0/5	1/5	0/5	0/5
Abscess	0/5	0/5	0/5	0/5	0/5
Dermal oedema	1/5	3/5	3/5	2/5	3/5
Dermal haemorrhage	0/5	1/5	1/5	0/5	0/5
Siderophages	0/5	0/5	0/5	0/5	0/5
Microscopic findings after recovery ^b					
Granulomatous inflammation	0/5	0/5	0/5	NM	1/5
Abscess	0/5	0/5	0/5	NM	0/5
Dermal oedema	0/5	0/5	0/5	NM	3/5
Dermal haemorrhage	0/5	0/5	0/5	NM	0/5
Siderophages	0/5	0/5	0/5	NM	2/5

M, male; F, female; NM, not measured.

* $P < 0.05$.

^a Based on final body weight; statistical analysis not performed.

^b Injection site only.

^c Small areas of haemorrhage in saline control; more marked in other groups with evidence of slight calcification; findings of very slight clarification among small numbers of animals only after 2 weeks recovery.

^d 1/5 animals had abscess at adjacent axillary lymph node at week 3.

reduction in some haematology measurements (haemoglobin, packed cell volume, mean cell count and mean cell haemoglobin) and albumin levels among rats treated at 0.5 ml per animal along with raised globulin levels at both 0.05 and 0.5 ml per rat. Other findings comprised increased spleen weight (10–12%) for both sexes and liver weight (12%) for males at the high dose level. None of these findings were seen after the 2 week recovery period. In light of no associated microscopic findings, the slight changes in the red cell parameters and increased liver weight were considered coincidental and of no toxicological relevance. At the terminal kill, there were macroscopic and microscopic findings suggestive of local irritation at the injection site of both control and treated animals. However, findings were fairly minimal among saline treated animals with cases of small areas of haemorrhage and dermal oedema. These findings were much more obvious in vaccine vehicle and vaccine-treated groups with evidence of haemorrhage and slight calcification at the injection site in some animals along with cases of granulomatous inflammation, abscess and dermal oedema and haemorrhage. One male dosed with a 0.5 ml showed an abscess in a single axillary lymph node and this finding was considered to be due to infection from the injection site spreading to the draining lymph node. There was no evidence that the severity of the injection site findings was greater when Pollinex® Quattro Ragweed was compared with the L-tyrosine control. Cases of granulomatous inflammation and dermal oedema (1/10 and 7/10 animals, respectively) as well as siderophages in 2/10 animals (indicative of an old haemorrhage) were seen after 2 weeks of recovery in rats previously treated at 0.5 ml per animal.

Genotoxicity

When tested in a *Salmonella typhimurium* reverse mutation assay at up to 3000 µg plate⁻¹, modified ragweed pollen extract showed no evidence of toxicity in terms of thinning of background lawn growth or any evidence of an increase in revertant numbers that could be considered as indicative of any mutagenic activity.

Local Tolerance Study

Not unexpectedly, modified ragweed pollen tyrosine adsorbate vaccine showed evidence of dose site reaction in the rat and dog (see Table 2). In the rat, single injections of 0.2 ml resulted in an acute, transient localised inflammatory reaction. Multiple injections of the same dose volume resulted in a more marked reaction with necrosis which resolved readily within 7–14 days when dosing is discontinued; no evidence of progressive changes was seen. Similarly in the dog, single or multiple subcutane-

ous injections of 0.5 ml resulted in a localised inflammatory reaction with rapid tissue regeneration.

Discussion

Ragweed allergy is well documented in both Europe and the United States and can affect over 10% of the overall population (Arbes *et al.*, 2005; Taramarcaz *et al.*, 2005; Wopfner *et al.*, 2005). As a form of treatment for this condition, allergy vaccination using allergoids is recognised as safe and efficacious. This paper reviews pre-clinical data available for a ragweed pollen allergoid with special reference to a new formulation, Pollinex® Quattro Ragweed.

The allergoids used in Pollinex® Quattro are pollen extracts chemically modified with glutaraldehyde which are potentially useful particularly in terms of reduced IgE reactivity whilst maintaining the ability of specific IgG induction (Moran and Wheeler, 1976; Moran *et al.*, 1977; Wheeler *et al.*, 1976). Adsorption of these allergoids to L-tyrosine to enhance both safety and efficacy has resulted in a range of allergen extract formulations with useful potential (Baldrick *et al.*, 2002b; Newland *et al.*, 1999; Wheeler *et al.*, 1982; Wheeler and Woroniecki, 2001; 2004). Clinical use of these vaccines (e.g. as Pollinex®) has shown a safe and efficacious profile over many years of human use (Bonifazi *et al.*, 1991; Drachenberg *et al.*, 2002; 2003; Ko *et al.*, 1987; Metzger *et al.*, 1981; Muehlethaler *et al.*, 1990; Palmer and Balacescu, 1977; Verstraeten and Wheeler, 1978). A further recent addition to some of the Pollinex® vaccines has been the immunostimulatory adjuvant MPL® (to encourage a skew in a Th1-like direction) whose safety and efficacy has been well documented elsewhere (e.g. Alving, 1993; Baldrick *et al.*, 2000b; Clay and Urban, 2005; Lodmell *et al.*, 2000; Moore *et al.*, 1999; Puggioni *et al.*, 2005; Thoelen *et al.*, 1998; Ulrich and Myers, 1995). A recent immunological study, employing a mouse model using a human serum albumin/tyrosine/MPL® combination, showed a favourable IgG1 and IgG2a antibody shift to a more Th1 biased response (Woroniecki *et al.*, 2005). Other rodent antibody induction studies using ragweed pollen MATA materials adjuvanted with MPL® have shown further evidence of a Th1 skewing effect (Wheeler *et al.*, 2001). A recent clinical study using Pollinex® Quattro Ragweed has demonstrated the vaccine to be well tolerated and to significantly reduce allergy symptoms (www.allergytherapeutics.com). Similar safety and efficacy findings have been reported for a counterpart vaccine using grass pollen (Drachenberg *et al.*, 2001; Mothes *et al.*, 2003; von Baehr *et al.*, 2005).

The preclinical studies reported here support safe clinical use of Pollinex® Quattro Ragweed by indicating a lack of toxicological findings. Single doses of a high ragweed allergoid concentration by the subcutaneous route in the

mouse and rat showed no effects at levels greatly above a typical clinical dose volume of 0.5 ml for these types of materials. Thus, on a weight-to-weight basis, a safety margin of about 2400-fold (mouse = 25 g) or about 240-fold (rat = 250 g) was achieved for a 60 kg person.

No signs of systemic toxicity were seen with a high ragweed pollen allergoid concentration following intramuscular (rat) or subcutaneous (dog) dosing with modified ragweed pollen tyrosine adsorbate vaccine on 10 occasions over 20 days. A possible immunostimulatory effect was seen in the rat as evidenced by slight increases in neutrophils and spleen weight. Thus, on a weight-to-weight basis, a safety margin of about 240-fold (rat = 250 g) or about 8-fold (dog = 7 kg) was achieved for a 60 kg person. Repeated clinical doses (a total of 4) of Pollinex® Quattro Ragweed will be used and potential adverse effects were examined by an exaggerated dosing regimen of seven subcutaneous doses to the rat over 3 weeks using this vaccine with a ragweed allergoid concentration of up to 6000 SU ml⁻¹. No signs of systemic toxicity were found and although not accompanied by any change in white cell count, the changes in albumin and globulin levels along with increased spleen weight may have been due to immunostimulatory activity considering that the modified pollen extract has activity *per se* and MPL® is an immunologically active adjuvant. Recent reviews of animal studies evaluating the potential toxicity of L-tyrosine and MPL® have highlighted consistent findings associated with stimulation of the immune system (notably increased white blood cell values and increased spleen weight) in the rat and dog (Baldrick *et al.*, 2002a; 2002b). Other recent studies with allergoid/MPL®/L-tyrosine vaccines (house dust mite allergoids from *Dermatophagoides pteronyssinus* and *D. farinae*, 50:50 mix) and Pollinex Quattro Grass vaccine (mixture of 12 common grass plus rye cereal pollen allergoids), respectively, administered as repeated subcutaneous doses to the rat, raised neutrophil (and total white cell) count and spleen weight and/or altered albumin and globulin levels (Baldrick *et al.*, 2002c; 2004). These findings were again largely related to the immunostimulatory components of the vaccine.

In the present study, the possibly real, limited signs of immunostimulation were not seen at the lower dose level of 0.05 ml per rat, nor following a 2-week non-dose period at the higher 0.5 ml per rat dose indicating a reversibility of the effects. On the basis of a 0.5 ml clinical dose volume, the dose levels used for Pollinex® Quattro Ragweed at 6000 SU ml⁻¹ and the high dose vaccine represent a safety margin of about 240-fold for a 60 kg person, based on a 250 g rat.

Although of very limited design, a reverse mutation bacterial assay showed that modified ragweed pollen extract gave no evidence of genotoxicity.

Local tolerance studies in the rat and dog with modified ragweed pollen tyrosine adsorbate vaccine showed

notable macroscopic and microscopic findings indicative of irritation of the dose site. However, these findings were not progressive and were reversible. In the repeat dose rat toxicity study reported here, findings at the injection site of control (L-tyrosine formulation) and Pollinex® Quattro Ragweed treated animals were also consistent with those of local irritation. Subcutaneous administration of a glutaraldehyde-modified house-dust mite vaccine or Pollinex® Quattro Grass (containing L-tyrosine plus MPL® adjuvant) in a local tolerance study in the rabbit and a repeat dose toxicity study in the rat also showed microscopic findings of irritation of the dose site (Baldrick *et al.*, 2002c; 2004). Interestingly, these findings showed evidence of recovery, unlike the present study which still showed microscopic changes after a 2 week recovery period.

A potential for local irritation effects with Pollinex® Quattro Ragweed is not unexpected as dose site reactions are often seen following clinical vaccination and can be related to the presence of an adjuvant. Indeed, aluminium salts (which have been used as adjuvants for many years in human vaccines) are known to produce local reactions in animals and clinically (Gupta *et al.*, 1995; Nagore *et al.*, 2001; Singh and O'Hagan, 1999; Vogel and Powell, 1995; Wheeler and Woroniecki, 2004). It is also well known that granulomas can occur after administration of aluminium adsorbants. Recent animal studies examining macrophagic myofasciitis associated with clinical use of vaccines containing aluminium salts have shown dose site infiltration of macrophages with intracytoplasmic crystalline inclusions in rats and monkeys (Gherardi *et al.*, 2001; Verdier *et al.*, 2005). The findings reported in the present investigation are likely to be related to the presence of L-tyrosine in the dose formulation and it has been pointed out elsewhere that L-tyrosine injections in animals results in local swelling and an inflammatory response associated with deposited material (probably related to the low solubility of L-tyrosine) (Baldrick *et al.*, 2002b). However such responses, as found with ragweed allergoid-containing vaccines, resolved rapidly, even after multiple injections and showed no evidence of a progressive nature. Clinical use of MATA vaccines has shown some evidence of local reactions (including swelling and/or erythema and itch) (Miller, 1976; Miller *et al.*, 1976; Palmer and Balacescu, 1977; Verstraeten and Wheeler, 1978; Drachenberg *et al.*, 2001; 2003). Several tolerability studies indicate that local injection reactions are comparable to other commonly used vaccine depot systems such as aluminium adsorbed extracts (Bonifazi *et al.*, 1991; Drachenberg *et al.*, 2003; Muehlethaler *et al.*, 1990; Symington *et al.*, 1977). Furthermore, no granulomas occur after administration of L-tyrosine adsorbates, which is attributable to the solubilisation and metabolism of the natural amino acid. Clinical data from the use of MATA vaccines containing MPL® (with 2% L-tyrosine and 50 µg ml⁻¹ MPL®) also indicate low

injection site reaction (Drachenberg *et al.*, 2002; Clay and Urban, 2005).

In conclusion, a specific preclinical safety evaluation strategy developed to support clinical use of Pollinex® Quattro Ragweed and comprising use of older single and repeat dose toxicity and local tolerance studies with modified ragweed pollen tyrosine adsorbate vaccine and a new repeat dose toxicity study in the rat with Pollinex® Quattro Ragweed has shown that there were no toxicological issues at levels greatly in excess to those proposed for clinical use. A not unexpected injection site reaction was seen throughout the studies which can largely be attributed to the presence and volume of L-tyrosine as a depot.

References

- Alving CR. 1993. Lipopolysaccharide, lipid A and liposomes containing lipid A as immunologic adjuvants. *Immunobiology* **187**: 430–446.
- Arbes SR Jr, Gergen PJ, Elliott L, Zeldin DC. 2005. Prevalences of positive skin test responses to 10 common allergens in the US population: results from the third National Health and Nutrition Examination Survey. *J. Allergy Clin. Immunol.* **116**: 377–383.
- Baldrick P, Richardson D, Elliott G, Wheeler AW. 2002a. Safety evaluation of monophosphoryl lipid A (MPL®): An immunostimulatory adjuvant. *Regul. Toxicol. Pharmacol.* **35**: 398–413.
- Baldrick P, Richardson D, Wheeler AW. 2002b. Review of L-tyrosine confirming its safe human use as an adjuvant. *J. Appl. Toxicol.* **22**: 333–344.
- Baldrick P, Richardson D, Wheeler AW. 2002c. Safety evaluation of a glutaraldehyde modified tyrosine adsorbed house dust mite extract containing monophosphoryl lipid A (MPL®) adjuvant: A new allergy vaccine for dust mite allergy. *Vaccine* **20**: 737–743.
- Baldrick P, Richardson D, Wheeler AW, Woroniecki SR. 2004. Safety evaluation of a new allergy vaccine containing the adjuvant Monophosphoryl Lipid A (MPL®) for the treatment of grass pollen allergy. *J. Appl. Toxicol.* **24**: 261–268.
- Bellinghausen I, Metz G, Enk AH, Christmann S, Knop J, Saloga J. 1997. Insect venom immunotherapy induces interleukin 10 production and a Th2- to Th1 shift, and changes surface marker expression in venom allergic subjects. *Eur. J. Immunol.* **27**: 1131–1139.
- Bonifazi F, Antonicelli I, Bilo MB, Pucci S, Taylor IH, Wheeler AW, Youtlen LJF. 1991. Hyposensitization therapy of *Parietaria*-sensitive patients with a tyrosine-adsorbed allergoid Pollinex *Parietaria* (Bencard *Parietaria*). *J. Invest. Allergol. Clin. Immunol.* **1**: 37–44.
- Bousquet J, Lockey RF, Malling HJ. 1998. WHO Position Paper. Allergen immunotherapy: Therapeutic vaccines for allergic diseases. *Allergy* **53**(suppl): 1–42.
- Clay M, Urban E. 2005. Specific immunotherapy using pollen allergoids adjuvanted with monophosphoryl lipid A over a three year period shows good efficacy and increased tolerability. Poster presented at British Society for Allergy and Clinical Immunology meeting, July 2005, Loughborough, UK. Abstract. *Clin. Exp. Allergy* **2005**; **35**: 1411.
- De Becker G, Moulin V, Pajak B, Bruck C, Francotte M, Thiriart C, Urban J, Moser M. 2000. The adjuvant monophosphoryl lipid A increases the function of antigen-presenting cells. *Int. Immunol.* **12**: 807–815.
- Drachenberg KJ, Proll S, Urban E, Woroniecki SR. 2002. Short-term immunotherapy using an allergy vaccine adjuvanted with monophosphoryl lipid A: a post-marketing surveillance study. *Int. Rev. Allergol. Clin. Immunol.* **8**: 219–223.
- Drachenberg KJ, Proll S, Urban E, Woroniecki SR. 2003. Single-course specific immunotherapy with mixed pollen allergoids: results of a multi-centre study. *Allergol. Immunopathol.* **31**: 77–82.
- Drachenberg KJ, Wheeler AW, Stuebner P, Horak F. 2001. A well-tolerated grass pollen specific allergy vaccine containing a novel adjuvant, monophosphoryl lipid A reduces allergic symptoms after only four preseasonal injections. *Allergy* **56**: 498–505.
- Durham SR, Till SJ. 1998. Immunologic changes associated with allergen immunotherapy. *J. Allergy Clin. Immunol.* **102**: 157–164.
- Ebner C. 1999. Immunological mechanisms operative in allergen-specific immunotherapy. *Int. Arch. Allergy Immunol.* **119**: 1–5.
- Gherardi RK, Coquet M, Cherin P, Belec L, Moretto P, Dreyfuss PA, Pellissier J-F, Chariot P, Authier F-J. 2001. Macrophagic myofasciitis lesions assess long-term persistence of vaccine-derived aluminium hydroxide in muscle. *Brain* **124**: 1821–1831.
- Gupta RK, Rost BE, Relyveld E, Siber GR. 1995. Adjuvant properties of aluminium and calcium compounds. *Pharm. Biotechnol.* **6**: 229–248.
- Hopkins M, Lees BG, Richardson DG, Woroniecki SR, Wheeler AW. 2001. Standardisation of glutaraldehyde-modified tyrosine-adsorbed tree pollen vaccines containing the Th1-inducing adjuvant, monophosphoryl lipid A (MPL®). *Allergol. Immunopathol.* **29**: 245–254.
- Ismaili J, Rennesson J, Aksoy E, Vekemans J, Vincart B, Amraoui Z, Van Laethem F, Goldman M, Dubois PM. 2002. Monophosphoryl lipid A activates both human dendritic cells and T cells. *J. Immunol.* **168**: 926–932.
- Jung CM, Prinz JC, Rieber EP, Ring J. 1995. A reduction in allergen induced FcεR2/CD23 expression on peripheral B cells correlates with successful hyposensitisation in grass pollinosis. *J. Allergy Clin. Immunol.* **95**: 77–87.
- Ko HS, Chen SJ, Jaworska E. 1987. IgG and IgE antibody responses to treatment with modified ragweed tyrosine adsorbate. *Ann. Allergy* **59**: 441–449.
- Lodmell DL, Ray NB, Ulrich JT, Ewalt LC. 2000. DNA vaccination of mice against rabies virus: effects of the route of vaccination and the adjuvant monophosphoryl lipid A (MPL®). *Vaccine* **18**: 1059–1066.
- Malling HJ. 2000. Minimising the risks of allergen-specific injection immunotherapy. *Drug Saf.* **23**: 323–332.
- Martin M, Michalek SM, Katz J. 2003. Role of innate immune factors in the adjuvant activity of monophosphoryl lipid A. *Infect. Immun.* **71**: 2498–2507.
- Metzger WJ, Dorminey HC, Richerson HB, Weiler JM, Donnelly A, Moran D. 1981. Clinical and immunologic evaluation of glutaraldehyde modified, tyrosine adsorbed short ragweed extract. A double blind, placebo controlled trial. *J. Allergy Clin. Immunol.* **68**: 442–448.
- Miller ACML. 1976. A comparative trial of hyposensitization in 1973 in the treatment of hay fever using Pollinex and Alavac-P. *Clin. Allergy* **6**: 551–556.
- Miller ACML, Hart AP, Tees EC. 1976. D. pteronyssinus-tyrosine adsorbate: biological and clinical properties. *Acta Allergol.* **31**: 35–43.
- Moore A, McCarthy L, Mills KHG. 1999. The adjuvant combination monophosphoryl lipid A and QS21 switches T cell responses induced with a soluble recombinant HIV protein from Th2 to Th1. *Vaccine* **17**: 2517–2527.
- Moran DM, Wheeler AW. 1976. Chemical modification of crude timothy grass extract. I. Antigenicity and immunogenicity changes following amino group modification. *Int. Arch. Allergy Appl. Immunol.* **50**: 693–708.
- Moran DM, Wheeler AW, Overall BG, Woroniecki SR. 1977. Chemical modification of crude timothy grass extract. III. The effect of glutaraldehyde-induced aggregation on antigenic and immunogenic properties. *Int. Arch. Allergy Appl. Immunol.* **54**: 315–321.
- Mothes N, Heinzkill M, Drachenberg KJ, Sperr WR, Krauth MT, Majlesi Y, Semper H, Valent P, Niederberger V, Kraft D, Valenta R. 2003. Allergen-specific immunotherapy with a monophosphoryl lipid A-adjuvanted vaccine: reduced seasonally boosted immunoglobulin E production and inhibition of basophil histamine release by therapy-induced blocking antibodies. *Clin. Exp. Allergy* **33**: 1198–1208.
- Muehlethaler K, Wuthrich B, Peeters AG, Terki N, Girard JP, Frank E. 1990. Hyposensitization in pollinosis. Results of a 3-year controlled study with 2 depot-allergoid grass pollen extracts: aluminium hydroxide-adsorbed allergoid and tyrosine-adsorbed allergoid. *Schweiz. Rundsch. Med. Prax.* **79**: 430–436.

- Nagore E, Martinez-Escribano JA, Tato A, Sabater V, Vilata JJ. 2001. Subcutaneous nodules following treatment with aluminium-containing allergen extracts. *Eur. J. Dermatol.* **11**: 138–140.
- Newland BJ, Lees BG, Wheeler AW. 1999. Advantages of L-tyrosine as a depot adjuvant for formulation of therapeutic allergy vaccines. Poster presented at British Society of Immunology & British Society for Allergy and Clinical Immunology, Joint 7th Annual Conference, Nov-Dec 1999, Harrogate, UK. *Immunology* **98** (suppl 1): 145.
- Palmer WR, Balacescu A. 1977. A multi-centre trial of pollen-tyrosine adsorbate. *Acta Allergol.* **32**: 44–57.
- Puggioni F, Durham SR, Francis JN. 2005. Monophosphoryl lipid A (MPL®) promotes allergen-induced immune response deviation in favour of Th1 responses. *Allergy* **60**: 678–684.
- Rolland JM, Douglass J, O'Hehir RE. 2000. Allergen immunotherapy: current and new therapeutic strategies. *Expert Opin. Invest. Drugs* **9**: 515–527.
- Singh M, O'Hagan D. 1999. Advances in vaccine adjuvants. *Nat. Biotechnol.* **17**: 1075–1081.
- Symington IS, O'Neill D, Kerr JW. 1977. Comparison of a glutaraldehyde-modified pollen-tyrosine adsorbate with an alum-precipitated pollen vaccine in the treatment of hay fever. *Clin. Allergy* **7**: 189–194.
- Taramarcz P, Lambelet C, Clot B, Keimer C, Hauser C. 2005. Ragweed (Ambrosia) progression and its health risks: will Switzerland resist this invasion? *Swiss Med. Wkly* **135**: 538–548.
- Thoelen S, Van Damme P, Mathei C, Leroux-Roels G, Desombere I, Safary A, Vandepapeliere P, Slaoui M, Meheus A. 1998. Safety and immunogenicity of a hepatitis B vaccine formulated with a novel adjuvant system. *Vaccine* **16**: 708–714.
- Ulrich JT, Myers KR. 1995. Monophosphoryl lipid A as an adjuvant. Past experience and new directions. In *Vaccine Design: The Subunit and Adjuvant Approach*, Powell MF, Newman MJ (eds). Plenum Press: New York; 495–524.
- Verdier F, Burnett R, Michelet-habchi C, Moretto P, Fievet-Groyné F, Sauzeat E. 2005. Aluminium assay and evaluation of the local reaction at several time points after intramuscular administration of aluminium containing vaccines in the Cynomolgus monkey. *Vaccine* **23**: 1359–1367.
- Verstraeten JM, Wheeler AW. 1978. A comparative study of an aqueous grass pollen extract and glutaraldehyde-treated grass pollen-tyrosine adsorbate in the treatment of pollenosis. *Clin. Allergy* **8**: 435–443.
- Vogel FR, Powell MF. 1995. A compendium of vaccine adjuvant and excipients. *Pharm. Biotechnol.* **6**: 141–228.
- Von Baehr V, Hermes A, von Baehr R, Scherf H-P, Volk H-D, Fischer von Weikersthal-Drachenberg KJ, Woroniecki S. 2005. Allergoid-specific T-cell reaction as a measure of the immunological response to specific immunotherapy (SIT) with a Th1-adjuvanted allergy vaccine. *J. Invest. Allergol. Clin. Immunol.* **15**: 234–241.
- Wheeler AW, Jenkins PM, Moran DM. 1976. Chemical modification of crude timothy grass extract. II. Class and specificity of antibodies induced by chemically modified timothy grass pollen extract. *Int. Arch. Allergy Appl. Immunol.* **50**: 709–728.
- Wheeler AW, Marshall J, Ulrich JT. 2001. A Th1-inducing adjuvant, MPL®, enhances antibody profiles in experimental animals suggesting it has the potential to improve the efficacy of allergy vaccines. *Int. Arch. Allergy Immunol.* **126**: 135–139.
- Wheeler AW, Moran DM, Robins BE, Driscoll A. 1982. L-Tyrosine as an immunological adjuvant. *Int. Arch. Allergy Appl. Immunol.* **69**: 113–119.
- Wheeler AW, Woroniecki SR. 2001. Immunological adjuvants in allergy vaccines: past, present and future. *Allergol. Int.* **50**: 295–301.
- Wheeler AW, Woroniecki SR. 2004. Allergy vaccines: new approaches to an old concept. *Expert Opin. Biol. Ther.* **4**: 1473–1481.
- White JF, Bernstein DI. 2003. Key pollen allergens in North America. *Ann. Allergy Asthma Immunol.* **91**: 425–435.
- Wopfner N, Gadermaier G, Egger M, Asero R, Ebner C, Jahn-Schmid B, Ferreira F. 2005. The spectrum of allergens in ragweed and mugwort pollen. *Int. Arch. Allergy Immunol.* **138**: 337–346.
- Woroniecki SR, Skinner M, Hobden T, Mikhlovskaya LI. 2005. Immunogenic effects of L-tyrosine compared to alum when combined with monophosphoryl lipid A and a model antigen: preliminary results. Poster presented at European Academy of Allergology and Clinical Immunology (EAACI) meeting, Munich, Germany. Abstract. *J. World Allergy Org.* 2005; suppl 1: 499.
- www.allergytherapeutics.com. Positive outcome of pivotal Pollinex® Quattro Ragweed study R204. www.allergytherapeutics.com/cgi-bin/search.cgi (accessed August 2006).
- www.emea.eu.int-a. European Medicines Agency: Committee for Medicinal Products for Human Use European Public Assessment Report for Fendrix. www.emea.eu.int/humandocs/PDF/EPAR/fendrix/8291305en6.pdf (accessed August 2006).
- www.emea.eu.int-b. Committee for Proprietary Medicinal Products: Note for Guidance on Preclinical Pharmacological and Toxicological Testing of Vaccines (CPMP/465/95, operational since 1998). www.emea.eu.int/pdfs/human/swp/046595en.pdf (accessed August 2006).
- www.hc-sc.ca. Health Canada Drug Product Database: Pollinex R. www.hc-sc.gc.ca/drugs2/product/p3409.html (accessed August 2006).
- www.pslgroup.com. Canada approves Melacine therapeutic vaccine for late-stage melanoma. www.pslgroup.com/dg1424a2.htm (accessed August 2006).
- www.who.int. World Health Organization: Nonclinical Evaluation of Vaccines — WHO Technical Report Series, No. 927, 2005. www.who.int/biologicals/publications/trs/areas/vaccines/nonclinical_evaluation/en/index.htm (accessed August 2006).