PRODUCTION OF CERVINE PROLACTIN ANTISERA: A COMPARISON OF ADJUVANTS

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In this study we immunised rabbits against a cervine prolactin (cPRL) immunogen and compared the use of Freund's adjuvant (FA) with two alternative oil-based adjuvants, to determine which was most effective at stimulating antibody production.

A cPRL-bovine thyroglobulin conjugate (cPRL-bTg) was prepared by the reaction of thiolated-cPRL with 4-dithiopyridine-activated bTg, and was administered to 15 white New Zealand rabbits in water-in-oil emulsions (0.6ml per rabbit) containing either FA, non-ulcerative Freund's adjuvant (NUFA) or an adjuvant (STM) composed of a mineral oil, Arlacel 85 and Tween 85 in the ratio of 8:1:1 (n=5 animals per group). The proportions of oily adjuvants and aqueous immunogen solution in the three emulsions were those recommended by the manufacturers (1:1, 2:1 and 1.25:1 respectively). Primary immunisation injections contained 90µg cPRL-bTg and were given s.c. in multiple sites in the back, and 3 booster injections (each of 75µg cPRL-bTg) were administered at 6 week intervals, as single doses i.m. in alternating hind limbs. Complete forms of FA and NUFA were used for primary injections, and incomplete (ie without killed mycobacteria) for subsequent treatments. Blood was sampled 12 days after each booster injection, and was allowed to clot overnight at room temperature. Sera were stored at -20°C until assayed for antibody titres (percentage binding of ^{125}I-ovine PRL in serum diluted 1:5000) using standard radioimmunoassay techniques. Rabbits were weighed weekly and were manually and visually assessed for the presence of swellings at the primary injection sites.

Rabbits in the STM group developed higher titres than those in the FA and NUFA groups ($p<0.001$). After the third booster injection the titres (mean±s.e.m.) were 45.3±4.8%, 8.0±4.3%, and 3.3±4.3% respectively. Swellings were detected at a greater percentage of FA injections sites (56%) 5 weeks after primary immunisations than at STM (17%) or NUFA (27%) sites ($p<0.01$). Those caused by STM were completely reabsorbed after a further 5 weeks, whereas after that time swellings were still present at 50% and 23% of FA and NUFA sites respectively. There was no significance difference between the groups in the rate of weight gain ($p>0.05$).

We conclude that STM is an effective replacement for FA in immunisations aimed at antibody production in rabbits. Its use is particularly to be recommended in view of animal ethics concerns about the side effects of FA. NUFA, like STM, caused less local tissue reaction than FA, but it was the poorest stimulant of antibody production in this experiment.