leno) y posteriormente se agrega el anticuerpo LT39, que únicamente reconoce la forma petamérica de CTB. Uno de lo objetivos del presente trabajo es la caracterización del modelo topológico propuesto para OmpC, mediante la inserción de diferentes péptidos de esta porina en el extremo amino terminal de CTB, que permitan establecer si estos péptidos se encuentran en regiones transmembranales o en regiones expuestas al medio externo; una estrategia similar fue empleada para establecer el modelo topológico de *Lam*B de *E. coli* (6). Por otra parte, también cabe destacar que el CTB es un excelente inmunoadyuvante, por ello radica la importancia a desarrollar un sistema que permita la expresión de péptidos unidos al CTB, por esto las fusiones genéticas ofrecen una buena alternativa en la obtención de altas cantidades de proteína. Finalmente, se tienen las siguientes conclusiones:

- Se construyó un vector que facilitó la fusión genética, expresión y rastreo de péptidos en el extremo amino terminal de la subunidad B de la toxina del cólera.
- Este vector (pCTBtet) se puede emplear para la expresión de cualquier otro péptido de interés biológico.

TOWARDS CLINICAL APPLICATION OF GAMMA INULIN ADJUVANTS

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Introduction

Gamma inulin (γ-iN) adjuvants (1) depend on an unbranched polyfructose with a mol wt of 8000-12000 (50-75 residues). It is a chemically well-characterised neutral polysaccharide, cheap, easy to handle and purify and very stable. Unlike the α and β polymorphs, γ-IN is insoluble at 37 °C and controls its sole biological activity. It only activates complement of the alternative pathway, opsonising antigens with C3b fragments. These react with C3 receptors on leukocytes, activating them in turn. Thus the action of γ-IN is well understood, seeming to dissect out a non-toxic part of the inflammatory response. It is a potent immune modulator, having vaccine adjuvant, antitumour and natural immunity activity. Adjuvant action is much higher if γ-IN is co-crystallised with aluminium hydroxide ('Algammulin', AG): such particles both adsorb antigen and activate complement.

Progress towards human clinical application of γ-IN and AG as vaccine adjuvants has taken the following directions.

Results and Discussion

Standard vs fine formulations of AG

Published work with AG has used the 'standard formulation', with hydrated particle diameters >2 μm and inulin: $Al(OH)_3$ ratios ~10:1. A commercially viable method now gives submicron particles ("fine formulation"). These diffuse faster from injection sites, are more active yet produce less local reaction.

High inulin: $Al(OH)_3$ ratio

Studies in C57 black mice (2) confirm that a high inulin: $Al(OH)_3$ ratio in AG greatly increases the emphasis on Th1 type antibodies (eg. IgG2a). The inulin: $Al(OH)_3$ ratio can be manipulated to emphasise either Th1 or Th2 responses as desired. Most applications need the Th1, CMI emphasis (high inulin: $Al(OH)_3$ ratio >30:1).

Clinical Trials

A small Phase I human clinical trial of AG standard formulation (3) used a recombinant human papillomavirus (HPV) type 16 E7 protein. All patients seroconverted and the human efficacy of AG is supported. Toxicity at high doses (25 mg s.c.) was minimal.

Commercial antigens

Several clinical antigens are enhanced by AG. AG (fine formulation, high-Th1 emphasis) created specific CTL activity with HPV E7 protein in mice (4). Diphtheria toxoid antigenicity was boosted by AG (2), especially for IgG2a, and the life of antibody was extended. AG produced 5-6 times more primary antibody to hepatitis B virus surface antigen than equivalent doses of $Al(OH)_3$ (5). Gamma-irradiated or live whole influenza virus plus γ-IN induced protection against heterotypic influenza virus challenge (6). AG plus bromelain-extracted influenza virus haemagglutinin enhanced influenza viral clearance and neutralising anti-body in homotypic virus challenge (7). AG bearing conjugates of peptides of *Plasmodium falciparum* merozoite surface antigen 2 enhanced neutralising antibody and survival in mice challenged with *P. chabaudi* (8, 9).

Conclusion

The promise of γ-IN adjuvants for clinical use has increased.

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