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TraT: A POWERFUL CARRIER MOLECULE FOR THE STIMULATION OF IMMUNE RESPONSES TO PROTEIN AND PEPTIDE ANTIGENS

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A number of integral membrane proteins (Imps) isolated from *Escherichia coli* have been examined for their ability to generate serum antibody responses in the absence of adjuvant. These proteins were found to stimulate high titers of serum antibody when injected into rabbits or mice in saline. The antibody titers elicited were not significantly increased by the addition of a powerful adjuvant such as IFA. Covalent conjugation of BSA, of the DNP group, and of a peptide Ag from *Plasmodium falciparum* to these protein carriers resulted in a significant enhancement of the immune response to the conjugated material in comparison with the response elicited when the immunogen was injected without adjuvant or was not conjugated to Imps. The antibody response to these conjugates could not be significantly increased by the addition of IFA. Thus, the Imps of *E. coli* represent powerful carrier molecules which, when injected into mice and rabbits, are not only capable of generating high titers of antibody to themselves, but also to molecules conjugated to them. Immunization with immunogens coupled to these proteins results in the production of high titers of antibody without the need for oil-based adjuvants, thereby avoiding the unwanted side effects of such adjuvants.

In order to protect an animal against an invading pathogen (bacterial, viral, or parasite) the animal can be vaccinated with the whole organism or with subunits of the pathogen, so as to elicit a protective immune response in the host. The immune response generated to such antigenic challenge can often be augmented by the coadministration of an immunopotentiating agent or adjuvant. The best of these agents are the depot type adjuvants such as CFA and IFA. These adjuvants are capable of increasing the antibody response after Ag injection some 50 to 100 times the level obtained when Ag is injected alone.

While adjuvants such as CFA and IFA can greatly enhance the immune response to an Ag, they suffer from the major disadvantage that when used with an Ag in an injectable form they can cause severe pain, fever, and abscess formation at the site of injection, a situation which renders them unsatisfactory for use in human or many veterinary vaccines (1).

We present data here on a class of molecules which overcome the disadvantages inherent in adjuvants such as CFA and IFA but which preserve their desirable adjuvanting activity. These molecules are represented by the Imps[2] of *Escherichia coli*, more specifically the *traT*, *ompF* and *ompA* gene products.

TraT is a major outer membrane protein isolated from the surface of IncF plasmid-carrying serum-resistant *E. coli* (2–5). OmpF is a major porin extractable from the outer membrane of *E. coli* (6–9), whereas OmpA is a heat-modifiable outer membrane protein responsible for phage sensitivity of some strains of *E. coli* (6, 10).

In this article we describe a method for the purification of TraT, OmpA, and OmpF from the outer membrane of *E. coli*. Initial studies are also described in which the immune response generated to these proteins injected alone has been compared with that generated by the simultaneous injection of the Imps mixed with IFA. Imps were also examined for their ability to stimulate antibody responses to proteins, peptides, and haptens which had been covalently linked to these carriers and injected into rabbits and mice.

MATERIALS AND METHODS

Isolation of TraT, OmpA, and OmpF Proteins

E. coli (strain BTA 11352 containing the plasmid cI857 as well as plasmid pBTA439, a derivative of plasmid pBR329 into which has been inserted a 6.0-kb *Eco*RI fragment of the R100 plasmid which contains the DNA sequence coding for TraT, expressed from the λ leftward promoter P_L) were grown in a fermenter at 30°C and induced at 42°C for 2 h. After induction, the cells were concentrated and washed (distilled water) in an Amicon DC10LA concentrator (0.1-μm hollow fiber cartridge). Cells were removed from the concentrator and the Imps extracted from the cells by the addition of a solution containing 0.2 M sodium acetate buffer, pH 2.5, 2% cetrimide (Sigma) in 20% EtOH plus 0.2 M $CaCl_2$ (final concentration). The extraction was allowed to proceed overnight at RT after which the bacteria were pelleted by centrifugation (17,000 × *g*, 20 min).

TraT and OmpF were precipitated from the supernatant by the addition of ethanol to 50% and centrifugation (4000 × *g*, 10 min). OmpA was then precipitated from the supernatant by the further addition of ethanol to 80% (final, v/v). All proteins were then resuspended in 1% Zwittergent, 20 mM sodium acetate buffer, pH 6.5, 20 mM EDTA and further purified by chromatography on DEAE-Sepharose (Pharmacia) in 20 mM sodium acetate buffer, pH 6.5, containing 0.1% Zwittergent (Calbiochem) and 20 mM EDTA. Proteins were eluted by using a linear gradient of 0 to 1 M NaCl in the loading buffer. Fractions containing the Imps were pooled, precipitated with ethanol, and resuspended in 10% SDS and further purified by size exclusion chromatography on S-300 Sephacryl (Pharmacia Fine

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[2] Abbreviations used in this paper: Imp, integral membrane protein; Omp, outer membrane protein; DNFB, dinitrofluorobenzene; RT, room temperature; CSP-Pep, B cell epitope from the circumsporozoite protein of *P. falciparum*; CSP-TE, T cell epitope plus the B cell epitope from the circumsporozoite protein of *P. falciparum*.

Chemicals) in 10 mM Tris·HCl, pH 8.8, containing 2% SDS, 20 mM EDTA.

Proteins purified by the above methods traveled as single bands when analyzed by SDS-PAGE (11) with m.w. of 36,000, 34,000, and 28,000 for OmpF, OmpA, and TraT, respectively, and were found to be free of contaminating LPS when subjected to SDS-PAGE and silver stained by the method of Tsai and Frasch (12) (Fig. 1). The TraT protein was found to be contaminated with less than 1.0 ng of LPS per mg of protein when tested in the *Limulus* amebocyte lysate assay (13, 14).

Pyrogenicity Testing of TraT

Purified preparations of TraT were tested for pyrogens by the Animal Resources Centre, Murdoch, Western Australia, according to the method described in the British and American Pharmacopoeia. The averaged results from three rabbits per test showed a complete absence of pyrogens in 500-μg samples of TraT.

Dinitrophenylation of Carriers

TraT, OmpA, and OmpF were dinitrophenylated according to the method of Little and Eisen (15). Briefly, carriers in 0.1 M carbonate buffer, pH 9.5, were reacted with a solution of 0.1 M DNFB (Sigma Chemical Co., St. Louis, MO) (in acetone) overnight at RT. Unreacted DNFB was removed by repeated precipitation of the Imps with ethanol and resuspension in 1% Zwittergent.

Preparation of Glutaraldehyde Conjugates

BSA (Sigma) was coupled to TraT, OmpA, and OmpF by using the two-step glutaraldehyde procedure of Avrameus et al. (16). Briefly, BSA was dissolved at 1 mg/ml in 0.1 M sodium phosphate buffer, pH 6.8, and was reacted with 0.2% glutaraldehyde for 2 h at RT. After overnight dialysis against 0.1 M Carb/bicarb buffer, pH 9.5, the glutaraldehyde-activated BSA was added to the Imps a molar ratio of 1:1 and reacted for 24 h at RT.

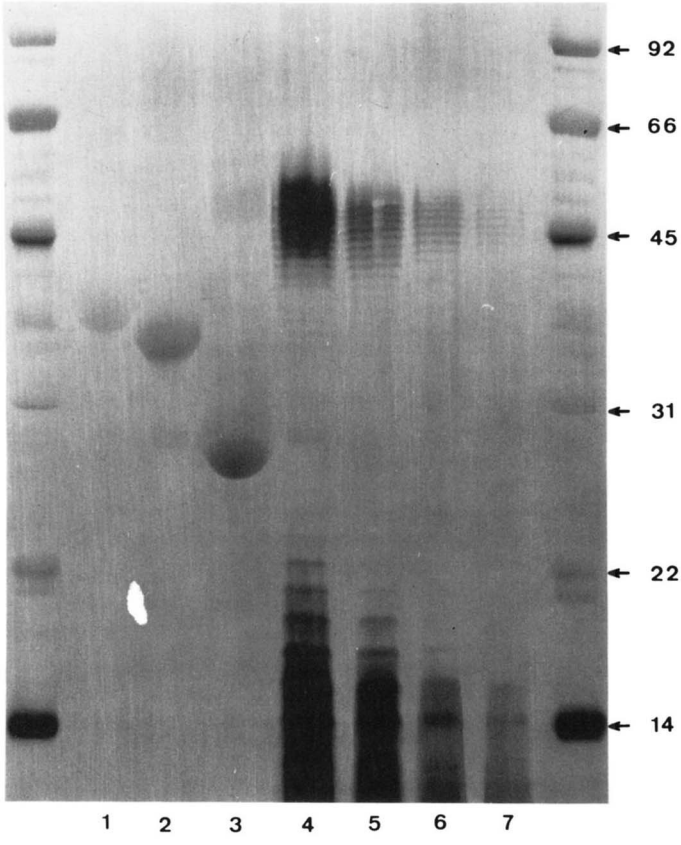

Figure 1. SDS-PAGE analysis of purified Ag. Purified proteins were examined by SDS-PAGE with a 12.5% polyacrylamide gel. After electrophoresis the gel was stained for LPS by using the method of Tsai and Frasch (12). Purified OmpF (*lane 1*, 50 μg), OmpA (*lane 2*, 50 μg), and TraT (*lane 3*, 40 μg) are shown. Different quantities of *E. coli* LPS were also electrophoresed (*lanes* 4 to 7; 10 μg, 3 μg, 1 μg, and 300 ng, respectively). Bio-Rad low m.w. markers are shown ($\times 10^{-3}$).

Synthesis of Peptides

A B cell epitope from the circumsporozoite protein of *Plasmodium falciparum* [NH_2-Cys-(Asn-Pro-Asn-Ala)$_4$-COOH] (CSP-Pep) (17, 18) as well as the CSP-Pep plus the T cell epitope from the same molecule [NH_2-(Asn-Pro-Asn-Ala)$_4$-Pro-Ser-Asp-Lys-His-Ile-Glu-Gln-Tyr-Leu-Lys-Lys-Ile-Lys-Asn-Ser-Ile-Ser-Cys-COOH] (CSP-TE) (19) were synthesized on an Applied Biosystems #430A peptide synthesizer. Both peptides were purified by chromatography on G-25 Sephadex (Pharmacia) in 10% acetic acid, followed by reverse phase HPLC on a VYDAC C-18 column with the use of a linear gradient of 5 to 60% acetonitrile in 0.1% trifluoroacetic acid.

Conjugation of cys-Peptides to TraT and OmpA

TraT (1 mg/ml in 50 mM sodium phosphate buffer, pH 7.4, 0.1% Zwittergent) and OVA (1 mg/ml in 50 mM PO_4 buffer, pH 7.4) were activated with a 10-fold molar excess of *m*-maleimido benzoic acid *n*-hydroxysuccinimide ester (MBS) (Sigma) for 20 min at RT. A 10-fold molar excess of CSP-Pep, or CSP-TE was dissolved at 1.0 mg/ml in distilled water, added to the activated proteins, and allowed to react overnight. Unreacted peptide was then removed from the TraT by precipitation with 80% ethanol and repeated washing in ethanol and from the OVA by extensive dialysis against PBS. Finally the TraT-peptide conjugate was resuspended in 1% Zwittergent.

Ag Administration

Rabbits. NZW rabbits (2 to 2.5 kg, 2 to 3 rabbits per group) were injected i.m. with 100 μg of Ag in 0.1 ml of sterile physiologic saline. Injections were performed on days 0 and 36. Weekly bleedings were obtained from the longitudinal ear vein and antibody titers were measured by a standard ELISA with TraT, OmpA, OmpF, BSA, DNP-sheep IgG, or CSP-Pep as coating Ag.

Mice. All mice were obtained from the Animal Resources Centre (Perth, Western Australia). Female C57BL/6J mice (H-2b) (18 to 22 g), were injected with 50 μg of Ag i.m. on days 0 and 14. Blood samples were taken from the retro-orbital plexus on day 21, allowed to clot, and stored frozen (−20°C) until required for antibody determination.

ELISA. The ELISA for the determination of antibody titers was performed as described previously (20, 21). Anti-DNP titers were measured by coating ELISA plates with dinitrophenylated sheep IgG. Anti-CSP-Pep responses were measured by coating the plates with the peptide (5 μg/ml). All other Ag were coated at 2 μg/ml in 0.1 M carbonate buffer, pH 9.5. Titers are expressed as the geometric mean of the reciprocal of the antiserum dilution which gave an ELISA reading of 0.5 after 45 min at 37°C.

In vitro T cell assay. Groups of three CBA/CaH mice (H-2k) were immunized s.c. with the CSP-TE peptide alone, conjugated to TraT, or mixed in CFA. After 10 days the mice were killed and their draining lymph nodes removed. The cells were dispersed to single-cell suspensions and plated in 96-well tissue culture plates at 5×10^5 per well in RPMI 1640 media containing 10% heat-inactivated FCS and 50 μM 2-ME. Triplicate wells were challenged with doses of 10, 20, or 50 μg of CSP-TE peptide or 2 or 50 μg of PHA. Cells were incubated in 5% CO_2 for 48 h after which 20-μl aliquots of the supernatants were removed and added to cultures of the IL-3-dependent cell line, FCDP1. After a further 24 h of incubation, all cultures were pulsed with 0.5 μCi [^{3}H]thymidine/well and cultured for a further 6 h before harvesting and counting. Results are expressed as the average number of counts from Ag or IL-3-stimulated wells minus counts from wells of unstimulated cultures.

RESULTS

Effect of adjuvant on the immune response to Imps. The immune response generated to the Imps injected alone was compared with that generated by the Imps injected with IFA (Fig. 2). i.m. administration of TraT in saline quickly elicited high titers of serum antibody to the immunizing agent (Fig. 2). In fact the titers generated by TraT in saline were only marginally increased (four- to eightfold) by injection of this Ag in IFA. No significant increase in titer was seen when mice or rabbits were injected with Imps mixed with carbopol, liposomes, alhydrogel, or muramyl dipeptide (results not shown). This is in direct contrast to the response generated by the soluble Ag, BSA. In this case a poor antibody response

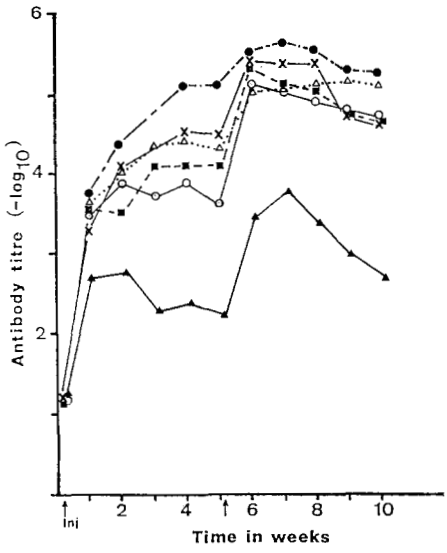

Figure 2. Effect of adjuvant on the immune response to Imps. Rabbits ($N = 3$) were injected i.m. with 100 μg of TraT (×), OmpA (■), OmpF (○), and BSA (▲) in saline. TraT (●) and BSA (△) were also injected i.m., mixed with IFA. Results are expressed as the mean of the reciprocal of the serum dilution that gave an ELISA reading of 0.5 after 45 min at 37°C.

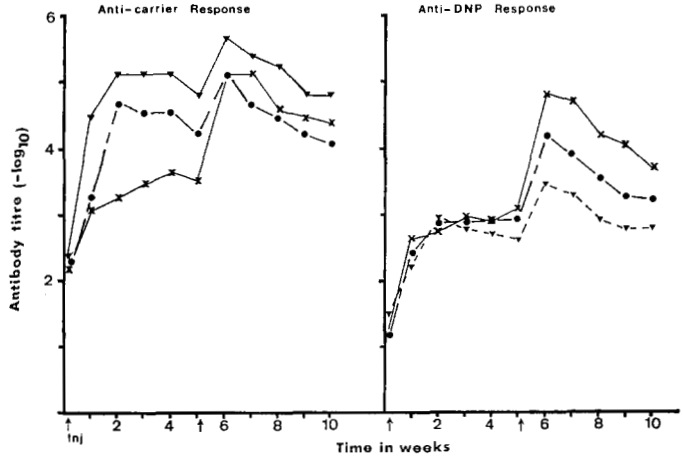

Figure 3. Examination of the adjuvant-carrier activity of Imps. Dinitrophenylated preparations of TraT (●), OmpA (×), and BSA (▼) were compared for their ability to stimulate anti-carrier (*left panel*) and anti-DNP responses (*right panel*) after i.m. injection into rabbits ($N = 3$). DNP-BSA was injected in IFA, whereas DNP-TraT and DNP-OmpA were injected in saline. Injection times are indicated by *arrows*.

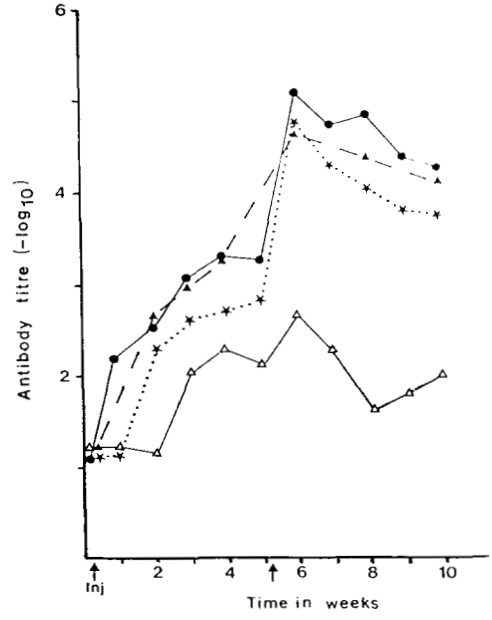

Figure 4. Stimulation of the immune response to BSA by conjugation to Imps. BSA was covalently cross-linked to TraT (●) or OmpA (★) with glutaraldehyde. Conjugates were injected in saline. BSA was suspended in saline and injected alone (△) or mixed with IFA (▲) into mice (five per group). The antibody response to BSA was determined as in Figure 2.

was generated to the Ag administered in saline, but was markedly increased (10- to 100-fold, depending upon the time after immunization) by injection of the Ag in IFA (Fig. 2).

Injection of OmpA or OmpF in saline also elicited high serum antibody titers. Thus, immunization with 100 μg of TraT, OmpA, or OmpF resulted in the generation of high antibody titers which were similar for each of the immunizing Ag.

At no stage did any of the animals injected with Imps show any adverse signs to the immunogens as judged by pyrogenicity in the rabbits, the presence of macroscopic lesions (*viz*: there was no evidence of granuloma formation, erythema, abscesses, or inflammatory lesions), regardless of whether the immunogen was given s.c., i.m., or i.p.

Examination of the adjuvant-carrier activity of Imps. The potential for TraT and OmpA to augment anti-hapten (DNP) and anti-protein (BSA) responses was examined. The two Imps were substituted with DNP by using DNFB or were cross-linked via glutaraldehyde to BSA (see *Materials and Methods*) and were injected i.m. into rabbits.

Conjugation of DNP or BSA to either TraT or OmpA had little effect on the generation of the anti-Imp response after immunization of rabbits (Fig. 3; cf Fig. 2). Injection of dinitrophenylated Imps resulted in a 4- to 16-fold increase in the anti-DNP response (for OmpA and TraT, respectively) above that observed after the injection of DNP-BSA mixed in IFA. Similarly, immunization with BSA covalently coupled to either TraT or OmpA resulted in a much higher antibody titer to BSA than when BSA was injected in saline (Fig. 4). In fact, the level of antibody titer generated was equivalent to that achieved by injection of BSA in IFA. Similar results have been achieved in both C57/B1 and in the LPS nonresponder strain, C3H/HeJ mice (results not shown).

Stimulation of an anti-CSP response with a CSP:TraT conjugate. In order to examine the adjuvanting effect of TraT on peptide Ag, a synthetic peptide derived from the CSP Ag of *P. falciparum* [NH_2-Cys(Asn-Pro-Asn-Ala)$_4$] was synthesized and conjugated to TraT and OVA.

Immunization of mice with CSP-Pep coupled to TraT resulted in the stimulation of an anti-CSP-Pep response which was greater than seen when the Ag was coupled to OVA and injected in IFA (Table I). Mixing the CSP-OVA with TraT resulted in only a small increase in antibody response to the peptide.

A similar enhancement of anti-peptide responses has also been seen with conjugates to peptide Ag from a number of other proteins such as the HIV gp120 protein and to the hepatitis B pre-S2 protein (results not shown).

Stimulation of a T cell response by CSP-TE:TraT conjugates. Conjugates of TraT and a CSP-T cell epitope (CSP-TE) were examined for their ability to prime CBA/CaH (H-2K) mice to generate a T cell response. Draining lymph node cells isolated from mice immunized with various preparations of CSP-TE were placed in culture

TABLE I

Stimulation of anti-peptide responses by injection of CSP-TraT conjugates[a]

Ag[b]	Antibody Response	
	Anti-TraT	Anti-CSP
CSP-TraT	316,227 ± 125,000	2,248 ± 750
CSP-OVA	106 ± 80	1 ± 0
CSP-OVA + IFA	27 ± 1	212 ± 170
CSP-OVA + TraT	405,280 ± 64,000	28 ± 26

[a] Mice ($N = 5$) received two injections of Ag i.m. on days 0 and 14. One week after the second injection, the animals were bled and the sera assayed for antibody activity against the appropriate Ag (see *Materials and Methods*).

[b] Ag dose was 20 μg. Conjugation procedure was *m*-maleimido benzoic acid *n*-hydroxysuccinimide ester.

and challenged with CSP-Pep, CSP-TE peptide, or PHA.

Cells isolated from mice immunized with the CSP-TE conjugated to TraT were readily stimulated in culture by the CSP-TE peptide and by PHA (Table II). The stimulation obtained was higher than that seen with cells isolated from mice immunized with the peptide in CFA. Thus the addition of 2, 10, 50 μg/ml of CSP-TE to CSP-TE-TraT-immunized cells resulted in a stimulation of 37,850, 46,700, and 96,140 cpm (respectively), whereas in the mice immunized with CSP plus CFA the stimulation was only 34,200, 34,245, and 59,000 (at the same dose levels). No stimulation was seen with cells from mice immunized with the CSP-TE peptide injected in saline or with nonimmunized mice. In no case were cells stimulated by the CSP Ag alone. This is to be expected as CBA/CaH mice of the H-2k haplotype are not able to respond to the T cell epitope in the CSP peptide and therefore require priming by the T cell epitope in the CSP-TE peptide (19). When the supernatants were removed from stimulated cell cultures and placed on cultures of the IL-3 dependent cell line, FCDP1, these cells were stimulated in a similar fashion to that observed with the primary cell cultures. As IL-3 is only released from stimulated T cells (22) this demonstrates that the stimulation observed in the primary cell cultures was in fact due to direct stimulation of the T cell population of cells.

DISCUSSION

There has recently been a great deal of progress in the isolation of protective protein Ag from many of the organisms responsible for infectious diseases of man and animal. Notable in this regard has been the identification of the protein Ag responsible for conferring protective immunity against malaria (17, 18, 19, 23), foot and mouth disease virus (24–26) and hepatitis B (27–31). Successful immunization of animals with many of these peptide and protein Ag has been achieved only by the use of adjuvants such as IFA and CFA. These depot-type adjuvants are, however, unsuitable for clinical use due to their propensity to create large lesions at the site of vaccine injection (1). This has led to a definite need to discover new adjuvants for the administration of proteins and their peptide substituents to the immune system of susceptible hosts.

There are a number of molecules which have been shown to augment the immune response to coadministered Ag. Notable among these are the viral membrane proteins or ISCOMS, which have been shown to greatly enhance the immune response to peptides when injected simultaneously with Ag (32–34). Similarly, injection of outer membrane vesicles of *Neisseria meningitidis* premixed with peptides possessing hydrophobic tails stimulated an enhanced immune response to the peptides (35). It must be noted, however, that no attempt was made to remove LPS during the preparation of the outer membrane vesicles of *N. meningitidis*. It is likely therefore that at least some of the stimulation seen with these vesicles may have been due to the mitogenic properties of the LPS (36) contained in the complexes.

In this study we have used preparations of individual outer membrane proteins which are free of detectable LPS (less than 1 ng LPS/mg of protein for TraT, OmpA, and OmpF), and have shown that these purified integral membrane proteins act as powerful immunostimulatory molecules both in eliciting antibody responses to the proteins themselves as well as to proteins or peptides covalently attached to them. Thus the i.m. injection of TraT, OmpF or OmpA resulted in the generation of high titers of antibody to the immunizing antigen (Fig. 1). This antibody response was not significantly enhanced by the addition of a strong adjuvant such as IFA. Similarly when these proteins were covalently linked to hapten (DNP), protein (BSA), or peptide (CSP) Ag and injected into mice or rabbits they were capable of stimulating antibody titers to these substituents similar to those generated when these molecules were administered in IFA.

In addition to the pronounced stimulation of antibody responses to a number of Ag, a conjugate of TraT with a

TABLE II

In vitro cell-mediated responses to CSP-TE-TraT immunized mice[a]

Immunogen	Proliferative Response				
	CSP-TE			CSP	PHA
	2 μg/ml	10 μg/ml	50 μg/ml	20 μg/ml	2 μg/ml
CSP-TE-TraT	37,850 ± 4,951	46,700 ± 17,273	96,140 ± 19,187	3,036 ± 2,950	65,600 ± 14,200
CSP-TE + CFA	34,200 ± 10,436	34,245 ± 14,463	59,000 ± 10,700	988 ± 7,500	67,400 ± 11,800
CSP-TE	191 ± 717	587 ± 412	644 ± 285	819 ± 425	14,872 ± 5,012
Saline + CFA	3,853 ± 5,235	3,837 ± 5,705	945 ± 6,003	425 ± 4,300	49,673 ± 14,300
	IL-3 Stimulation				
CSP-TE-TraT	4,818 ± 3,608	6,120 ± 2,368	10,247 ± 1,788	543 ± 3,108	2,639 ± 2,338
CSP-TE + CFA	3,917 ± 1,551	3,876 ± 2,349	6,084 ± 1,503	557 ± 2,483	2,520 ± 2,167
CSP-TE	100 ± 154	47 ± 190	29 ± 271	129 ± 407	290 ± 302
Saline + CFA	470 ± 1,069	477 ± 2,019	865 ± 1,459	488 ± 1,112	1,179 ± 1,383

[a] Data represent cpm of stimulated cultures minus background counts of unstimulated cultures. Counts are presented for the mean of triplicate samples ±95% confidence limits of the difference between means of stimluated minus unstimulated cultures. PHA controls received 2 μg/ml for the primary cell cultures; however, cells used for stimulation of the IL-3-dependent cell line, FCDP1, received 50 μg/ml PHA.

known T cell epitope (CSP-TE) led to the stimulation of an active cellular immune response to the CSP-T cell epitope which was comparable to that elicited when CSP-TE was injected in CFA, an adjuvant traditionally used to stimulate strong cellular responses. The mitogenic response generated in vitro was shown to be due to stimulation of T cells as the culture supernatants from these cells were able to stimulate the IL-3-dependent cell line FCPD1.

Despite the large immune response generated by the Imps, at no time were any lesions noted at the site of injection of the Imps either in mice or rabbits (results not shown). This is in contrast to the effects seen after injection of IFA or CFA, which are known to induce large lesions at their injection sites.

The mechanism whereby these Imps act as immunostimulants is unknown, although it has been shown previously that a number of preparations of outer membrane proteins can act as potent B cell mitogens. Thus proteosomes formed from outer membrane vesicles were mitogenic for B cells (37–39) and enhanced the immune response to peptides inserted noncovalently, into the proteosomes via hydophobic tails. However, each of these preparations was undoubtedly contaminated with significant quantities of LPS, a known B cell mitogen.

Bessler and others (40–43) have shown that proteins I and II (OmpF and OmpA) as well as Braun's lipoprotein are all polyclonal B cell activators. In contrast, we have been unable to demonstrate any mitogenic activity in our preparations of OmpA, OmpF or TraT (purified as described in the *Materials and Methods*) when tested in the in vitro assay described by Bessler and co-workers (results not shown). It is therefore probable that the mitogenic activity described by those authors was due to residual contamination by LPS (stated by the authors to be less than one percent).

The mitogenic activity of Braun's lipoprotein has been postulated to reside in the lipid portion of the molecule (40, 42). Covalent linkage of the synthetically prepared lipid tail to a number of peptides enhanced the immune response to the conjugated peptides. Hopp and other workers have also demonstrated enhanced immunogenicity of Ag following covalent conjugation to various lipids (28, 35, 44–46). TraT has also been shown to be a lipoprotein (5) and so it is conceivable that the lipid portion of this molecule may be responsible for the adjuvant activity shown by the TraT conjugates described above. However, OmpA and OmpF which lack this lipid component also have potent adjuvanting activity. Furthermore, oversubstitution of any of the Imps with DNP, or over cross-linking to BSA results in a reduction in the adjuvant activity of these molecules, thus making it unlikely that the possession of the lipid component in TraT is the major reason for its adjuvanticity.

The use of Imps as adjuvants has significant advantages over the use of mineral-oil adjuvants (IFA and Montanide), ISCOMS, or outer membrane vesicles of *N. meningitidis*. First, the Imps are easy to purify from nonpathogenic strains of *E. coli* without the copurification of LPS (a known contaminant of the outer membrane vesicles). Second, the Imps themselves can be used as carriers for the generation of anti-hapten and anti-peptide responses, and third, the injection of the Imps with Ag does not result in the formation of deleterious lesions at the site of injection.

Although the peptides used in this study were covalently conjugated to the Imps, it should be possible (with rDNA technology) to insert the DNA sequence encoding these and other peptides into the gene coding for the Imp, and thereby obtain a recombinant protein expressing the sequence of the peptide, while maintaining the adjuvant properties of the Imps.

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