tic chromosomal translocation, t(18;X)(q11:q11), the breakpoint on chromosome X has been assigned clearly at Xp11.2 (*39*). The *elk*-1 gene may be a candidate for involvement in this translocation. *elk*-2 also maps to a chromosome region, 14q32, which may be near characteristic translocation breakpoints. Chromosome band 14q32, the site of the IgH and the putative oncogene TCl-1 (*31, 40*) is frequently rearranged in B and T cell tumors. B cell neoplasias exhibit chromosomal rearrangements within the IgH locus at 14q32.3, whereas in T cell neoplasias with translocation in 14q32, the 14q32 breakpoint usually occurs centromeric to the IgH locus (*31, 41*). The human proto-oncogene AKT-1, a homolog of the viral oncogene v-*akt*, has also been assigned to chromosome band 14q32 centro meric to the IgH locus (*42*). It remains to be seen whether *elk* or related sequences play a role in the pathogenesis of these malignancies.

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Immune Response to Cholera Toxin Epitope Inserted in *Salmonella* Flagellin

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Bacterial flagella are potent immunogens and aromatic-dependent (*aro*) *Salmonella* as live vaccines evoke humoral and cellular immune responses. Such strains expressing epitopes of protective antigens as inserts in flagellin would provide a novel way to vaccinate against diseases caused by unrelated pathogens. A synthetic oligonucleotide specifying an epitope of cholera toxin subunit B was inserted in a *Salmonella* flagellin gene. The chimeric flagellin functioned normally and the epitope was expressed at the flagellar surface. Parenteral administration to mice of an *aroA* flagellin-negative strain of *S. dublin* expressing the chimeric flagellin gene evoked antibody to cholera toxin.

RECENT DEVELOPMENTS IN VACcine construction include the use of synthetic peptides of relevant antigens from various pathogenic microorganisms (*1*); the construction of *Salmonella* strains made nonvirulent by irreversible mutations and therefore safe for use as live vaccines (*2*); and the use of these avirulent strains as carriers of cloned genes that specify foreign proteins to obtain an immune response to heterologous protective antigens (*3*), in much the same way as vaccinia virus carrying foreign genes has been used to confer protection against, for instance, rabies (*4*). We have combined these approaches by inserting a synthetic oligonucleotide into a cloned *Salmonella* flagellin gene; mice given a *Salmonella* live vaccine expressing the recombinant flagellin gene showed an immune response to the inserted epitope.

The flagellin gene used, *H1-d*, determines the phase-1 flagellar antigen, *d*, of *Salmonella muenchen*, a protein of 509 amino acids; its sequence includes a "hypervariable segment" of about 350 bp with no more than 30% amino acid identity to the corresponding sequences in *Salmonella* flagellar antigens *i*, *c*, and *a* (*5, 6*). Epitopes of flagellar antigens *i* (*7*) and *d* (*8*) have been identified in the hypervariable regions of genes *H1-i* of *S. typhimurium* and *H1-d* of *S. muenchen*. A chromosomal Eco RI fragment containing *H1-d*, originally cloned in plasmid pBR322 (*5*), was transferred to pUC19, a plasmid lacking Eco RV sites, to produce plasmid pLS405; the in vitro deletion of an Eco RV fragment gave plasmid pLS408, with a 48-bp deletion in the hypervariable region (Fig. 1). This deletion reduces but does not abolish flagellar function (as inferred from the motility of a flagellin-negative strain carrying pLS408) and removes one or more epitopes of antigen *d* (*8*). The remaining Eco RV site of pLS408 allows the blunt-end insertion of oligonucleotides.

The epitope chosen for expression in flagellin, peptide CTP3, consists of residues 50 to 64 of the B subunit of cholera toxin (*9*); it elicits both polyclonal (*10*) and monoclonal (*11*) antibodies that bind to the peptide and bind and neutralize cholera toxin. A synthetic 45-bp, double-stranded oligonucleotide specifying CTP3, with codon usage corresponding to that in sequenced *Salmonella* flagellin genes (and with a new Cla I site to facilitate analysis) was inserted at the unique Eco RV site of plasmid pLS408 (Fig. 1). We transformed competent cells of a flagellin-deficient *Escherichia coli* strain, CL447 (*12*), and selected for ampicillin-resistant colonies. The oligonucleotide insert improved flagellar function, as judged by the rate of spread in semisolid medium of clones with the insert.

Recombinant plasmids were sequenced by means of a 15-bp primer located 30 bp downstream from the insertion point. Plasmids found to have a single complete copy of the 45-bp sequence in correct orientation

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(or with 9 bp missing from one or the other end) were transferred to *Salmonella* hosts, in which the cloned flagellin genes are better expressed than in *E. coli* CL447. The chosen plasmids were used to obtain ampicillin-resistant transformants from *S. typhimurium* LB5000, a restriction-negative and modification-proficient strain (*13*) that is nonflagellate because of mutation *flaA66*, which abrogates expression of the flagellin genes. Phage P22 HT105/1 *int* was used to transduce the now-modified plasmids to *S. typhimurium* SL5676, which has a deletion at *H2-1,2* (the gene specifying the phase-2 flagellar antigen of this species) and a Tn*10*-generated deletion or deletion-inversion at *H1-i* that does not affect the function of adjacent *fla* genes. The ampicillin-resistant transductants obtained had functional flagella, as shown both by rapid-spreading growth in semisolid medium and by translational motility of nearly all the bacteria of log-phase broth cultures examined by low-power, dark-field microscopy. Protein immunoblots of lysed bacteria or disaggregated flagella showed that a single band bound both rabbit antibody to flagella antigen *d* (*14*) and mouse monoclonal antibody to CTP3 (MAb TE33) (Fig. 2). MAb TE33 immediately immobilized and rapidly agglutinated bacteria of strain SL5676 made motile by pLS411, the plasmid with the insert-bearing flagellin gene, but had no effect on the same strain made motile by plasmids with the wild-type gene or on that with the 48-bp deletion. Exposure of the foreign epitope at the surface of the flagella was detected by gold immunolabeling (Fig. 3) of the flagella of Formalin-fixed bacteria, with MAb TE33 as the first antibody.

For tests of immunogenicity we replaced the phase-1 flagellin gene, *H1-g,p* of aromatic-dependent live-vaccine *S. dublin* strain SL1438 (*3*) with a flagellin allele inactivated by a transposon, *H1-i*::Tn*10*; as *S. dublin* is monophasic, the resulting strain, SL5928, was nonmotile but became motile when transformed with plasmids containing either the wild-type, the deletion, or the chimeric form of *H1-d*, just as observed for the flagellin-negative *S. typhimurium* host, SL5676. The pUC-derived plasmids are stable in the live vaccine strain used, as shown by the ampicillin resistance of all of more than 100 colonies from a bacterial suspension after two passages in broth without ampicillin and by the ampicillin resistance of all colonies recovered from mouse livers at autopsy. We immunized C57BL/6 mice with three intraperitoneal injections of 5×10^6 bacteria, either Formalin-killed or live, at 7-day intervals. A week after the last injection the mice were bled and their sera were tested by enzyme-linked immunosorbent assay (ELISA) for reactivity with CTP3 peptide or whole cholera toxin (Fig. 4). We detected antibody to the inserted epitope in all the sera; all sera reacted as strongly with cholera toxin as with the CTP3 peptide.

Epitopes of gp120 of human immunodeficiency virus type 1 (HIV-1) (*15*), of *Plasmodium berghei* circumsporozoite protein (*16*), of an epitope of the M protein of *Streptococcus pyogenes* type 5 (*17*), and of two epitopes of surface proteins of hepatitis B virus (*18*) have also been expressed with this system. In all of these investigations an immune response to the foreign epitope was generated in mice, rabbits, or guinea pigs after administration of either an *aroA* live vaccine strain carrying the recombinant flagellin gene or killed flagellate bacteria or flagella of such a strain. Thus, the hypervariable region of *H1-d* is a useful site for the insertion of foreign epitopes into the flagel-

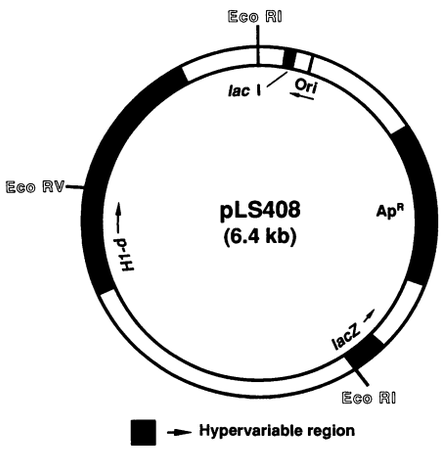

Fig. 1. Structure of plasmid pLS408 and the base sequence of the synthetic oligonucleotide used as the insert. This plasmid was derived from pUC19 by the insertion, at its Eco RI site, of a fragment that includes gene *H1-d* from *S. muenchen* ATCC8388, originally cloned in pBR322 (*5*) as pLS402, followed by the in vitro deletion of an Eco RV fragment, of 48 bp, in the hypervariable, antigen-determinant segment of *H1-d*. The oligonucleotide used, shown at the bottom, was designed to specify peptide CTP3 with codon usage according with that of sequenced *Salmonella* flagellin genes (*5, 6*) and with a Cla I restriction site; it was made by annealing two 30-base synthetic oligonucleotides and filling in the single-strand regions by Klenow fragment treatment. The product was blunt-end ligated to a phosphatase-treated Eco RV digest of pLS408.

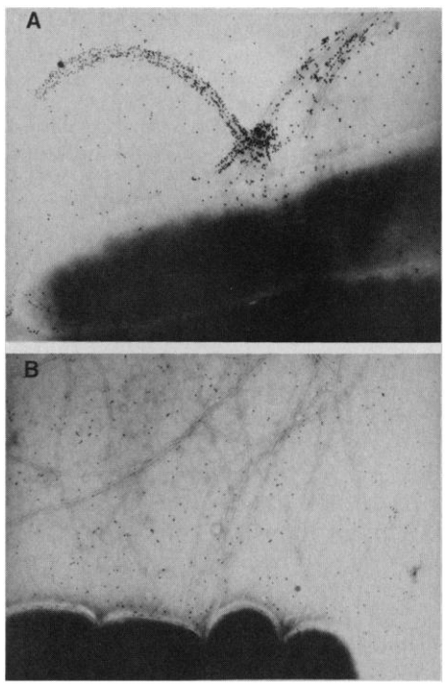

Fig. 3. Immunogold labeling of the CTP3 epitope at the surface of the flagella. Strain SL5676 harboring either plasmid pLS411, which has the complete CTP3 insert (**A**), or plasmid pLS408, with the in vitro deletion but not the insert (**B**), were labeled by treatment with MAb TE33 and gold-conjugated goat antibody to mouse IgG (Janssen) for electron microscope visualization (×30,000).

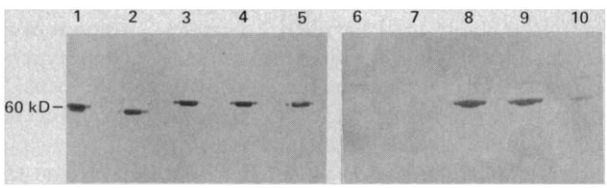

Fig. 2. Protein immunoblot analysis of *Salmonella typhimurium* SL5676 harboring various flagellin plasmids. Bacterial extracts were reacted with rabbit antibody to flagellar antigen *d* (lanes 1 through 5) or with MAb TE33 (anti-CTP3) (lanes 6 through 10). Lanes 1 and 6, pLS402 (parent plasmid); lanes 2 and 7, pLS408 (the in vitro deletion mutant); lanes 3 and 8, pLS411 (complete CTP3 insert); lanes 4 and 9, pLS415 (insert lacks the first 9 bp of the CTP3-specifying oligonucleotide); lanes 5 and 10, pLS416 (insert lacks the last 9 bp of the CTP3-specifying oligonucleotide). TE33 is an immunoglobulin G1 (IgG1) mouse MAb to a peptide (residues 50 to 64) of cholera toxin subunit B (*11*). TE33 antibodies from ascites fluid of BALB/c mice were purified by precipitation with 45% ammonium sulfate (twice) and chromatography on a DEAE-Sephacel column. Bacterial protein extracts were subjected to electrophoresis on a 10% SDS-polyacrylamide gel and blotted onto nitrocellulose sheets, which were probed with the indicated antibodies and then with peroxidase-conjugated goat antibody to rabbit IgG (for antiflagellin) or peroxidase-conjugated goat antibody to mouse IgG (for TE33). The absence of amino acids 50 to 52 of the epitope in pLS415 (lane 9) did not affect the binding of MAb TE33, but the absence of amino acids 62 to 64 (lane 10) reduced binding of this monoclonal antibody.

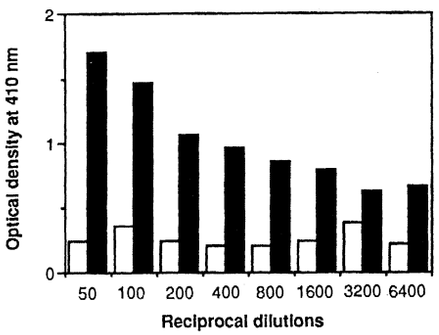

Fig. 4. Antibody response of five mice immunized with SL5929, a *Salmonella dublin* live vaccine strain that expresses the chimeric flagellin genes; □, before immunization, ■, after immunization with SL5929. Reactivity of mouse sera with whole native cholera toxin was measured by solid-phase ELISA (*9*), with peroxidase-conjugated goat antibody to mouse IgG (TAGO). Mice were injected intraperitoneally three times, at weekly intervals, with 5×10^6 Formalin-killed bacteria; sera were collected 7 days after the last injection. The bars represent the mean optical density for sera from five mice (SE <15% for all dilutions).

lar antigen, and the altered *Salmonella* strains, when used as vaccines, can cause the production of antibodies specific for the foreign epitope (*19, 20*). Some inserts three times as long as the 16–amino acid deletion are compatible with flagellar function (*18*). This flexibility indicates that it may be feasible to insert several epitopes of a protective antigen (at one or more sites), to increase the chance of an immune response, despite the probability that a given epitope will be ineffective in some subjects of an outbred population.

Some in-frame inserts did not confer motility on a flagellin-negative host; we are investigating what constraints exist for retention of function. In results to be presented elsewhere, we provide evidence that an epitope placed in the flagellar filament protein can elicit a cellular immune response, as shown by proliferative response of cultured spleen lymphocytes to the relevant peptide (*21*). The ability to (i) synthesize peptides for the identification of antigenic and protective epitopes from pathogens, (ii) insert the corresponding oligonucleotides into flagellin genes, and (iii) use safe and effective live vaccine strains of *Salmonella* that induce cellular and humoral immunity makes possible the construction of vaccines for protection against infections for which immune prophylaxis is presently not feasible.

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19. The advantage of this approach is exemplified by the fact that although in our experiments CTP3 expressed within the flagellin protein induced antipeptide response, CTP3–β-galactosidase hybrid protein in *E. coli* did not lead to detectable production of antipeptide antibody, although a "priming" immune response could be obtained. Even the presence of the OmpF signal peptide at the amino terminus of the hybrid protein (which would have made the fusion protein a component of the bacterial outer membrane) did not lead to an immune response against CTP3. C. O. Jacob, M. Leitner, A. Zamir, D. Salomon, R. Arnon, *EMBO J.* **4**, 3343 (1985).
20. After the completion of our work we saw the report by G. Kuwajima *et al.* [*Bio/Technology* **6** (no. 9), 1080 (1988)] on the insertion of an oligonucleotide specifying an 11-residue epitope of egg-white lysozyme at the site of a deletion in the central region of the flagellin gene, hag, of *E. coli*; the chimeric flagellin filaments bound antibody to a lysozyme fragment but their injection into guinea pigs did not evoke antibody able to bind the lysozyme fragment.
21. S. M. C. Newton, L. Rosenberg, B. A. D. Stocker, unpublished observations.

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Heritable Allele-Specific Differences in Amounts of apoB and Low-Density Lipoproteins in Plasma

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Low-density lipoprotein (LDL) concentrations correlate with risk of coronary heart disease, and genetic variation affecting LDL levels influences atherosclerosis susceptibility. The principal LDL protein is apolipoprotein B (apoB); apoB is not exchangeable between lipoprotein particles and there is only one apoB per LDL particle. Plasma LDL therefore consists of two populations, one containing apoB derived from the maternal and one from the paternal apoB alleles. Products of the apo*b* gene with high or low affinity for the MB-19 monoclonal antibody can be distinguished, and this antibody was used to identify heterozygotes with allele-specific differences in the amount of apoB in their plasma. A family study confirmed that the unequal expression phenotype was inherited in an autosomal dominant manner and was linked to the apo*b* gene locus. Significant apoB genetic variation affecting plasma LDL levels may be more common than previously appreciated.

APOLIPOPROTEIN B (APOB) IS A constituent of several types of lipoprotein particles and exists in two forms, B-100 and B-48. The B-100 protein, which is made primarily in the liver, is virtually the only protein in low-density lipoprotein (LDL) and mediates the interaction of LDL with its receptor (*1–4*). Defects in the apo*b* gene have been described that result in diminished plasma apoB in the disorder hypobetalipoproteinemia (*5–8*). As a consequence, homozygotes have little or no plasma LDL cholesterol, whereas heterozygotes have approximately 30% of normal levels (*5*). These observations suggest that other defects in the apo*b* gene might also influence LDL cholesterol levels.

We now report that there are heritable differences in the contribution of specific apo*b* alleles to plasma apoB concentrations.

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