

**MONOCLONAL ANTIBODY WHICH
AGGLUTINATES *E. COLI* HAVING THE CS4-CFA/I
FAMILY PROTEIN**

**CROSS REFERENCE TO RELATED
APPLICATIONS**

[0001] This application is a continuation of U.S. patent application Ser. No. 08/905,046, filed 1 Aug. 1997, abandoned, listing Frederick J. Cassels, Andrew Lees, and Richard Schuman as inventors, which is herein incorporated by reference in its entirety.

**ACKNOWLEDGMENT OF GOVERNMENT
SUPPORT**

[0002] This invention was made by employees of the United States Army. The government has rights in the invention.

FIELD OF THE INVENTION

[0003] This invention relates to a monoclonal antibody to a consensus peptide of the formula:

VEKNITVTASVDPTIDLLQADGSALPSAVALTYSPA. (SEQ ID NO:1)

[0004] The monoclonal antibody of the invention binds exclusively to the sequence

SAVALTYSPA. (SEQ ID NO:2)

BACKGROUND OF THE INVENTION

[0005] The effect of *E. coli* in mammals is dependent on the particular strain of organism. Many beneficial *E. coli* are present in the intestines. Since the initial association of *E. coli* with diarrheal illness, five categories of diarrheagenic *E. coli* have been identified and are presently recognized: enterotoxigenic (ETEC), enteropathogenic (EPEC), enterohemorrhagic (EHEC), enteroaggregative (EAggEC), and enteroinvasive (EIEC). These categories are grouped according to characteristic virulence properties, such as elaboration of toxins and colonization factors and/or by specific types of interactions with intestinal epithelial cells. ETEC are the most common of the diarrheagenic *E. coli* and pose the greatest risk to travelers. *E. coli* of the family CS4-CFA/I are some of the more common enterotoxigenic *E. coli*. There is need for vaccines which are specific against this class of *E. coli* that give rise to antibodies that cross-react with and cross-protect against the more common members of the CS4-CFA/I family. Six members of this family of ETEC fimbrial proteins are CFA/I, CS1, CS2, CS4, CS17 and PCF 0166. ETEC are responsible for high infant mortality in developing countries, with an estimate that almost 800,000 deaths per year are due to these organisms. These organisms also cause illness in adult travelers to regions where the disease is endemic.

[0006] Colonization factor antigens (CFA) of ETEC are important in the initial step of colonization and adherence of the bacterium to intestinal epithelia. In epidemiological studies of adults and children with diarrhea, CFA/I is found in a large percentage of morbidity attributed to ETEC. The CFA/I is present on the surfaces of bacteria in the form of

pili (bimbriae), which are rigid, 7 nm diameter protein fibers composed of repeating pilin subunits. The CFA/I antigens promote mannose-resistant attachment to human brush borders with an apparent sialic acid sensitivity.

[0007] A study of proteins in *E. coli* belonging to the CS4-CFA/I family resulted in the finding that the N-terminal region of the protein maintains a high degree of sequence identity between members of this group. Immunological evidence shows that cross-reaction exists between members of the family CS4-CFA/I.

[0008] Cassels, et al. have identified a consensus peptide of 36 amino acids which acts as an immunogen raising antibodies against the proteins of all members of the *E. coli* family CS4-CFA/I. The region of the protein represented in the subunit encompasses known linear B- and T-cell epitopes of CFA/I. The consensus peptide has a high level of homology to strains bearing six different colonization factors. The consensus peptide is of the formula:

VEKNITVTASVDPTIDLLQADGSALPSAVALTYSPA. (SEQ ID NO:1)

DESCRIPTION OF THE INVENTION

[0009] It is the purpose of this invention to identify a monoclonal antibody raised to the consensus peptide of Cassels and which will agglutinate all bacteria bearing CS4-CFA/I family proteins.

[0010] Preparation of the Immunogen:

[0011] A: Iodoacetylation of tetanus toxoid:

[0012] To 0.64 ml of a composition containing 18.9 mg/ml (12 mg) of tetanus toxoid (TT) (obtained from SmithKline Beecham) was added 5×HEPES buffer (75 μ l of 0.75 M HEPES, 5 mM EDTA, pH 7.3). The TT was labeled with a 40 fold molar excess of N-hydroxysuccinimidyl iodoacetate (32 μ l of 0.1 mM in dimethylformamide). After two hours, the protein was desalted on 2 P6 cartridges (BioRad) in series, equilibrated with HEPES buffer (0.15M HEPES, 1 mM EDTA, pH 7.3). The void volume fraction was concentrated to about 0.7 ml using a MACROSEP™ 50 device (Filtron Corp).

[0013] B: Reduction of peptide:

[0014] About 10 mg of peptide consensus peptide of the formula

CVEKNITVTASVDPTIDLLQADGSALPSAVALTYSPA (SEQ ID NO: 3)

[0015] was solubilized in 1.1 ml HEPES buffer containing 100 μ l acetonitrile and reduced by the addition of solid dithiothreitol to a final concentration of 0.5M. After 1 hour the peptide was desalted in two parts on a 1×50 cm G10 column (Pharmacia), equilibrated with acetate buffer (10 mM sodium acetate, 0.1 M NaCl, 2 mM EDTA and 0.02% sodium azide at pH 5) and run at 1 ml/min. The void volume fractions were pooled.

[0016] Ellman's reagent (G. L. Ellman, Arch. Biochem. & Biophys., 82:70 (1959)) was used to determine that the peptide was reduced to a thiol.