Heteroimmunization to the Capsular Polysaccharide of *Haemophilus influenzae* Type b Induced by Enteric Cross-Reacting Bacteria

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Cross-reacting *Escherichia coli* strains Easter and 89 and *Bacillus pumilis* fed to newborn rabbits and *E. coli* fed to adult rhesus monkeys did not exert untoward reactions. The *E. coli* regularly colonized the newborns' intestinal tract from 1 to 7 weeks. High doses of *E. coli* were necessary to colonize adult primates. Colonization occurred in fewer newborn rabbits and lasted only 1 to 3 weeks with *B. pumilis*. Colonized newborn rabbits and adult rhesus had an active *Haemophilus influenzae* Type b (HITB) immune response. In the rabbit, colonization resulted in accelerated induction of immunoglobulin (Ig) M-, IgA-, and IgG-producing cells in the spleen, mesenteric lymph nodes, and Peyer's patches after HITB challenge. *E. coli*-fed and control newborn primates were naturally colonized with nasopharyngeal and enteric cross-reacting bacteria and both groups rapidly developed HITB antibodies in the absence of the homologous organisms. Human newborn stool cultures, taken at the time of discharge from the nursery, showed a 0.9% carriage rate for cross-reacting *E. coli*. These “carrier” infants acquired HITB antibodies more rapidly than their age-matched “noncarrier” controls.

*Haemophilus influenzae* Type b (HITB) is the leading cause of endemic bacterial meningitis and an etiologic agent of other serious infectious diseases in the United States and in many other countries (12, 13, 20, 23, 25, 26, 35, 39, 43-45). Because they have lost their transplacentally acquired antibodies and have not yet developed antibody synthesis, individuals older than 3 months and younger than 6 years constitute the most susceptible age group (2, 5, 6, 12, 13, 26, 31, 33, 38). The specificity of these protective serum antibodies is mostly anticapsular (2, 3, 5, 6, 28, 29, 31, 33, 35, 38).

The wide prevalence of anticapsular antibodies in the normal adult population is difficult to explain by asymptomatic carriage of the homologous organism since its occurrence in normal infants and children is low and it is rarely found in adults (10, 12, 15, 35, 42, 44). In addition, a similar age-related development of anticapsular antibodies was observed in laboratory rabbits and primates without detection of HITB (37).

Utilizing the antiserum agar technique (10, 27), *Escherichia coli* and several gram-positive bacteria containing polyribitol phosphate as their cell wall teichoic acid were found to cross-react with the capsular polysaccharide of HITB (7, 16, 36). In an earlier report it was found that feeding of two cross-reacting *E. coli* strains accelerated the formation of HITB antibodies in newborn laboratory rabbits (24). At 8 weeks of age the fed animals had higher levels of HITB antibodies than controls. An active heteroimmune response was shown by the higher serum anticapsular antibodies of the *E. coli*-fed animals after challenge with living HITB organisms. These findings suggested that immunization against HITB diseases in humans could be achieved by neonatal feeding of enteric nonpathogenic, cross-reacting bacteria. Accordingly, more detailed and comparative studies of the relation between these organisms as inhabitants of the gastrointestinal tract and the development of HITB antibodies in laboratory rabbits, rhesus monkeys, and human infants were undertaken.

(These data were presented in preliminary form to the Society for Pediatric Research, San Francisco, Calif., May 1973, and at the 4th International Convocation on Immunology, Buffalo, N.Y., June 1974.)

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MATERIALS AND METHODS

Bacteria. The antigenic and immunogenic properties of the cross-reacting E. coli O75:K100:H5, strains Easter and 89, and Bacillus pumilis (Sh-18), media, and growth conditions, and intravenous challenge with HITB (strain Rab) have been described (7, 36). The cross-reacting antigen, previously designated Kf147, has been assigned the nomenclature K100 and the W.H.O. test strain is f147/51.

Antiserum agar. The preparation of the antiserum agar with tryptic soy broth (Difco) for stool cultures and of Levinthal media (BHI, Difco) for nasopharyngeal cultures utilizing burro HITB antiserum has been described (7, 36).

Animals. Newborn hybrid rabbits were raised and maintained in our laboratory. Newborn rhesus (Macaca mulatta) raised at the Primate Research Center, University of California, Davis, were maintained with their mothers throughout the experiment. The E. coli-fed and saline-fed (controls) animals were housed separately. The adult primates (1 to 2 years) fed strain Easter were housed in separate rooms at the Food and Drug Administration and were kept two to three animals per cage. The primates fed strain 89 ranged from 2 to 3 years of age and were housed in individual cages in the same room at the National Institute of Child Health and Human Development.

The organisms for feeding were prepared from a 4- to 5-h culture. The animals were fed 1 g of sodium bicarbonate before feeding of the bacteria to prevent acid denaturation during their passage through the stomach (11, 32).

Antibody assay. The HITB capsular polysaccharide and its tyraminated derivative were prepared for radioimmunocassay of capsular antigens as described (15, 31). The sensitivity of the assay was 0.05 μg of antibodies per ml and the reproducibility was ±12% in the range of 0.05 to 1.8 μg/ml. Sera withdrawn for routine laboratory assays from infants attending the outpatient department of Charlotte Memorial Hospital, and maternal and cord sera to be discarded from the blood bank of Charlotte Memorial Hospital and the Jacobi Hospital of Albert Einstein College of Medicine, Bronx, N.Y., were used as controls.

Plaque-forming cells (PFCs). Direct and anti-immunoglobulin-induced PFCs were assayed with sheep erythrocytes coated with HITB polysaccharide. PFCs were assayed in the spleens, mesenteric lymph nodes, and Peyer's patches of rabbits by a glass-slide variation of the hemolysis-in-gel test, as modified for pneumococcal polysaccharide by Baker et al. (8). The goat anti-rabbit immunoglobulin antiserum was kindly supplied by Susan Craig and John Cebra, Johns Hopkins University.

Antigen-induced proliferation (AIP). HITB capsular polysaccharide was added to cell suspensions taken from spleen, mesenteric lymph nodes, and Peyer's patches and was incubated with tritiated thymidine (2H, New England Nuclear Corp.). Incorporation of the isotope was assayed with a microtiter plate technique originally proposed by Harrison et al. (16, 17).

E. coli serotypes. The O and H antigens of the halo-producing E. coli isolated from stool cultures were serotyped by George Hermann, Center for Disease Control, Atlanta, Ga., and by Ida and Frits Ørskov, International Escherichiae Reference Centre (WHO), Statens Seruminstitut, Copenhagen, Denmark.

RESULTS

Neonatal rabbits. Each organism (10⁹) was fed to three to five litters of four to eight newborns. No difference in growth or mortality was observed in any of the E. coli-fed groups as compared to the controls. Table 1 shows that colonization, as detected by stool cultures on antiserum agar, was most successful with E. coli Easter (100%), was 80% for strain 89, and was 52% for B. pumilis Sh-18. Colonization for strains Easter and 89 lasted from 2 to 7 weeks in most animals. Most positive cultures showed a predominance of the halo-producing E. coli. In contrast, Sh-18 colonization lasted 1 to 3 weeks and eight of the positive animals showed only one positive stool culture during this period.

Many other cross-reacting bacteria, such as Staphylococcus aureus coagulase positive and several other bacillus species, were observed in the stool or nasopharyngeal cultures at least once in all animals. At 8 weeks of age, the highest anti-type b antibodies were in the controls (average 1.14 μg/ml, range 0 to 12). This high average level at this age was due to four animals in one litter of a mother with a serum anti-type b antibody level of 28.2 μg/ml.

There was no difference in the PFCs or AIP among Easter, Sh-18-fed, and control groups at 8 weeks of age. The 89-fed animals showed an increase in PFCs of the three immunoglobulin classes. However, this PFC increase was not accompanied by an increase in serum anti-type b antibodies or AIP.

After intravenous challenge with HITB, all fed animals showed significant increases of anti-type b antibodies, PFCs, and AIP. Injection of HITB in the controls did not result in increased PFCs or AIP in any tissue studied. The E. coli-fed animals showed marked increment in all three assays as compared to the controls. The widest range of induced reactivity was observed in the Sh-18-fed group. The immune response of the Sh-18-fed animals was related to the presence of positive stool cultures, as the average serum anti-type b antibodies after HITB challenge was 3.73 μg/ml in the colonized as compared to 1.65 μg/ml in the fed but noncolonized group.

An increase of PFCs of the three immunoglobulin classes was observed 5 days after HITB challenge in the spleen, mesenteric lymph...
Table 1. Heteroimmune response to Haemophilus influenzae type b capsular polysaccharide induced by cross-reacting enteric bacteria fed to neonatal rabbits

<table>
<thead>
<tr>
<th>Bacteria fed</th>
<th>No. of animals</th>
<th>No. colonized</th>
<th>Anti-type b (μg of Ab/ml)</th>
<th>Plaque-forming cells</th>
<th>³H incorporated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Spleen</td>
<td>MLN</td>
</tr>
<tr>
<td>Prechallenge (8 weeks)</td>
<td></td>
<td></td>
<td></td>
<td>IgM</td>
<td>IgG</td>
</tr>
<tr>
<td><em>E. coli</em> (Easter)</td>
<td>18</td>
<td>18</td>
<td>0.12 (0-0.95)</td>
<td>1.8</td>
<td>1.5</td>
</tr>
<tr>
<td><em>E. coli</em> (89)</td>
<td>14</td>
<td>11</td>
<td>0.15 (0-0.32)</td>
<td>94</td>
<td>64</td>
</tr>
<tr>
<td><em>B. pumilis</em> (Sh-18)</td>
<td>23</td>
<td>12</td>
<td>0.23 (0-1.1)</td>
<td>0.9</td>
<td>0.2</td>
</tr>
<tr>
<td>Saline (controls)</td>
<td>25</td>
<td>0</td>
<td>1.14 (0-12.1)</td>
<td>36</td>
<td>11</td>
</tr>
<tr>
<td>Postchallenge (5 days)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>E. coli</em> (Easter)</td>
<td>15</td>
<td></td>
<td>1.84 (0.22-10.2)</td>
<td>338</td>
<td>135</td>
</tr>
<tr>
<td><em>E. coli</em> (89)</td>
<td>9</td>
<td></td>
<td>2.74 (0.25-13.9)</td>
<td>647</td>
<td>86</td>
</tr>
<tr>
<td><em>B. pumilis</em> (Sh-18)</td>
<td>16</td>
<td></td>
<td>2.5 (0-10.5)</td>
<td>322</td>
<td>101</td>
</tr>
<tr>
<td>Saline (controls)</td>
<td>16</td>
<td></td>
<td>0.35 (0.12-6.2)</td>
<td>21</td>
<td>14</td>
</tr>
</tbody>
</table>

*Abbreviations: ND, Not done; MLN, mesenteric lymph node; PP, Peyer's patches; Ig, immunoglobulin; Ab, antibodies.

*Many animals in this group had cross-reacting bacteria, especially _S. aureus_, in rectal and nasopharyngeal cultures using antiserum agar. The serum anti-type b antibodies, PFCs (plaques/10⁶ nucleated cells), and AIP (³H incorporation) are expressed as averages. At 8 weeks of age, several rabbits in each experimental group were sacrificed and the remaining animals were injected with live 5 x 10⁶ HITB organisms intravenously. These animals were sacrificed 5 days later for analysis.
nodes, and Peyer's patches in all three fed groups compared to controls. The highest levels of PFCs were observed in the animals fed strain 89. Comparable results were observed for the AIP assay.

**Adult primates.** In the first experiment, 20 adult rhesus, ages 1 to 2 years, were fed 10⁸ strain Easter (Table 2, A). Five controls, housed in a separate room, were fed saline. Only four of the 20 fed animals had a single positive stool culture within the next month. None of the fed animals became ill and their weight gain was comparable to that of the controls for the following 2 months. Most of the animals in both groups had detectable levels of anti-type b antibodies prior to the feeding. The average prefeeding anti-type b antibody level was 0.28 \( \mu g/ml \), which gradually rose in E. coli-fed animals to 0.58 \( \mu g/ml \) during the next 3 months. During this period, the controls changed from 0.10 to 0.36 \( \mu g/ml \). Using a paired t test, antibody values of the fed animals for week 12 were significantly higher than their preimmune levels (\( \Delta = 0.283, \sigma = 0.387, P < 0.01 \) by the two-sided test). The antibody increase of the controls during the same period is not statistically significant (\( \Delta = 0.028, \sigma = 0.111, P > 0.5 \)). However, when compared to the controls, the increase in the fed animals is light and not significant (\( P = 0.16 \)). In the next experiment (Table 2, B), five adult primates were fed 10¹¹ Easter. None of the animals had adverse symptoms such as diarrhea or weight loss. Four of the five fed animals showed positive stool cultures up to 12 weeks after feeding. In contrast to the controls, all of fed animals had at least a fourfold rise in serum anti-type b antibodies. This elevation in anti-type b antibodies in the fed animals was maximum at 3 weeks and slightly declined in 3 months. The controls showed a slight elevation in anti-type b antibodies from a preimmune average of 0.24 to 0.36 \( \mu g/ml \). The antibody increase from the preimmune levels at 12 weeks was significant at \( P < 0.02 \) for both fed and control animals (\( \Delta \) fed = 0.456, \( \sigma \) fed = 0.252, \( \Delta \) controls = 0.094, \( \sigma \) control = 0.049). However, the increase in the fed animals was significantly higher compared to the controls (\( P < 0.02 \)). Two of the control animals showed a cross-reactive E. coli in their stool cultures at 6 to 8 weeks.

The adult primates fed 10¹¹ E. coli strain 89 differed from the animals fed Easter in that they were 8 or more years old and their average prefeeding antibodies were 0.79 \( \mu g/ml \) (Table 2, C). Three of the five fed animals were colonized for 4 to 6 weeks and one of the controls had a cross-reacting E. coli in its stool cultures from week 4 to 6. During this period, it reacted with an anti-type b antibody level from 0.18 to 0.42 \( \mu g/ml \). The fed animals responded from a preimmune level of 1.04 to a peak level at 1 week of 3.52 \( \mu g/ml \). This peak level declined to 2.0 \( \mu g/ml \) at 8 weeks. The controls showed no rise at 1 to 2 weeks and only a slight rise from 0.54 to 0.72 \( \mu g/ml \) after 8 weeks. The antibody increase of the fed animals in this group at 8 weeks is not significant (\( \Delta = 0.715, \sigma = 0.634, P \approx 0.12 \)). However, at the peak of their response, at 1 week there is a significant rise (\( \Delta = 2.215, \sigma = 1.343, P < 0.05 \)). The controls did not show an increase at that time (\( \Delta = -0.288, \sigma = 0.132 \)) and the response of the fed animals compared to the controls is statistically significant (\( P < 0.05 \)). It should be noted that during the course of these two experiments many bacteria with HITB cross-reacting antigens were detected in the nasopharynx and stools of both fed and control groups, which may explain the slight antibody rise observed in the controls.

**Newborn primates.** Four newborns were fed 10⁸ and 14 were fed 10¹¹ strain Easter, and 14 were fed 10¹¹ strain 89. Eight controls were fed saline. As was observed with newborn rabbits and adult primates, no toxic effects as expressed by diarrheal disease, weight loss, or slowed growth and development were observed in either the fed or control groups. Bimonthly nasopharyngeal and rectal cultures of all the animals did not reveal HITB but did show many cross-reactants in nasopharyngeal and/or rectal cultures at least once in the ensuing 4 months after feeding. The most commonly encountered cross-reacting organism was S. aureus.

All newborn primates fed 10⁸ and 10¹¹ Easter and 10 of the 14 animals fed 10¹¹ strain 89 were colonized for 1 to 10 weeks after the feeding. After an initial decline in the level of passively acquired antibodies, there was an anti-type b antibody rise in all the animals commencing at age 3 to 4 months. This age-related development of anti-type b antibody is comparable to that detected for other primates and domesticated swine (unpublished data). Two of the 14 animals fed strain 89 reacted with comparatively high levels of serum anti-type b antibodies (8.9 and 10.4 \( \mu g/ml \)) at 2 to 3 weeks of age. However, there was no difference in the average serum anti-type b antibodies of all groups including the controls at 6 to 12 months of age (average 0.61 ± 0.45 \( \mu g/ml \)).

**Natural occurrence of cross-reacting E. coli in human newborns.** Rectal cultures were taken from 2,627 newborns at their day of discharge from the nursery at Charlotte Memorial Hospital, Charlotte, N.C., from January
Table 2. Serum *Haemophilus influenzae* type b antibodies in adult primates (*Macaca mulatta*) fed cross-reacting *Escherichia coli* strains Easter and 89

<table>
<thead>
<tr>
<th>Expt no.</th>
<th>Dose of bacteria fed</th>
<th>No. of animals</th>
<th>Preimmune</th>
<th>1 week</th>
<th>2 weeks</th>
<th>3 weeks</th>
<th>4 weeks</th>
<th>8 weeks</th>
<th>12 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Easter (10⁹)</td>
<td>20</td>
<td>0.28</td>
<td>0.44</td>
<td>0.44</td>
<td>0.43</td>
<td>0.54</td>
<td>0.46</td>
<td>0.58</td>
</tr>
<tr>
<td></td>
<td>No. colonized</td>
<td></td>
<td></td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Saline</td>
<td>5</td>
<td>0.10</td>
<td>0.19</td>
<td>0.20</td>
<td>0.17</td>
<td>0.26</td>
<td>0.23</td>
<td>0.36</td>
</tr>
<tr>
<td></td>
<td>No. colonized</td>
<td></td>
<td></td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>B</td>
<td>Easter (10¹¹)</td>
<td>5</td>
<td>0.26</td>
<td>0.60</td>
<td>0.64</td>
<td>0.90</td>
<td>0.82</td>
<td>0.75</td>
<td>0.68</td>
</tr>
<tr>
<td></td>
<td>No. colonized</td>
<td></td>
<td></td>
<td>1</td>
<td>3</td>
<td>4</td>
<td>3</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Saline</td>
<td>5</td>
<td>0.24</td>
<td>0.23</td>
<td>0.24</td>
<td>0.22</td>
<td>0.32</td>
<td>0.36</td>
<td>0.36</td>
</tr>
<tr>
<td></td>
<td>No. colonized</td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>C</td>
<td>89 (10¹¹)</td>
<td>4</td>
<td>1.04</td>
<td>3.52</td>
<td>1.59</td>
<td>1.74</td>
<td>1.75</td>
<td>2.0</td>
<td></td>
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<tr>
<td></td>
<td>No. colonized</td>
<td></td>
<td></td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>1</td>
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<tr>
<td></td>
<td>Saline</td>
<td>4</td>
<td>0.54</td>
<td>0.43</td>
<td>0.54</td>
<td>0.69</td>
<td>0.57</td>
<td>0.72</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

*Three groups of adult primates were fed 10⁹ and 10¹¹ strain Easter and 10¹¹ strain 89. Their nasopharynx and stool were cultured for cross-reacting organisms with H1TB antiserum agar. The serum antibodies are expressed as the average and the range of values is in parentheses.*
1972 through March 1974 and plated onto HITB antiserum agar. Cross-reacting E. coli were found in 23 of the cultures (approximately 0.9%). All the organisms were O75 serotype and none of the carriers showed any sign of diarrheal or invasive disease at the time of their discharge from the hospital. No unusual medical findings have been detected in these babies to date. Sixteen of the 23 carriers were available for continued study and serum HITB antibodies were assayed at various intervals up to 2 years. Fifteen of the 16 carriers had detectable HITB antibodies on all occasions. In contrast, 16 of 52 (30%) infants and children, ages 2 to 24 months, did not have detectable HITB antibodies. It should be emphasized that controls were not cultured for HITB or cross-reacting E. coli or other organisms during the study. Their carriage of cross-reacting E. coli is expected to be 1% as in the general population. The difference between the two groups is statistically significant (Yates corrected chi square = 17.11, \( P < 0.005 \)). It is of interest that 4 of the 16 carriers had no detectable cord HITB antibodies. At age 2 to 4 months, their serum HITB antibodies were 0.34, 0.22, 0.32, and 0.21 \( \mu g/ml \), suggesting active synthesis at this age.

DISCUSSION

The concept that "natural" antibodies and, hence, protective immunity to invasive diseases caused by HITB (and possibly to other encapsulated bacteria) may be due to antigenic stimulation by enteric cross-reacting bacteria has gained support from our findings (10, 24, 30, 35). Most adult rabbits and primates (M. mulatta) have serum antiscapsular antibodies without detectable carriage of HITB (24). Rabbits were shown to have a variety of cross-reacting bacteria including E. coli, bacilli, staphylococci, and various gram-negative organisms. The rapid development of antiscapsular antibodies in neonatal rhesus revealed was related to the frequent finding of cross-reacting bacteria in the absence of demonstrable HITB in these animals. In all fed and control animals, stool and nasopharyngeal S. aureus was detected in at least one sample. S. aureus, coagulase positive, contains polyribitol phosphate as a component of its cell wall teichoic acid and has been shown to induce HITB capsular antibodies in rabbits and burros after intravenous injection (7). Obviously, S. aureus, coagulase positive, fed to newborns is not a suitable organism for consideration as an immunizing agent to HITB.

Colonization with cross-reacting E. coli was observed in 0.9% of normal human newborns at 4 days of age. As observed with the animals deliberately fed the cross-reacting bacteria at birth, this natural colonization was transient and seemed to have no effect upon the health and development of the newborn. Thus, consistent with the in vitro assays showing no enteropathogenicity characteristics, naturally occurring or deliberate colonization with high concentrations of these bacteria seems to be harmless. In experiments to be published we have found that stool cultures from healthy adults yield the cross-reacting E. coli O75-K100:H5 with the same frequency as observed in the newborns.

Three parameters of the immune response to the HITB capsular polysaccharide were shown to have been stimulated by neonatal feeding of cross-reacting bacteria. At 8 weeks of age no significant difference could be discerned between experimental and controls. However, 5 days postchallenge with live HITB there was an enhancement of PFCs, AIP, and serum antibody responses in the fed animals. An interpretation of these data is that feeding and colonization activates (primis) susceptible cells that are not detectable with these assays prior to challenge. Injection with a sublethal dose of live HITB resulted in lymphoid cell division and antibody synthesis. Spleen and mesenteric lymph nodes and Peyer's patches responded, suggesting that feeding stimulated localized as well as and splenic immunoglobulin (Ig) M-, IgA-, and IgG-producing cells. Thus, assays of serum anti-type b antibodies may not be a sensitive indication of the HITB immune status of young rabbits and possibly humans. The relative proportion of PFC immunoglobulin classes in the infant is comparable to the reported overall pattern (1, 9).

A comparison between the two cross-reacting E. coli and B. pumilus as potential immunogens reveals differences: the highest rate of colonization was induced by E. coli Easter and the lowest with Sh-18. However, the highest levels as well as the widest range of anti-type b antibodies were observed in the Sh-18 animals. Further studies with combinations of those organisms are planned to determine the most effective heteroimmunogen.

Spontaneous acquisition of cross-reacting bacteria is a probable mechanism for synthesis of natural and protective antibodies to at least several pyogenic bacteria. Gastrointestinal colonization of human newborns by E. coli O83 has been accomplished by neonatal feeding without ill effect by Lodinova et al. (21, 22). Protective immunity to pneumococci was induced by feeding the homologous organism to rats (34). Heteroimmunization to the human blood group B
substance has been achieved by feeding of cross-reacting E. coli to human adults and infants (19, 40). This colonization resulted in the synthesis of serum and intestinal antianti- bodies to the E. coli O antigen earlier than in age-matched controls. The conditions for feeding and colonizing adult human volunteers with pathogenic E. coli have been characterized (11). This information forms the basis for feeding cross-reacting E. coli to assay their heteroimmunogenicity for HTIB in adult humans. Other enteric nonpathogenic bacteria with cross-reacting antigens to pathogenic encapsulated bacteria have been isolated and characterized. Use of these cross-reacting bacteria to feed and colonize newborns may be a general method for preventative immunization against invasive bacterial diseases (19, 21, 22, 34, 35).

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