Anatomical and Immunological Responses of Rabbit Gallbladders to Bacterial Infections

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To study the sequential morphological and immunological response of the rabbit gallbladder to bacterial infection and to compare the inflammatory responses with different pathogens, gallbladders were infected with Streptococcus faecalis and two strains of Escherichia coli, one of which produced enterotoxin. Gallbladder infection was produced either by intravenously injecting bacteria into rabbits with a small liver infarct or by injecting bacteria directly into the gallbladder of normal rabbits. The percentage of gallbladders infected intravenously with a nonenterotoxigenic E. coli strain was 86% at 1 week, 70% at 3 weeks, and 15% at 6 weeks. Epithelial necrosis and leukocyte infiltration were prominent 1 week after infection. At 3 and 6 weeks after infection, there was crypt distortion and increased mucus secretion in the epithelium as shown by periodic acid-Schiff staining. The lamina propria was infiltrated with mononuclear cells, many of which were plasma cells. Myofibroblasts (contractile fibroblasts) were also identified on transmission microscopy. In addition to these changes, toxigenic E. coli produced subepithelial capillary dilation in the villus core. Morphological changes (excluding toxin-associated changes) were related to the duration of infection rather than to the specific species of infecting bacteria. Infected gallbladders studied by immunofluorescence had a greater than 50-fold increase in plasma cells compared with control cells. In addition, the number increased with the duration of infection. Immunoglobulin A cells were the major cell type in gallbladders infected by intravesical injection, whereas immunoglobulin G cells predominated in gallbladders infected intravenously. The gallbladder appears to mount a local immune response to bacterial infection.

Over 400,000 cholecystectomies are performed per year (unpublished data for 1979 from the National Hospital Discharge Survey, National Center for Health Statistics), and 4 to 10% of these operations are due to acute acalculous cholecystitis (16, 43). The significance of this disease is further exemplified in the pediatric age group in which 40% of the cholecystectomies are a result of acute acalculous cholecystitis (17). Classically, acute acalculous cholecystitis was associated with acute infections, in particular, typhoid fever (32), scarlet fever (38), and leptospirosis (2). More recently, it has been encountered in patients with extensive burns and massive trauma in association with bacteremia (31, 37).

There are multiple factors which precipitate the onset of acute acalculous cholecystitis. Glenn suggests that bacteremia, in the presence of associated biliary stasis, may be one of the important etiological factors irrespective of the specific organism or its site of origin (16).

Although the histological changes of acute acalculous cholecystitis in humans have been described, the pathogenic process has not been established. Some animal studies have attempted to examine the sequential response of the infected gallbladder to specific organisms, such as Salmonella typhi (5, 9, 29), Vibrio cholerae (24, 36), or Streptococcus species (33). The histological changes in the rabbit gallbladder infected in typhoid bacilli and Streptococcus species encompass the range of changes reported in the infected human gallbladder (9, 29, 33). In humans, biliary infection is most commonly caused by normal aerobic microflora (3, 26). In these studies, bacterial cholecystitis was produced either by direct injection of bacteria into the gallbladder (by needle or indwelling catheter) (24, 29, 36) or by systemic infection of bacteria in numbers sufficient to produce endotoxin shock (5, 9, 10, 29, 33). The resulting infection was temporary and associated with an

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inflammatory infiltrate of the gallbladder muco-
sa (29, 36).

The morphological changes found in gallblad-
ders infected with either S. typhi, Streptococcus
species, or V. cholerae have been assumed to be
specific for these organisms (27, 29, 33, 36).
However, it is unlikely that the gallbladder mu-
cosa responds specifically to different bacterial
organisms. Rather, the gallbladder, an analog of
the intestine, might respond to bacterial infec-
tion by mounting a local immune response.
Although biliary transport of immunoglobulin A
(IgA) immunoglobulin has recently been dem-
onstrated (4), the ability of the biliary tract itself
to produce a local immune reaction has not been
shown. The morphological events which occur
after bacterial infection of the gallbladder might
be in part related to the stimulation of local
immunity by bacterial antigens.

To further elucidate the sequential changes in
the infected gallbladder, a rabbit model was used
to describe the pathological process and the
immune response of the gallbladder to bacterial
infection. To determine whether the gallbladder
responds stereotypically to bacterial infection,
gallbladders were infected with three bacterial
agents: Escherichia coli, Streptococcus faecalis,
and E. coli H10407, which is enterotoxin pro-
ducing. Because E. coli H10407 simulates chol-
era infection and produces higher infection rates
in our model, it was used to observe changes in
the gallbladder secondary to toxin production.

MATERIALS AND METHODS

Experimental design. Gallbladder infection was
produced by two methods. In method 1, a sterile liver
infarct was produced by cautery and then infected by
an intravenous injection of bacteria. This method
atraumatically produces a high rate of associated gal-
bladder infection and avoids morphological changes in
the gallbladder caused by direct trauma or by endotox-
in shock. This indirect method of gallbladder infection
was used for all of the morphological studies and some
of the immunological studies. Immunological changes
were also examined by method 2, in which gallblad-
ders were infected by direct injection of bacteria into
the gallbladder lumen. Both methods used coccidia-
free New Zealand white female rabbits weighing 1.5 to
2.0 kg.

Liver infarction and intravenous infection. Rabbits
were shaved, pretreated with atropine, and anesthe-
tized with ether before operation. At laparotomy,
electrocautery was performed on a lobe of the liver to
produce a burn 1 cm in diameter. Incisions were
closed by layers with 3-0 chromic suture and skin
staples. After 24 h, an inoculum of bacteria was
injected via the marginal ear vein, as described below
(bacteremia was not present 24 h after inoculation as
evidenced by sterile blood cultures). Animals were
sacrificed after 1, 3, or 6 weeks. This procedure
produced infected bile in most animals. Control ani-
imals were separated into two groups: (i) cauterized
rabbits inoculated intravenously with saline and sacri-
ficed 1, 3, or 6 weeks later, and (ii) sham-operated
(noncauterized) animals injected with a bacterial in-
oculum and examined 1 week later. Rabbits with liver
infarcts who were infected experimentally, but whose
bile was not infected at the time of sacrifice (1, 3, and 6
weeks post-inoculation), also functioned as a control
group. In this group, however, it was not determined
whether biliary infection had never taken place or had
been present and then spontaneously cleared.

Infection by direct gallbladder injection. Each rabbit
was anesthetized with ether, the gallbladder was ex-
posed, and the bacterial inoculum was injected in a
volume of 0.15 ml with a 26-gauge needle. Leakage
was controlled by applying mild pressure. At 15 min
after inoculation, 1 ml of ear blood was taken for
culture. Control rabbits had saline injected into the
gallbladder. The experimental and control rabbits
were sacrificed 1 week after inoculation. Gallbladder
bile was aspirated, and the colony-forming units
(CFU) per milliliter were determined by dilution.

Bacteria. Three strains of bacteria were used: (i) HS,
a noninvasive nontoxicogenic E. coli strain isolated from
normal human feces (11); (ii) H10407, a well-character-
ized strain (11, 39) of enterotoxigenic E. coli (positive
for heat-labile and heat-stable enterotoxin) isolated in
Dacca, Bangladesh, from a patient with diarrhea; and
(iii) S. faecalis, isolated from a patient with enterococ-
cal vertebral osteomyelitis. All bacteria were washed
two times in saline before injection.

Rabbits were infected intravenously with 1-ml vol-
umes of $1 \times 10^9$ to $4 \times 10^9$ CFU of E. coli strains or $1 \times 10^9$ to $2 \times 10^9$ CFU of S. faecalis. Rabbits were
infected by direct injection into the gallbladder with E.
coli H5 at a dose of either $1 \times 10^9$ or $1 \times 10^5$ CFU
in each of four rabbits.

Tissue processing, bacterial quantification, and histo-
pathology. Rabbits were rapidly sacrificed at various
times with intravenous phenobarbital, and tissue spec-
imens were obtained aseptically. After the bile was
aspirated for bacterial culture, the gallbladder was
removed, and a longitudinal section was taken from
the free surface for histology. Sections of liver infarct
and normal liver were dissected and homogenized in
40 ml of saline; 10-fold dilutions were plated on
MacConkey agar and phenylethyl alcohol plates, and
viable bacteria were counted. Gram-negative bacteria
were identified by the triple sugar iron reaction and
serogglutination. S. faecalis colonies were identified by
Gram smear and a positive reaction in Pfizer
selective enterococcus broth.

Gallbladder specimens were cut lengthwise; one
piece was fixed in 10% buffered Formalin, and another
portion was used for fluorescent-antibody studies.
Paraffin sections of Formalin-fixed tissue were then
stained with hematoxylin and eosin or periodic acid-
Schiff. Rabbit granulocytes all have large granules and
stain eosinophilic with hematoxylin and eosin. The
term heterophile, employed by Bloom and Fawcett (6)
to describe rabbit granulocytes, is not used in this paper.

Ten gallbladders, examined by scanning and trans-
mission electron microscopy, were fixed by vascular
perfusion with a solution containing 2% formaldehyde
and 2.5% glutaraldehyde, buffered with 0.04 M potas-
sium phosphate (22). Perfusion fixation maintains the
gallbladder in an expanded state and minimizes mor-
phological artifacts caused by a collapse of the gal-
bladder before fixation (30). After 5 min of perfusion,
RESULTS

Microbiological results. Both infarcted liver tissue and bile obtained from cauterized rabbits inoculated intravenously with saline were sterile. Injection of *E. coli* H10407 into sham-operated noncauterized rabbits frequently produced biliary infection (five of seven rabbits, 71%). However, only one of seven and none of five sham-operated controls inoculated with *E. coli* HS and *S. faecalis*, respectively, had biliary infection.

The number of rabbits with infected bile decreased significantly with time (Table 1). The CFU per milliliter of bile from gallbladders still infected at 3 and 6 weeks were comparable to the CFU per milliliter of bile from infected gallbladders studied over 1 week.

**Histology.** All control animals (saline injected and sham operated) without biliary infection had histologically normal gallbladders. The normal histology of the rabbit gallbladder has been previously well described (20, 41). We also noted droplets positive for periodic acid-Schiff staining in the apex of some epithelial cells. Also, scanning microscopy showed, between the rugal folds, small pits resembling those of the guinea pig gallbladder (Fig. 1).

**Rabbits with infected infarcts and sterile bile.** Gallbladders of rabbits with culture-positive infarcts whose bile contained less than 10 CFU/ml were normal when examined 1 week after inoculation. However, among animals with culture-positive infarcts and noninfected bile, at 3 and 6

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**TABLE 1. Bacterial infection of liver infarcts and gallbladder bile in cauterized rabbits**

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Days of infection</th>
<th>No. of rabbits with infected infarcts/total no. of rabbits</th>
<th>Infected infarct mean bacterial count (log CFU/μl)</th>
<th>No. of rabbits with infected bile/total no. of rabbits</th>
<th>Infected bile mean bacterial count (log CFU/ml)*</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em> HS</td>
<td>7–10</td>
<td>14/14</td>
<td>7.7 ± 0.5</td>
<td>12/14</td>
<td>6.4 ± 1.8</td>
</tr>
<tr>
<td></td>
<td>20–24</td>
<td>13/14</td>
<td>5.1 ± 1.1</td>
<td>11/14</td>
<td>6.5 ± 1.9</td>
</tr>
<tr>
<td></td>
<td>42–44</td>
<td>8/13d</td>
<td>4.5 ± 1.5</td>
<td>2/13d</td>
<td>7.0 ± 0.8</td>
</tr>
<tr>
<td><em>E. coli</em> H10407</td>
<td>7–10</td>
<td>5/5</td>
<td>7.2 ± 0.3</td>
<td>4/5</td>
<td>8.4 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>20–24</td>
<td>2/2</td>
<td>6.3 ± 0.1</td>
<td>2/2</td>
<td>8.2 ± 0.1</td>
</tr>
<tr>
<td><em>S. faecalis</em></td>
<td>7–10</td>
<td>9/11</td>
<td>7.0 ± 1.8</td>
<td>6/11</td>
<td>7.7 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>20–24</td>
<td>10/11</td>
<td>7.0 ± 0.3</td>
<td>4/10</td>
<td>5.2 ± 1.6</td>
</tr>
<tr>
<td></td>
<td>42–44</td>
<td>3/9d</td>
<td>4.5 ± 0.5</td>
<td>1/9d</td>
<td>7.7 ± 1.0</td>
</tr>
<tr>
<td>Infarct alone</td>
<td>7–10</td>
<td>0/5</td>
<td></td>
<td>0/5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20–24</td>
<td>0/4</td>
<td></td>
<td>0/4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>42–44</td>
<td>0/5</td>
<td></td>
<td>0/5</td>
<td></td>
</tr>
</tbody>
</table>

* Sham-operated noncauterized control rabbits intravenously inoculated with bacteria and sacrificed 1 week after infection had the following results: *E. coli* HS, one of seven animals and *S. faecalis*, none of five had infected bile; in contrast, five of seven sham-operated rabbits inoculated with *E. coli* H10407 had infected bile with a mean bacterial count of 7.2 ± 0.5 log CFU ml.
* Values are means ± standard deviation.
* Values are means ± standard deviation of eight rabbits.
* There was a significant difference $P < 0.05$ (chi-square test) between the number of infected infarcts and bile at 7 to 10 and at 42 to 44 days in rabbits inoculated with either *E. coli* HS or *S. faecalis*.
weeks after inoculation, 2 of 8 and 6 of 19 gallbladders, respectively, had mild lamina propria lymphocytic infiltration, increased lymphocyte infiltration, cuboidal metaplasia of the epithelium, and crypt distortion. The gallbladders of the remaining animals resembled those of uninfected controls.

**Rabbits with infected gallbladders.** Gallbladders infected with *E. coli* HS and *S. faecalis* were similar 7 to 10 days after inoculation. Acute inflammatory changes predominated in gallbladders infected for 1 week. Prominent findings were focal necrosis with adjacent cuboidal metaplasia of the normal columnar epithelium (Fig. 2) and marked infiltration of the surface epithelium with heterophiles. Occasionally, the infiltration was severe enough to produce epithelial cysts filled with clumps of eosinophilic leukocytes. There was an increase in droplets positive for periodic acid-Schiff staining in the epithelial cells without marked crypt distortion. In one gallbladder infected with *E. coli* HS, the inflammatory changes were so severe that they were associated with submucosal vessel throm-
FIG. 3. Scanning micrograph of a gallbladder 1 week after infection with *E. coli* HS. The normally confluent hexagonal epithelial cell outline has a cobblestone appearance due to marked variation in the size and shape of the epithelial cells. There is increased pitting and hyperplastic hillocks (H) of the surface epithelium. The villus core is packed with cells (arrow) (×360). Note the contrast with the scanning micrograph of a normal gallbladder (Fig. 1).

bosis with coagulation necrosis of the mucosa. The lamina propria, normally containing only scattered fibroblasts and mononuclear cells, was now infiltrated with heterophiles, mononuclear cells, and plasma cells. This was reflected on scanning microscopy (one gallbladder infected with *E. coli* HS) as marked distension and flattening of the mucosal folds associated with the infiltration of the core of the villus with inflammatory cells (Fig. 3). The following points are of interest to the morphologist. The microvilli were not significantly changed. Electron microscopy revealed cells with the appearance of myofibroblasts in the lamina propria. These cells have previously been reported in contracting granulation tissue (34). Bacteria were not seen in the sections studied by transmission microscopy.

Gallbladders infected with *E. coli* H10407 (an enterotoxin-producing strain) examined 1 week after inoculation had the changes described above, but in addition, were characterized by marked enlargement of subepithelial capillaries with interstitial edema of the villi (Fig. 4). The enlarged capillaries did not appear damaged. On scanning microscopy, this was reflected as tensely swollen rugae in which the epithelial cells were stretched out, flattened, and whose intervening cell margins were poorly defined. Further detail showed the presence of increased pits and normal microvilli. Transmission elec-
FIG. 5. Scanning micrograph of a gallbladder 3 weeks after infection with E. coli HS. An immature lymphoid follicle (L) is present in the lamina propria and intestinal metaplasia of the epithelium (arrow) is present (×180).

Transmission micrographs (not shown) demonstrated an increased number of apical epithelial mucus droplets and widened lateral intracellular spaces, a condition observed in gallbladders which are actively transporting fluid (12, 23).

At 3 weeks after inoculation, gallbladders infected with S. faecalis, and E. coli HS no longer had epithelial necrosis. The predominant changes were evidence of increased mucus secretion and an inflammatory infiltrate. This infiltrate was more intensive and contained a greater proportion of mononuclear cells compared with the infiltrate of gallbladders infected for 1 week. Epithelial hyperplasia and crypt distortion was noted (Fig. 5). Epithelial cells filled with periodic acid-Schiff-stained granules were especially prominent in the epithelial crypts with intestinal metaplasia. Intraepithelial leukocytes were increased as were plasma cells and other mononuclear cells in the lamina propria. The muscularis and submuscularis were thickened and showed increased mononuclear infiltration. As noted at 1 week, the myofibroblasts in the lamina propria were still present on electron microscopy (Fig. 6).

In gallbladders infected with E. coli H10407, marked capillary distension remained 3 weeks after inoculation, but was less prominent than after 1 week. Except for the increase in subepithelial capillary size, gallbladders infected with E. coli H10407 were similar to E. coli HS-infected gallbladders (Fig. 7).

Rabbits with infected bile at 6 weeks showed pathological changes similar to the rabbits with infected bile at 3 weeks. However, there was increased crypt distortion and intestinal meta-

FIG. 6. Transmission electron micrography of the lamina propria of a gallbladder examined 3 weeks after infection with E. coli HS. Two myofibroblasts (M) containing numerous fine filaments and dense bodies are present and appear to be contracting as evidenced by the irregular wavy cell outline (×1,030).
plasia, a higher degree of lymphocytic infiltration in the walls of the tissue, and more extensive lymphoid follicle formation in the subepithelial layers in the rabbits with infected bile at six weeks. Also, perivascular lymphocytic cuffing of blood vessels and lymphocyte epithelial transmigration were more prevalent than observed in the rabbits with infected bile at 3 weeks.

**Fluorescent-antibody studies from intravenously infected rabbits.** No differences in counts of antibody-containing cells were found between specimens immediately fixed and those washed for 18 h and then fixed. These results have been combined and treated as a single unit. Gallbladders from cauterized controls injected with saline had very few cells containing IgG, IgA, and IgM. Similar results were found in noninfected gallbladders of sham-operated rabbits injected intravenously with *E. coli* or *S. faecalis* (Table 2). Four rabbits intravenously injected with *E. coli* HS had culture-positive liver infarcts with culture-negative bile (noninfected gallbladders); gallbladders from these animals had few plasma cells. Increased counts occurred as the interval between inoculation and sacrifice increased.

All intravenously infected gallbladders had high counts of immunoglobulin-containing cells. Gallbladders from rabbits with bile and the infarct cultures positive for *E. coli* had a significantly increased plasma cell population compared with noninfected controls (Table 2). The number of IgG cells exceeded the number of IgA cells, and the number of IgM cells was the smallest. The number of plasma cells of all immunoglobulin types was higher 3 weeks after infection than 1 week after infection. Gallbladder IgG/IgA cell ratios did not significantly change with an increased duration of infection.

No correlation was found between serum antibody titers of *E. coli* HS and the number of plasma cells present in gallbladders infected with this organism. Six rabbits with bile infected with *E. coli* HS, sacrificed 1 week after inoculation, had a geometric mean serum antibody titer to *E. coli* of $1:320 \pm 1:24$. A similar group of eight rabbits with infected bile sampled 3 weeks after inoculation had a mean serum antibody titer of only $1:38 \pm 1:38 (P < 0.01)$. Saline-injected controls and rabbits with infected infarcts but uninfected bile were examined 6 weeks after inoculation. Both groups had mean serum antibody titers below 1:20.

*S. faecalis*-infected gallbladders also had a
predominance of IgG-containing cells. Total counts tended to be lower than rabbits infected similarly with *E. coli*.

Fluorescent-antibody studies from intraluminally infected gallbladders. In animals given 1 × 10⁶ CFU of *E. coli* HS intravesically, bacteremia (>10⁵ CFU/ml) was present in all animals sampled 15 min after injection. In the lower dose group, 5 × 10⁵ CFU of, only two of four rabbits were bacteremic 15 min post-inoculation. Both of these rabbits had less than 15 CFU of bacteria per ml cultured from their blood. No difference was found in the number or the proportion of immunoglobulin cell types in the gallbladders of rabbits infected with the high- or low-dose inoculum.

At 1 week post-infection with *E. coli* HS, gallbladders infected intravesically had more IgA and fewer IgG cells than did gallbladders infected by the intravenous route (Table 2). IgA cells were the major immunoglobulin cell type in gallbladders infected by intravesical injection, whereas IgG cells predominated in gallbladders infected intravenously (Table 2).

**DISCUSSION**

Gallbladders were infected without direct trauma or systemic shock by first infarcting the liver before intravenous injection of bacteria. Confusion caused by anatomical changes in the gallbladder occurring during the production of infection was thereby avoided. This model also allowed biliary colonization to occur with species and strains of bacteria, such as *E. coli* HS and *S. faecalis*, which normally would not produce biliary infection after a nonlethal dose.

Gallbladders were infected with *S. faecalis*, *E. coli* HS, and *E. coli* H10407, which produces enterotoxin. The same histological changes, except for those related to toxin production, were observed with each bacterial species. Although not reported here, infection with other bacterial species and strains (*S. typhi*, *V. cholerae*, and three strains of *E. coli*) produced similar changes. The observed histological and morphological changes were consistent with changes which occur secondary to inflammation. In addition, the gallbladder appears to respond to bacterial infection with a local immune response which probably accounts for the uniformity of the observed histological change.

Specific histological changes were related to the duration of infection and not to the type of bacterial agent. Initially, all three bacterial agents produced epithelial necrosis and a heterophilic inflammatory response. The pathological lesions observed at 3 and 6 weeks were similar and were associated with (i) distorted crypts with increased mucus secretion, and (ii) mononuclear cell infiltration. Gallbladders infected with an enterotoxigenic producing strain of *E. coli* also had subepithelial capillary enlargement and villus edema.

Like Wienstock and Bonneville (41), we saw apical secretory vesicles in normal rabbit epithelial cells; other investigators have noted their absence (20). Gallbladders examined 3 and 6 weeks after infection had a marked increase in mucus secretion compared with the mucus se-

<table>
<thead>
<tr>
<th>GROUP</th>
<th>INOCULUM*</th>
<th>ROUTE</th>
<th>NO. OF RABBITS SAMPLED</th>
<th>CULTURE OF INFRACT</th>
<th>CULTURE OF BILE</th>
<th>MEAN NO. OF CELLS PER FIELD (×400)</th>
<th>MEAN IGG/IgA RATIO†</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL</td>
<td>Saline</td>
<td>Systemic</td>
<td>4</td>
<td>−</td>
<td>−</td>
<td>1.0</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td><em>E. coli</em> HS</td>
<td></td>
<td>5</td>
<td>NA</td>
<td>0.4</td>
<td>2.8</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td><em>E. coli</em> HS</td>
<td></td>
<td>2</td>
<td>+</td>
<td>0.4</td>
<td>0.8</td>
<td>0.4</td>
</tr>
<tr>
<td>INFECTED BILE</td>
<td><em>E. coli</em> HS</td>
<td>Systemic</td>
<td>14*</td>
<td>+</td>
<td>+</td>
<td>77 ± 56 16 ± 17</td>
<td>1.95 ± 1.13</td>
</tr>
<tr>
<td></td>
<td><em>E. coli</em> HS (1 × 10⁵)</td>
<td></td>
<td>4</td>
<td>+</td>
<td>45 ± 28 19 ± 17</td>
<td>0.66 ± 0.28</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>E. coli</em> HS</td>
<td>Intravesical</td>
<td>4</td>
<td>NA</td>
<td>58 ± 13 94 ± 20</td>
<td>0.64 ± 0.18</td>
<td></td>
</tr>
</tbody>
</table>

* *E. coli* HS, except where noted, was injected at a concentration of 1 × 10⁶ CFU. All rabbits were sampled 1 week after infection.

† Values are means ± standard deviation.

‡ The ratio of IgG to IgA plasma cells for each gallbladder was calculated, and the mean ratio ± standard deviation of each group of gallbladders was then determined. The ratio of IgG to IgA for gallbladders inoculated intravesically with *E. coli* HS is 0.65 ± 0.22. This ratio is significantly lower (p < 0.05) than the ratio 1.95 ± 1.13 found in gallbladders of rabbits inoculated with *E. coli* HS systemically and sacrificed at 1 week.

‡NA. Not applicable.

© Includes two rabbits with liver infarcts who were intravenously injected with 5 × 10⁵ CFU of bacteria on the day of operation.

ND, Not done.
cretion from noninfected gallbladder. This reaction may be a nonspecific response to inflammation.

Myofibroblasts present in contracting granulation tissue (15, 34) were seen in infected gallbladders and appear to be part of the inflammatory response of the gallbladder.

Gallbladders infected with E. coli H10407 and V. cholerae (personal observation) produced secretory changes that have also been described in gallbladders actively transporting fluid (23). Cholera toxin injected into rabbit gallbladders activates adenyl cyclase and increases the transport of water and solute (28). Presumably, the same mechanism is responsible for the changes seen in gallbladders infected with E. coli H10407.

Bacterial infection or immunization of the gallbladder may provide a useful model in which to study local immunity. The normal (coccidia-free) rabbit gallbladder and its effluent bile are sterile. The mucosa contains only a few plasma cells, and as a result of this low cellular background, changes in the population of plasma cells due to immunization or infection are readily visible and easily measured.

The rabbit gallbladder appears to respond to bacterial infection with a local immune response. Evidence of this is the sequence of the inflammatory reaction, the presence of IgA plasma cells, the development of lymphoid follicles and lymphocytic cuffing around vessels, and the uniformity of the pathological response of gallbladders to different bacteria.

Bacterial infection provoked a vigorous plasma cell response in the gallbladder mucosa. Gallbladders infected by intravenous inoculation had more IgG than IgA plasma cells. In contrast, IgA plasma cells predominated in gallbladders infected intravesically.

Antigen applied to mucosal surfaces stimulates a local immune response which is primarily IgA in character (40). Rabbits with infected gallbladders had a proliferating antigen (bacteria) in contact with the gallbladder epithelium, resulting in an increase of IgA plasma cells. In chronic nonbacterial cholecystitis of humans, most plasma cells found in the gallbladder are also of the IgA type (8, 18).

We also found an increase in IgG cells in the gallbladders of rabbits infected by both the intravenous and intravesical routes. Systemic bacteremia and probable sensitization of the spleen and other nongut lymphoid tissue followed injection with both methods. It was not determined whether IgG cells in the gallbladder originated there or in peripheral lymph nodes and the spleen.

Biliary infection was short-lived and decreased sharply by 6 weeks. Similar results have been found when S. typhi or V. cholerae were used to infect normal rabbit gallbladders (29, 36).

The mechanism by which the biliary tract clears bacteria is, however, unclear. In rats, immunization of the intestine with bacteria led to the production of specific IgA antibody which was transported across the rat hepatocytes and then concentrated in the bile (19). IgA has been shown to interfere with the attachment of bacteria to epithelial receptors (42) and to facilitate bacterial agglutination (14). Also, as suggested by this study and studies with V. cholerae (24), biliary clearance of bacteria may occur through the development of a local immune response and the associated production of specific antibody. Evidence for the latter mechanism is the demonstration by immunofluorescence of plasma cells in E. coli HS-infected gallbladders which specifically bind E. coli HS antigen (personal observation). Whether either of the above mechanisms is responsible for biliary bacterial clearance still remains to be demonstrated.

As the reaction of the normal rabbit gallbladder to infection has been described, it is now possible to use this model to investigate the more complex interrelationship of calculi, infection, and the consequent immunopathology.

ACKNOWLEDGMENTS

The advice of D. Nalin, R. B. Sack, N. Pierce, F. Koster, and M. Schuster is appreciated. Additional support for this study came from K. Schutz and J. B. Phu and D. Sara.

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