PATHOGENESIS OF HEPATIC GRANULOMAS IN TURKEYS INFECTED WITH STREPTOCOCCUS FAECALIS: VAR. LIQUEFACIENS

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Received 8 February 1971

SUMMARY

The pathogenesis of hepatic granulomas in turkeys has been studied by reproducing the lesions experimentally with Streptococcus faecalis var. liquefaciens isolated during a field outbreak of turkey hepatic granulomas in Colombia. The 170 turkey poults (Bronze) used were 4 weeks old. Groups of poults were inoculated intravenously or orally with 0.1 ml of a 24-hour culture of Str. faecalis var. liquefaciens at a dilution of $3 \times 10^2$ on the MacFarland Nephelometer Standard 10. The oral route of inoculation reproduced a disease most similar to the naturally occurring disease.

Clinically, the acute phase of infection was characterized by a high mortality rate in the first to seventh days but only sporadically thereafter. The septicemic phase produced the formation of septic thrombi which localized in various organs, producing infarction with heterophilic infiltration. Once the septicemic phase of the problem passed, the disease was manifested primarily as hepatic granulomas. The subacute and chronic phase was characterized by a focal hepatitis initiated primarily as a focal necrotic cholangial lesion. The biliary epithelium had hyperplastic to degenerative processes which participated in the formation of biliary thrombi. Granulomas were characterized by focal areas of necrosis surrounded by Langhans-type giant cells, and macrophages.

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INTRODUCTION

Streptococcal infections in turkeys are considered a limiting factor in established turkey-producing countries as well as in countries where the poultry industry is developing. The primary losses are attributed to a high mortality rate in poults and a loss in reproductive capacity of adult turkeys when the granulomatous processes localize in the reproductive organs. Also contributing to the overall loss are carcass and visceral condemnations. The significance of *Streptococcus faecalis* in the pathogenesis of lesions in birds is still uncertain. As a result, studies were initiated to determine the pathogenesis of the granulomatous lesions in turkeys (Fig. 1) initiated by a strain of *Streptococcus faecalis* var. *lactis* isolated in pure culture from infected turkeys in a field outbreak in Colombia. This outbreak produced a mortality rate of 20%. With this strain, the disease has been reproduced experimentally in turkeys (Fig. 2).

LITERATURE REVIEW

The literature is scant on streptococcal infections of turkeys. The major portion of the work reviewed herein is directed toward the pathogenesis of the granulomas initiated by mycobacteria and fungi in the viscera of birds.
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Volkmar (22) stated that most of the streptococcal diseases of turkeys are chronic and that the bacteria, when grown in vitro, rapidly loses its virulence. He also considered that rodents could be natural reservoirs of the disease. Oehme (18) and Magnuson, cited by Volkmar (16), mentioned that streptococcal diseases in poultry are produced by heterogeneous nonpathogenic species of streptococci which become virulent when introduced into birds. Gross and Domermuth (9) reproduced valvular endocarditis in chickens and turkeys with Staphylococcus aureus or Pasteurella multocida isolated from naturally occurring cases and with strains of Str. faecalis isolated from the intestines of apparently normal chickens. They stated that the birds survived long enough to develop necrotic foci with a heavy accumulation of heterophils surrounded by macrophages followed by giant-cell formation. They found that 3 bacterial species produced the same lesion histologically and that the "environment" could be a more important factor in the development of the lesions than the bacterial species isolated.

![Liver from a turkey inoculated orally with an extract from a hepatic granuloma obtained from a turkey in a field outbreak (68P234). Str. faecalis var. liquefaciens was isolated from this lesion. Note caseous necrosis (A), zone of histiocytes (B) zone of lymphocytes (C). Four weeks postinoculation. H & E, 50x.](image-url)
Table 1. Percentage composition of the diets used in a study of hepatic granulomas in turkeys.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Diet A</th>
<th>Diet B*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>44.40</td>
<td>61.30</td>
</tr>
<tr>
<td>Sesame meal</td>
<td>14.00</td>
<td>9.80</td>
</tr>
<tr>
<td>Soybean oil meal</td>
<td>34.00</td>
<td>33.50</td>
</tr>
<tr>
<td>Fish meal</td>
<td>2.00</td>
<td>4.00</td>
</tr>
<tr>
<td>Alfalfa meal</td>
<td>6.00</td>
<td>2.00</td>
</tr>
<tr>
<td>Bone meal</td>
<td>1.20</td>
<td>1.20</td>
</tr>
<tr>
<td>Chlorm carbonate</td>
<td>0.40</td>
<td>0.80</td>
</tr>
</tbody>
</table>

Table legend: *Sterile diet.

Jortner and Helmboldt (12) used various intravenous doses of *Str. faecalis* isolated from a natural case of valvular endocarditis to reproduce the disease. From 16 of 30 adult chickens, Domermuth and Gross (7), in discussing the pathogenicity of *Str. faecalis*, reported that it is primarily pathogenic for poultry, causing an acute septicemia in young birds and a bacterial endocarditis in adult birds. They stated that birds inoculated with *Str. faecalis* var. *liquefaciens* died between 5 and 21 days postinoculation (average 14 days).

Moore and Gross (17) showed that a gram-positive filamentous strict anaerobic bacillus, classified as a member of the genus
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*Catenaabacterium*, was the causative agent of hepatic granulomas in turkeys. They considered *Str. faecalis* var. *liquefaciens* as an assisting agent. Hepatic granulomas were produced by intravenous injection of *Catenaabacterium*, or by oral administration of both *Catenaabacterium* and *Str. faecalis* var. *liquefaciens*. The authors mentioned that, with the oral route of inoculation, the intestinal lesion produced by *Str. faecalis* was necessary for the invasion of *Catenaabacterium*. They could not reproduce the granulomas when only *Catenaabacterium* was used with the intestine intact.

**MATERIALS AND METHODS**

One hundred and seventy Bronze poults were hatched, maintained in brooder cages in isolation units, and fed Diet A (Table 1) until 4 weeks old. Then they were changed to a sterile Diet B (Table 1) and inoculated by various routes with a strain of *Str. faecalis* var. *liquefaciens* isolated from hepatic granulomas in turkeys during a field outbreak. The strain used possessed the cultural and biochemical characteristics mentioned in Bergey's Manual (3). All poults were then maintained for the duration of the experi-

![Fig. 4. Liver of a poult inoculated orally with *Str. faecalis* var. *liquefaciens* and killed 2 weeks PI. Granulomas (arrow).](image-url)
ment in batteries within isolation units in the Laboratorlo de investigaciónes Medicas Veterinarias (LIMV-ICA), Bogota, Colombia.

Because the organism might possibly lose its virulence in culture media, it was maintained and the pathogenicity increased by making 6 subcutaneous serial passages in turkeys before each experimental inoculation in the initial and pathogenesis studies.

For the initial study, 30 pouls were divided into 3 groups: Group 1, intravenous inoculation, 10 pouls; Group 2, oral inoculation, 10 pouls; and Group 3, uninoculated controls, 10 pouls. The 20 inoculated pouls received 0.1 ml of a 24-hour culture of Str. faecalis var. liquefaciens standardized to $3 \times 10^8$ against the MacFarland Nephelometer Standard 10 (2). Groups of pouls were killed at various intervals to determine the most appropriate experimental design.

For the pathogenesis study, 140 pouls were divided into the following groups: Group 1, twenty pouls killed immediately to eliminate the possibility of previous granulomatous processes;

Fig. 5. Inflammatory reaction in a liver of a poult inoculated orally with Str. faecalis var. liquefaciens and killed 6 days Pl. Macrophages and numerous cells with various nuclear degenerative changes. H & E, 800x.
Group 2, intravenous inoculation, 30 poults; Group 3, one oral inoculation, 30 poults; Group 4, repeated oral inoculation every 2 days, 30 poults; and Group 5, uninoculated controls, 30 poults. The 90 uninoculated poults received 0.1 ml of a 24-hour culture of *Str. faecalis* var. *liquefaciens* diluted $3 \times 10^6$ on the MacFarland Nephelometer Standard 10.

After inoculation the turkeys were examined daily. All poults were killed by separating the atlanto-occipital articulation. Samples for aerobic culture on blood agar were collected from the liver, bile, spleen, and granulomas. All granulomas were cultured anaerobically on blood agar in Brewer anaerobic cylinders. Three (3) poults from each of Groups 2, 3, 4, and 5 were killed weekly for 10 weeks. Tissues for histopathology were collected from lungs, heart, proventriculus, ventriculus, duodenum, pancreas, ileum, liver, spleen, kidney, bursa of Fabricius, and brain, fixed in neutral buffered 10% formalin, and processed by the paraffin technique. Sections were cut at 4 µ and routinely stained with hematoxylin and eosin (H & E). Selected sections were stained with Brown-Brenn Gram stain for bacteria (13).

Fig. 6. Liver from a poult inoculated orally with *Str. faecalis* var. *liquefaciens* and killed 3 weeks PI. Biliary thrombi (arrow) with hydropic degeneration of the surrounding hepatic cells. H & E, 800X.
RESULTS

Initial study. To reproduce the disease, the initial study demonstrated that it was necessary to increase the virulence of the organism by making six rapid serial passages in turkeys using a dose of 0.1 ml dilution of $3 \times 10^8$. The first trial with the dose of 0.1 ml dilution of $3 \times 10^8$ gave insignificant results.

PATHOGENESIS STUDY

The 20 uninoculated poultcs (100%) killed were bacteriologically negative and were histopathologically negative for granulomatous processes. *Str. faecalis* var. *liquefaciens* was not isolated from any control animal.

*Str. faecalis* var. *liquefaciens* was recovered from the liver, spleen, and bile of 90% of the inoculated turkeys. Other than the *Str. faecalis* isolated, all aerobic cultures from granulomatous lesions were negative.

For clarity and brevity the periodic sampling periods are described under the acute, subacute, and chronic phases, which re-

![Liver of a poult inoculated orally with *Str. faecalis* var. *liquefaciens* and killed 3 weeks PI. Note the affected biliary (A) and vascular (B) structures. H & E, 800X.](image-url)
spectively correspond to 1 week postinoculation, 2 and 3 weeks postinoculation, and 4 to 10 weeks postinoculation.

**Acute phase. Clinical manifestations.** The turkeys were depressed, anorrhectic, shaking, and hovering in the corner of the cage. The feathers were ruffled and had lost their brilliance. Some turkeys had movements of the head suggesting involvement of the central nervous system.

Death loss was greatest on the 6th day postinoculation. Thereafter, losses were sporadic, with the surviving turkeys somewhat depressed.

**Gross lesions.** There was congestion of the subcutaneous tissues and serous membranes. The liver was enlarged and had a greenish discoloration. The pericardial sac was congested, and a fibrinous pericarditis was observed in 2 turkeys. The spleen was always enlarged.

**Microscopic lesions.** The liver was congested, and the hepatic sinusoids were dilated with erythrocytes and heterophils. Bacterial colonies were also observed in areas of heterophil accumulations.

Fig. 8. Liver from a poult inoculated orally with *Str. faecalis* var. liquefaciens and killed 8 weeks PI. Note hydropic degeneration of biliary epithelium. H & E, 800X.
Scattered throughout the parenchyma were necrotic foci with an associated acute inflammatory reaction (Fig. 3) and hyperplasia of Kupffer cells. The bile canaliculi were thrombosed with fibrin and cellular debris, accompanied by an acute pericanalicular hydropic change. The spleen was congested, and hyperplasia of the reticuloendothelial cells was prominent.

The heart was congested and had hemorrhagic foci. In some cases, fibrinous exudate was observed on the superficial surface of the pericardium, and in other cases infarction produced by septic thrombi was found. The central nervous system had focal areas of congestion and hemorrhage. In one case there was a large hemorrhagic infarction. In two cases there was a meningitis composed primarily of heterophils. The inflammatory reaction of the meninges had penetrated into the brain, producing a distinct perivascular cuffing. Satellitosis and neuronophagia were observed along with the heterophilic meningoencephalitis. Congestion and focal hemorrhages were found in the lungs and kidneys.

Subacute and chronic phase. Clinical manifestation. No alterations were observed clinically.

Fig. 9. Liver from a poult inoculated orally with *Str. faecalis* var. *liquefaciens* 3 weeks PI. Focal hydropic degeneration, necrosis and thrombi (arrow). H & E, 800X.
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**Gross lesions.** The liver was normal in size and color but had whitish foci of varied size throughout the liver (Fig. 4). The largest focal lesion was approximately 1 cm in diameter.

**Microscopic lesions.** The primary lesion of the liver was hyperplasia of lymphocytes in the focal lymphatic nodules. Some nodules contained epithelioid cells, while in some a necrosis was beginning (Fig. 5). The bile ducts in many areas were obstructed with fibrin and cellular debris adjacent to (Fig. 6) and in the larger obstructions. There was hyperplasia of the biliary epithelium, with hydropic degeneration of the hepatocytes (Figs. 7, 8). Found also in the parenchyma were hydropic degeneration (Figs. 6, 9) and focal areas of coagulation necrosis with an inflammatory filtrate composed primarily of heterophils. Most of the necrotic masses were ringed by zones of giant and epithelioid cells. In many of these lesions it was possible to distinguish the participation of the focal lymphatic nodules (Figs. 10, 11). No fibrous capsule formation was observed.

The spleen of all inoculated turkeys had hyperplasia of the...

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**Fig. 10.** Liver from a poult inoculated orally every 2 days with Str. faecalis var. liquefaciens and killed 2 weeks PI. Note the heterophil infiltration and giant cells. H & E, 820X.
reticuloendothelial cells. In the heart and spleen there were granulomas similar to those described in the liver. Sections with granulomas were stained with Brown and Brenn stain, demonstrating the presence of a gram-positive coccus consistent with \textit{Str. faecalis} (Fig. 12).

Table 2 presents the gross and microscopic lesions obtained from 140 turkeys. Incidence of granulomas differed markedly between the oral route and intravenous routes of inoculation. Granulomas were found consistently when the oral route was used.

**DISCUSSION**

The acute focal necrotizing hepatitis and focal hyperplasia of Kupffer cells in the intravenously inoculated turkeys appeared not to progress to a granulomatous reaction, whereas in the orally inoculated turkeys the granulomatous reaction was marked (Table 2). This suggests that \textit{Str. faecalis} needs some other factor or factors present in the intestinal tract in order to stimulate the production of hepatic granulomas. In all probability, the natural

![Liver from a poult inoculated orally with Str. faecalis var. liquefaciens and killed 2 weeks PI. Note the granuloma formation accompanied by hyperplasia of the lymphatic tissue. H & E, 820x.](image-url)
Table 2. Percentage of hepatic granulomas observed in 140 poult's used in the pathogenesis study of hepatic granulomas of turkeys produced by *Streptococcus faecalis* var. *liquefaciens*.

<table>
<thead>
<tr>
<th>Route of inoculation</th>
<th>Gross lesions</th>
<th>Microscopic lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial sacrifice</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Intravenous</td>
<td>0 (0%)</td>
<td>1 (8.3%)</td>
</tr>
<tr>
<td>One oral inoculation</td>
<td>14 (80.0%)</td>
<td>19 (86.8%)</td>
</tr>
<tr>
<td>Repeated oral inoculation</td>
<td>7 (38.8%)</td>
<td>15 (80.0%)</td>
</tr>
<tr>
<td>Control</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

The route of infection is the oral route. The difference in incidence of lesions observed with repeated oral or single oral inoculations is difficult to explain. Just why the percentages of lesions produced was higher with one inoculation than with repeated inoculation is not known.

Because of the possibility that *Str. faecalis* in culture loses its pathogenicity (22), rapid subcutaneous serial passages in

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Fig. 12. Liver from a poult inoculated orally on days one and three with *Str. faecalis* var. *liquefaciens* and killed 4 days PI. Note macrophages with phagocytosed streptococci (arrow). Brown and Brenn, 800X.
turkeys were made to maintain the pathogenicity. This possibly was one of the primary factors responsible for the success of granuloma reproduction in turkeys. We are in agreement with Domermuth and with Oehme (18) and Magnuson (16), who stated that the streptococcal diseases in birds are produced by various streptococci after they become pathogenic within the bird. The manner in which this change in the streptococci is produced is not a part of this study, but the necessity of an enterohepatic route is obvious. Once the streptococci arrive at the liver, they are either phagocytosed by the Kupffer cells or they gain entrance to the biliary circulation where the biliary thrombi are produced. The possibility also exists that an ascending infection by the streptococci occurs, producing later biliary thrombi.

In the work of Moore and Gross (17), the causative agent of hepatic granuloma was thought to be Catenabacterium. They could not reproduce the disease via the oral route, which is felt to be explained as a necrotic hepatitis produced by an anaerobe which formed the initial lesion.

The initial lesion in the acute phase of the granuloma is a focal necrotic area which may become: 1) focal necrosis of the hepatocytes produced by the action of toxins and/or bacterial enzymes; or 2) indirect focal hepatic necrosis produced by biliary thrombi or proliferative-degenerative process (4,5,6,10,11,14,15,20) resulting from either biliary localization from the hepatic circulation or ascending infection.

The focal necrotic areas in the liver are rapidly infiltrated by primarily heterophils (Fig. 1), and at times the necrotic tissue is circumscribed by a zone of histocytes and giant cells around the areas of necrosis (8,9,21). In turkeys, this is probably a characteristic reaction as giant cell formation is very rapid. In this study, giant cell formation was observed 4 days postinoculation. Focal areas of necrosis were immediately surrounded by multinucleated Langerhans-type giant cells and lymphocytes. It is our opinion that the primary initial lesion was produced by the inoculated Str. faecalis var. liquefaciens.

The proliferative and degenerative lesions of the bile ducts could be produced by a virulent organism eliminated by this route especially since Str. faecalis was isolated from the bile in 90%
Granulomas from *Streptococcus faecalis* of the inoculated turkeys. This is in agreement with the hypothesis of Popper (19), who considered that the ductal epithelial reaction could be caused by an antigenically abnormal bile and recognized as an irritant. Such could be the case in the present study.

The mechanism of bacterial entrance into the biliary circulation has not been determined; the close proximity of blood vessels and bile ducts may play an important part (Fig. 5). The degenerative and proliferative lesion of the bile duct as shown in Figs. 5 and 6 may terminate in necrosis, constituting the initial inflammatory lesion which initiates the granulomatous process. It is suggested in this study that there is direct participation by the bile ducts in the degenerative processes.

When Figs. 5, 6, 7, and 8 are examined it is appreciated that biliary obstruction is followed by hydropic degeneration of the surrounding hepatocytes (1,14) and other types of hepatocellular degeneration such as hyaline degeneration (4,6,10,11), which later necrose (4,5,11,14,20). If we consider the concept that bile can have a cytotoxic action, caused by the primary protein denaturation of the hepatocytes and/or bile regurgitation, producing necrosis by the bile salts (5,6,10,14), we have a new factor to add to the process of necrosis. Also, Benacerraf (1) mentioned that the action of bacterial endotoxins appeared to be toxic for Kupffer cells, producing an acute inflammatory response. This response would then proceed to a granulomatous reaction, either by the action of the products of inflammation or an immunologic mechanism or possibly a combination of both.

It is understood that use of the word pathogenesis implies more areas of study than we have covered. With the present morphologic study several possibilities of a lesion mechanism have been presented which warrant further detailed study of streptococci in turkeys.

REFERENCES


