COMPARATIVE PATHOGENICITY OF ACTINOMYCES SPECIES IN MICE

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SUMMARY. The comparative pathogenicity of different species of Actinomyces was studied in a susceptible weanling-mouse model. After the intraperitoneal injection of strains of Actinomyces israeli, A. naeslundi, A. viscosus and Arachnia propionica, numerous abscesses developed in the intestine, mesentery, liver, and at the site of injection. Lesions were not produced by A. odontolyticus. A. naeslundi and A. viscosus produced acute lesions that resolved after a few weeks. Abscesses produced by rough strains of A. israeli and Arach. propionica persisted and led to a slowly progressive chronic infection. Viable organisms were always recovered from the lesions. Spread of the lesions by extension into other areas, including the thoracic cavity, led to the death of the animal after approximately 1 year. This study demonstrated a clear difference in the pattern of infection produced by the different species of Actinomyces as well as Arach. propionica.

INTRODUCTION

Actinomyces israeli and A. bovis are generally considered to be the principal aetiological agents of classical actinomycosis in man and cattle respectively (Slack and Gerencser, 1975). However, there is evidence indicating that other species of Actinomyces and actinomyces-like organisms can cause infections of this type. A. naeslundi, A. viscosus and A. odontolyticus as well as Arachnia propionica have been isolated from human actinomycotic lesions (Buchanan and Pine, 1962; Coleman, Georg and Rozzell, 1969; Georg et al., 1972; Mitchell, Hintz and Haselby, 1977). In addition, these other species can induce experimental actinomycosis in certain laboratory animals (Coleman and Georg, 1969; Georg and Coleman, 1970).

Brown and von Lichtenberg (1970) studied the pathogenicity of A. israeli in susceptible weanling mice. They observed a pattern of infection in this experimental model that was similar to the course of other chronic infections. Initially there was an acute phase of growth and expansion of the lesions, which lasted for 1–6 weeks. This was followed by a static period during which some of the animals aborted the infection. Most of the animals then entered a prolonged chronic phase characterised by host–parasite balance with slow growth of the lesions. Previously, Geister and Meyer
M. J. BEHBEHANI AND H. V. JORDAN

(1951) had described a similar pattern of infection for experimental actinomycosis in mice.

The purpose of the present investigation was to study the comparative pathogenicity of different species of oral Actinomyces by means of the susceptible weanling-mouse model. This work was based on the premise that differences between the species would become more evident if considered in relation to the pattern of experimental infection described above for the prime pathogen, A. israeli.

MATERIALS AND METHODS

Animals. Male, CD-1, Swiss mice, approximately 21 days old and weighing 10–15 g were obtained from Charles River Breeding Laboratories (Wilmington, MA). The animals were housed in polypropylene mouse cages with pine-sawdust bedding. Distilled water and Purina Mouse Chow (Ralston Purina Co., St Louis, MO) were provided ad libitum.

Bacterial strains. The following strains were obtained from the Forsyth Dental Center culture collection: A. israeli 12102, 12103, 12597 (ATCC reference strains), and W2319 (from Dr June M. Brown, Center for Disease Control, Atlanta, GA); A. viscosus M100 (Jordan, Keyes and Bellack, 1972), T14Vi, T14Av (Hammond, Steel and Peindl, 1976) and W1838 (Dr June M. Brown); A. naeslundii N16 (Jordan et al., 1972) and 12104 (ATCC reference strain); A. odontolyticus 17982 and 17929 (ATCC reference strains); and Arach. propionica 14157 (ATCC reference strain).

Strains were revived from the lyophilised state and stock cultures were maintained in fluid thioglycollate medium (BBL, Cockeysville, MD). They were subcultured into Trypticase Soy Broth (TSB; BBL) and incubated at 37°C in an atmosphere of 80% N₂, 10% CO₂ and 10% H₂, in Brewer Anaerobic Jars (BBL). This regimen was used throughout the experiments. The incubation period was 4 days for A. israeli and 48 h for other strains. Cells were harvested by centrifugation, washed twice with sterile physiological saline and resuspended in a volume of saline equal to that of the sedimented cells as recommended by Brown and von Lichtenberg (1970).

In one experiment an attempt was made to standardise the inocula to a uniform optical density (OD). Cell suspensions were homogenised by repeated passage through a hypodermic needle (26½ gauge) and, for a 1 in 10 dilution of the original inoculum, adjusted to an OD of 0.230.

To determine the minimal infectious dose for A. viscosus strain M100 and A. israeli strain 12102, serial tenfold dilutions were prepared from the cell suspension and groups of mice given injection of 0.5 ml of the neat, 1 in 10, 1 in 100, 1 in 1000 and 1 in 10 000 dilutions. In addition, half of the A. israeli suspension was homogenised before dilution by repeated passage through a hypodermic needle to compare homogenised and non-homogenised inocula.

Estimate of viable counts of suspensions. The suspensions were homogenised by 10 passages through a hypodermic needle, and suitable tenfold dilutions plated out on Trypticase Soy Agar (TSA; BBL) with 5% defibrinated sheep blood. The plates were incubated for 7 days at 37°C in an atmosphere of 80% N₂, 10% CO₂ and 10% H₂ in Brewer anaerobic jars.

Infection of animals. Mice were given an intraperitoneal injection of 0.5 ml of the relevant bacterial suspension or 0.5 ml of TSB; non-homogenised bacterial suspension was used unless otherwise stated. At varying intervals, groups of animals were killed in a CO₂ chamber and necropsy was performed to determine the presence and location of the lesions. Randomly selected lesions were removed aseptically, homogenised in saline in a Ten Broeck tissue grinder and plated out in duplicate on TSA with and without 5% sheep blood. The plates were incubated at 37°C for 7 days in an atmosphere of 80% N₂, 10% CO₂ and 10% H₂ in Brewer anaerobic jars.

RESULTS

Comparison of A. israeli and A. viscosus

Of the 30 animals infected with A. israeli strain 12102, 11 (37%) died within 6 days.
PATHOGENICITY OF ACTINOMYCES IN MICE

Table I
Comparative pathogenicity of A. viscosus strain M100 and A. israeli strain 12102 in mice

<table>
<thead>
<tr>
<th>Species</th>
<th>Strain no.</th>
<th>Inoculum (cfu)</th>
<th>Number of animals with lesions/number of animals examined at intervals (weeks) after infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. viscosus</td>
<td>M100</td>
<td>$1.2 \times 10^9$</td>
<td>6/6* 4/6 5/6 3/6 0/6</td>
</tr>
<tr>
<td>A. israeli</td>
<td>12102</td>
<td>$1.2 \times 10^9$</td>
<td>4/4* 4/4* 4/4* 3/3* 4/4*</td>
</tr>
</tbody>
</table>

* Viable organisms isolated from all lesions tested.
† Of animals in this group, 30% died during the first week.

All 30 animals given injections of *A. viscosus* strain M100 survived. Table I shows the proportions of animals with lesions at different sampling times and also the recovery of viable organisms from the lesions.

At 1 week, abscesses were present in the liver, intestine and mesentery and at the site of injection in all the animals examined. Lesions were present on the spleen in all the *A. israeli*-infected animals but only sporadically in the *A. viscosus*-infected animals. Viable organisms could be obtained from all abscesses cultured at this time.

At 3 weeks, animals infected with *A. viscosus* appeared to be resolving the infections and viable *A. viscosus* could not be cultured from the lesions. All animals inoculated with *A. israeli* were infected and lesions yielded viable organisms.

When the experiment was terminated at 31 weeks the remaining *A. viscosus*-infected animals were free from lesions and there were fatty tissue deposits in the abdominal cavity. All the *A. israeli*-infected animals had large abscesses involving most of the abdominal organs and the diaphragm and a heavy infiltrate which fused most of the organs. *A. israeli* was cultured in large numbers ($>10^6$ colony forming units) from all the organs sampled.

The organs of control animals examined at each time interval always appeared normal.

*Comparison of A. israeli and A. naeslundi*

Of the 60 animals infected with *A. israeli* strain 12102, seven died in the first month compared with six in the *A. naeslundi* group. Table II shows that eight out of nine animals infected with *A. naeslundi* N16 and examined 2 weeks later had abdominal abscesses in the liver, spleen, mesentery and at the site of injection. The lesions varied in size from 1 to 15 mm (average 2 mm). Viable organisms were recovered from the lesions.

All animals infected with *A. israeli* strain 12102 and examined after 2 weeks had abscesses in the abdominal organs. They were generally larger than those caused by *A. naeslundi* strain N16 and ranged in size from 4 to 7 mm. Culture of the abscesses yielded viable organisms.

After 32 weeks a few lesions still persisted in three out of 10 animals infected with *A. naeslundi*. However, viable organisms were not recovered. Up to 20 abscesses per
animal were detected in those infected with *A. israeli* and in some cases the lesions had penetrated the diaphragm and invaded the pleural cavity. The spleen was greatly enlarged and in a few cases draining fistulae of the abdomen were observed.

The experiment was terminated at 58 weeks because the *A. israeli* infections had reached the terminal stage in most animals. Nine out of 13 animals in this group had multiple lesions and most of the organs were fused by connective-tissue adhesions. The infected animals were lethargic and moved with difficulty because of abdominal distension. Up to 20 ml of fluid could be collected from some animals and *A. israeli* was grown in large numbers from all lesions sampled. At this time animals given injections of *A. naeslundi* were free from lesions.

**Comparison of nine strains of Actinomyces and Arachnia propionica**

The pathogenicity of nine additional strains of *Actinomyces* and a strain of *Arach. propionica* was compared in groups of 30 mice, with inocula standardised to the same optical density. Table III shows that the three strains of *A. israeli* and *A. viscosus* and
the single strains of *A. naeslundi* and *Arach. propionica* induced lesions in most of the animals in the groups examined after 2 weeks. Viable organisms were recovered from all lesions cultured at this time. Lesions were not found in any of the animals infected with either strain of *A. odontolyticus*.

After 6 weeks the infection appeared to have aborted in many of the animals. A smaller proportion of animals was infected and there were fewer lesions per animal. At this time a difference began to emerge in the infections caused by *A. israeli* and *Arach. propionica* compared with those caused by *A. viscosus* and *A. naeslundi*. All animals given injection of *A. viscosus* strain T14Av and *A. naeslundi* strain 12104 were free from lesions at this time. *A. israeli* strains 12103, W2319, 12597, *A. viscosus* strain T14Vi and *Arach. propionica* strain 14157 could be grown from the lesions.

After 12 weeks, 60% of the remaining animals given injections of *A. israeli* strain W2319 and 50% of the animals given injections of *Arach. propionica* strain 14157 had active infections. Viable organisms were recovered from the lesions in both cases. All other animals examined were negative at this time except for a single lesion found in an animal infected with *A. viscosus* strain W1838. This abscess did not yield viable organisms.

The infectivity of individual strains was not necessarily related to the size of the infecting dose when expressed as colony-forming units (cfu)/ml. Dosages of *A. israeli* strain W2319 and *Arach. propionica* strain 14157, the only two strains that induced persistent chronic infections in this experiment, were very low compared with the other strains. The highest number of colony-forming units was recorded for *A. israeli* strain 12597, a strain that produced smooth colonies and smooth nongranular growth in broth. This strain did not induce a persistent infection.

*Comparison of homogenised versus non-homogenised inocula and determination of minimal infectious dose*

The comparative pathogenicity resulting from graded infectious doses of *A. viscosus* strain M100 and *A. israeli* strain 12102 as well as the effect of inoculum dispersal are shown in Table IV.

Most of the animals receiving undiluted inoculum died within 48–72 h. Animals receiving an inoculum diluted 1 in 10 showed some symptoms of illness and animals receiving an inoculum diluted 1 in 100 or more appeared normal at this time. Initial infectious patterns, as measured by the proportion of animals infected and the average number of lesions per animal, were similar at 2 weeks for the group of animals that received $2\cdot1 \times 10^8$ cfu/ml of *A. viscosus* or $1\cdot6 \times 10^8$ cfu/ml of dispersed *A. israeli* strain 12102. It appeared that the primary infection caused by *A. israeli* was no more severe than that caused by *A. viscosus* when roughly comparable inocula were used. However, as in the previous experiments the *A. israeli* infection, when established, progressed to the persistent chronic stage after 6 weeks while the *A. viscosus* infections were resolved.

*A. israeli* appeared to be more infectious when the inoculum was in the aggregated state. This is true whether the subgroups are compared on the basis of viable cfu in the inoculum or between comparable dilutions (e.g., 1 in 10, 1 in 100). At the next higher dilution (1 in 1000) lesions developed only in animals given the nondispersed inoculum.
Table IV
Comparative infectivity of different dosages of A. viscosus and A. israeli and the effect of inoculum dispersion

<table>
<thead>
<tr>
<th>Species (strain no.)</th>
<th>Inoculum</th>
<th>Dilution</th>
<th>Proportion of animals infected</th>
<th>Average lesions per animal</th>
<th>Proportion of animals infected</th>
<th>Average lesions per animal</th>
<th>Proportion of animals infected</th>
<th>Average lesions per animal</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. viscosus (M100)</td>
<td>10⁰</td>
<td>2.1 × 10⁹</td>
<td>6/10</td>
<td>2.17</td>
<td>3/10</td>
<td>1.00</td>
<td>0/10</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>10¹</td>
<td>2.1 × 10⁸</td>
<td>2/10</td>
<td>1.90</td>
<td>0/10</td>
<td>1.5*</td>
<td>0/10</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>10²</td>
<td>2.1 × 10⁷</td>
<td>0/10</td>
<td>3</td>
<td>0/10</td>
<td>...</td>
<td>0/10</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>10³</td>
<td>2.1 × 10⁶</td>
<td>0/10</td>
<td>...</td>
<td>0/10</td>
<td>...</td>
<td>0/10</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>10⁴</td>
<td>2.1 × 10⁵</td>
<td>0/10</td>
<td>...</td>
<td>0/10</td>
<td>...</td>
<td>0/10</td>
<td>...</td>
</tr>
<tr>
<td>A. israeli (12102)</td>
<td>10⁰</td>
<td>1.6 × 10⁹</td>
<td>10/10</td>
<td>2.50*</td>
<td>10/10</td>
<td>2.40*</td>
<td>8/10</td>
<td>9.12*</td>
</tr>
<tr>
<td>inoculum dispersed</td>
<td>10¹</td>
<td>1.6 × 10⁸</td>
<td>4/10</td>
<td>2.66*</td>
<td>4/10</td>
<td>1.75*</td>
<td>2/10</td>
<td>2.00*</td>
</tr>
<tr>
<td></td>
<td>10²</td>
<td>1.6 × 10⁷</td>
<td>0/10</td>
<td>...</td>
<td>0/10</td>
<td>...</td>
<td>0/10</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>10³</td>
<td>1.6 × 10⁶</td>
<td>0/10</td>
<td>...</td>
<td>0/10</td>
<td>...</td>
<td>0/10</td>
<td>...</td>
</tr>
<tr>
<td>A. israeli (12102)</td>
<td>10¹</td>
<td>1.5 × 10⁹</td>
<td>8/9</td>
<td>58.75*</td>
<td>10/10</td>
<td>45.70*</td>
<td>8/9</td>
<td>26.75*</td>
</tr>
<tr>
<td>inoculum aggregates</td>
<td>10²</td>
<td>1.5 × 10⁸</td>
<td>9/10</td>
<td>3.77*</td>
<td>7/10</td>
<td>3.00*</td>
<td>5/10</td>
<td>2.40*</td>
</tr>
<tr>
<td></td>
<td>10³</td>
<td>1.5 × 10⁷</td>
<td>3/10</td>
<td>1.33*</td>
<td>0/10</td>
<td>...</td>
<td>0/10</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>10⁴</td>
<td>1.5 × 10⁶</td>
<td>0/10</td>
<td>...</td>
<td>0/10</td>
<td>...</td>
<td>0/10</td>
<td>...</td>
</tr>
</tbody>
</table>

Although viable organisms were recovered, it is not known whether these lesions would have persisted if the three animals had not been killed after 2 weeks.

Although dispersal of the A. israeli inoculum through a fine needle resulted in a tenfold increase in the number of colony-forming units, darkfield examination of the inoculum revealed that aggregates of cells still remained.

Discussion

Previous attempts to demonstrate pathogenicity of Actinomyces in various experimental animals have not been uniformly successful (Emmons, 1935; Bibby and Knighton, 1941; Slack, 1942; Hazen, Little and Resnick, 1952; Howell et al., 1959). Rosebury, Epps and Clark (1944) provided an extensive review of the early literature on experimental actinomycosis in laboratory animals and concluded that certain essential factors in the pathogenicity of the disease had not been defined.

More consistent results were later obtained with the mouse as an experimental animal. Meyer and Verges (1950) reported that mice were susceptible to actinomycotic infections and emphasised the importance of using young mice weighing 10–15 g because older animals were often completely resistant. Other workers have demonstrated that species of Actinomyces other than A. israeli can produce abscesses in mice (Coleman and Georg, 1969; Georg and Coleman, 1970; Georg et al., 1972). Experimental periods in these studies generally did not exceed 3 weeks, thus pathogenicity was not studied in relation to the chronic stage of the disease. In one experiment, animals infected with A. naeslundii showed signs of resolving the infection at 8 weeks (Coleman and Georg, 1969). These workers reported that lesions produced...
in mice by *A. naeslundi* were similar to those produced by *A. israeli* and concluded that *A. naeslundi* had a similar pathogenic potential in man.

The present studies illustrate that even though chronic actinomycotic lesions can be maintained in mice, generally without any obvious signs of debilitation, the disease can be lethal if it is allowed to proceed for an extended period of time. This exceeded 1 year in some animals. Although previous workers (Meyer and Verges, 1950; Coleman and Georg, 1969) mentioned the desirability of observing animals for longer periods, these did not exceed 2 months and deaths resulting directly from the chronic infections were not reported. Brown and von Lichtenberg (1970) observed infected mice for as long as 8 months and reported that none of the animals died of actinomycosis.

Brown and von Lichtenberg (1970) also observed that weanling mice, 21–23 days old, were susceptible to infection by *A. israeli* and the size of the bacterial aggregates was important. Strains of *A. israeli* that grew in rough aggregates in liquid media regularly produced lesions, whereas smooth strains or homogenised inocula from rough strains did not. Specific details of the method of homogenisation or the extent of the reduction of the aggregates were not given. The smooth strain of *A. israeli* (ATCC12597) tested in the present study did not induce a chronic infection in the test animals. However, the attempt to eliminate the infectivity of a rough strain of *A. israeli* by homogenisation of the inoculum was not successful. The method of homogenisation did not reduce the inoculum to a completely disaggregated state.

The difficulty of trying to equalise infectious dosages of the different strains of *Actinomyces* is illustrated in the third experiment. Even though cell mass was standardised by the use of suspensions of equivalent density, the number of infectious units determined on the basis of viable colony-forming units varied greatly among the different strains. Conversely, standardisation of inocula on the basis of infectious units results in the delivery of unequal amounts of bacterial mass to the different experimental groups. Stringent homogenisation of the inocula by any technique that would be adequate for total reduction of bacterial aggregates changes a potentially important characteristic of the individual species and thus may obscure important differences in pathogenicity. Determination of the role of bacterial aggregates in the initiation and persistence of actinomycotic lesions will require more study.

Infectious dosages of *A. israeli* suggested by Brown and von Lichtenberg (1970) and used in the present work appear to represent the upper limit of inoculum size that can be used in this model. In some of the experiments with *A. israeli* strain 12102, this dosage could not be tolerated by the animals and a large percentage of them died. The results of the last experiment indicate that a 1 in 10 dilution of the inoculum infected most of the animals without causing excessive mortality. A 1 in 100 dilution of the inoculum resulted in a reduction in the proportion of animals that became infected and in the numbers of lesions per animal. At dilutions above this the infectivity of the inoculum became insignificant. It was observed in preliminary experiments that variations in the age or weight of the test animals significantly influenced the minimal infectious dose.

The present studies indicate that differences in pathogenicity between the different *Actinomyces* species are much less pronounced during the initial acute stage of the infection, particularly when the infecting dosages are adjusted to contain similar numbers of dispersed colony-forming units. Even during this stage, however, there is still a tendency for the *A. viscosus* and *A. naeslundi* infections to be less severe.
The most striking differences in the pathogenicity for the mouse of the different Actinomyces species were seen in the chronic stage of the infection. Two strains of A. israeli and a strain of Arach. propionica were able to survive the critical transition period after the acute phase. In a large percentage of the animals these species established a slowly progressive chronic infection from which viable organisms were continuously recovered. The other species apparently lacked the virulence to survive the critical transitional stage of the infection. It is interesting that A. odontolyticus showed little evidence of infectivity in the present study, even in the acute stage, which agrees with the earlier observations of Georg and Coleman (1970).

The patterns of acute and chronic infection observed in mice appear to reflect a fundamental difference in the way the host responds to infection by A. israeli compared with other Actinomyces species. The transition stage of the acute infection (Geister and Meyer, 1951; Brown and von Lichtenberg, 1970) apparently represents a critical time of mobilisation of host defences as well as consolidation of the lesions.

Acute and chronic forms of actinomycosis are recognised in man (Arnott and Ritchie, 1949; Glahn, 1950; Bentley and de Vries, 1973), but the relationship to experimental disease patterns seen in the mouse is not clear. Bentley and de Vries (1973) believe that an acute form of the infection does occur and that it can be indistinguishable from other acute pyogenic abscesses unless a bacteriological diagnosis is made. They have pointed out that chronic cervicofacial actinomycosis is now quite rare, probably due to the widespread use of antibiotics in infections after dental extractions.

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PATHOGENICITY OF ACTINOMYCES IN MICE


