ASPERGILLOSIS INVOLVING BONE

ASPERGILLOSIS (ASPERGILLUS NIDULANS)
INVOLVING BONE

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Infections of man with aspergillus organisms have been reported with ever-increasing frequency within the past two decades, the overwhelming majority of cases being caused by Aspergillus fumigatus. The following account of aspergillosis in a 6-yr-old boy is unusual in that the responsible agent was A. nidulans and it is unique in that the infection was associated with extensive destruction of bone.

Case report

A 6-yr-old boy was admitted to the Royal Belfast Hospital for Sick Children on 12 Aug. 1962 with an 8-wk history of increasing listlessness, anorexia, loss of weight and breathlessness on exertion. Investigations at another hospital 2 wk previously had revealed a moderate iron deficiency anaemia, left upper lobe consolidation as shown by chest radiography, and negative Mantoux tests.

The patient, who had always been small for his age, lived in the country but not on a farm. When 1 yr old he had been severely ill with a Salmonella dublin sepsicaemia (Boyle, 1958). Twelve months later he received treatment in hospital for a large submandibular abscess; staphylococci were isolated from the pus, but no fungi were seen in biopsy sections examined in retrospect.

Clinical examination. A very small pale child weighing 27½ lb. (12·5 kg.). Liver and spleen palpable. Numerous enlarged posterior cervical lymph-glands. Dullness on percussion and diminished breath sounds over both lung apices.

Haemoglobin 6·8 g. per 100 ml. Red cell count 4·43 x 10⁶ per c.mm. White cell count 5700 per c.mm. (polymorphs 59 per cent.). Erythrocyte sedimentation rate 60 mm. per hr (Westergren). Kveim test negative. Mantoux test negative. Total serum proteins 7·1 g. per 100 ml. Serum electrophoresis, raised γ-globulin (1·82 g. per 100 ml.). Chest radiograph, resolving consolidation in the left upper and midzones with fine punctate shadowing of the right base.

Diagnoses considered but discarded on the basis of investigations included sarcoidosis, tuberculosis and idiopathic pulmonary haemosiderosis.

Treatment and progress. After the patient's anaemia had been corrected with 2 pints (1130 ml.) of packed cells, a lung biopsy was performed through a left thoracotomy incision. At operation the left upper lobe was found to be shrunken and fibrotic, and to have adhesions to the chest wall. When an attempt was made to strip the lung from the chest wall, pus oozed out. The possibility of actinomycosis was suggested and a portion of the lung and some pus were sent to the Mycology Laboratory. The patient was started on 2 mega units of benzylpenicillin and 2 g. of sulphadimidine daily; treatment was discontinued after 7 days. Neither fungi nor bacteria were isolated from the biopsy specimens. The biopsy showed little recognisable pulmonary tissue; there was fibrosis and a considerable infiltration of chronic inflammatory cells, which in a few places formed granulomata with giant cells.

A few days after operation, radiographs of the cervical spine appeared normal. During the next 10 days the patient's condition deteriorated. He developed an upper dorsal kyphosis and held his neck extended. He complained of severe upper abdominal girdle pains; both plantar reflexes became extensor and ankle clonus was elicited. Radiographs of chest and spine now showed extensive destruction of ribs and thoracic vertebrae (D1–D8). There was also evidence of bilateral paravertebral abscess formation. A paravertebral needle biopsy sent for histological and microbiological investigation revealed an acute inflammatory reaction associated
with scattered collections of giant cells and mononuclears. Branching septate hyphae were seen on direct microscopy and *A. nidulans* was isolated in abundance in culture.

Amphotericin B (Fungizone) was given by inferior vena caval drip, 3 mg. daily, increasing to 6 mg. Each day’s dose was added to 60 ml. of 5 per cent. dextrose containing 1 mg. hydrocortisone and this was given slowly during 6 hr. Chlorpheniramine (Piriton) 4 mg. 6-hourly and dimenhydrinate (Dramamine) 15 mg. were given to counteract toxic effects. The patient’s condition deteriorated and he died 8 days later.

**Post-mortem findings**

The pathological findings of interest were confined to the lungs, thoracic vertebrae and ribs, spinal cord and spleen.

**Lungs.** The right lung lay free, but the left was bound closely to the parietal pleura by dense fibrous adhesions. The upper lobe was almost totally converted to fibrous tissue and it was honeycombed with cavities 1–5 cm. in diameter containing yellowish-brown pus. There was marked congestion of the right lung and the left upper lobe with a diffuse increase in fibrous tissue thoughout. In these areas a regular alveolar pattern could be distinguished. The major bronchi contained mucopurulent material. The hilar and paratracheal lymph-glands were moderately enlarged.

**Histology.** Widespread destruction of the alveolar pattern has occurred and the normal tissue is replaced with broad fibrous bands. Within the fibrous stroma, numerous cavities lined with granulation tissue, including many multinucleated foreign-body giant cells, are present. There is a moderate lymphocytic infiltration throughout. Granulomata composed of groups of giant cells are also found scattered through the fibrous tissue. Staining by the periodic acid-Schiff method demonstrates discrete branching septate hyphae that resemble elements of *Aspergillus* and lie closely adjacent to and within the cytoplasm of the giant cells. Ziehl-Neelsen staining excludes the presence of acid-fast bacilli.

Sections taken from other areas of the lung show congested alveoli with small foci of bronchopneumonia. Intra-alveolar haemorrhage has occurred in places and an occasional isolated giant cell is noted. There is no cavitation in these areas. The hilar and paratracheal lymph-glands show only reactive changes.

**Vertebrae and ribs.** The thoracic vertebrae (D1–D8) were soft and carious with complete destruction of the spinous processes. On section, yellowish pus exuded from the marrow. Several of the mid-thoracic vertebrae had collapsed and small paravertebral pockets of pus lay beneath the parietal pleura. The infective process had extended to involve the corresponding ribs, which showed nodular thickening throughout their course.

**Histology.** Numerous granulomata composed of clusters of giant cells, similar to those found in the lung, are present throughout the vertebral and rib marrow. Small abscesses composed of aggregates of polymorphs with surrounding haloes of giant cells are also present. Hyphal fragments are frequently observed at the centre of a micro-abscess. These are 3–5 μ in diameter and up to 60 μ in length; they are often “coralloid”, due to the presence of irregular lateral or central swellings. Some hyphae are less deeply stained, have poorly defined cell walls, and are apparently undergoing dissolution. The appearance of the fungus in lung and vertebral sections is similar. Staphylococci are not demonstrable in Gram-stained preparations.

**Spinal cord.** In the mid-thoracic region a large mass of carious bone and purulent material had been extruded into the epidural space by the vertebral collapse, and had compressed the spinal cord.

**Spleen.** This was moderately enlarged (200 g.) and had a smooth capsule. On section the pulp had a homogeneous appearance. The malpighian corpuscles could not be distinguished. Histologically, the pulp is very cellular with an excess
of polymorphs in the sinusoids. There is marked congestion and a few colonies of staphylococci are seen. The malpighian corpuscles are small but discrete.

Microbiological findings

Aspergillus nidulans was the only organism isolated from the child during his last illness. It was isolated in quantity from paravertebral pus during mycological investigations and on another occasion it was isolated on Löwenstein-Jensen medium during culture of the pus for tubercle bacilli. Colonial and microscopic morphology, including the formation of perithecia and ascospores, corresponded closely to that of \textit{A. nidulans} (Eidam) Wint. Isolations of \textit{A. nidulans} and a critical comparison of the hyphae present in biopsies from the paravertebral lesion and left lung suggested that this organism might have played an active role in the disease.

At necropsy, however, initial findings were apparently more consistent with a multiple aetiology. Eight specimens of pus from lesions of vertebrae, ribs and lungs yielded four potential pathogens. \textit{Staphylococcus aureus} was cultured from all 8 specimens, \textit{Candida albicans} from 5, \textit{A. nidulans} from 6 and \textit{A. fumigatus} from 3. The role of these organisms has been evaluated as follows.

\textit{Staphylococcus aureus}. This is the commonest cause of osteomyelitis and it was the only micro-organism isolated from all the necropsy specimens. There was strong evidence, however, that the staphylococcus appeared and became disseminated only in the last stages of illness. Thus (1) it had not been isolated before death, (2) it was of a type (all isolates typable at 1 routine test dose and shown to be identical with phage type 52/52A/79/80/7/42E/81) that is not normally of outstanding virulence or invasiveness and that has not been associated with outbreaks of sepsis in this area, and (3) it was not seen on microscopic examination in any biopsy or post-mortem tissue section except those of necropsy material from the spleen. It was therefore concluded that the staphylococcus had produced a widespread terminal infection, but was not involved in the formation of lung or bone lesions.

\textit{Candida albicans}. This was also disregarded as a primary pathogen because it had not been isolated from clinical material during life and because it was absent from tissue sections.

\textit{Aspergillus}. Since \textit{A. fumigatus} is the cause of the majority of aspergilloses and since this fungus was isolated from post-mortem material, it might have been regarded as the most likely cause of the disease. Serological evidence, however, indicated otherwise. Immuno-electrophoretic studies of the patient's serum made by Dr J. Pepys and Miss Joan L. Longbottom suggested specificity for \textit{A. nidulans}, rather than for \textit{A. fumigatus}. The serum gave no noticeable precipitation with antigen prepared from \textit{A. fumigatus}, but, with an \textit{A. nidulans} antigen, it gave a diffuse pattern of precipitation which resembled that found when the sera of patients with pulmonary mycetoma due to \textit{A. fumigatus} are tested with an extract of \textit{A. fumigatus}. The serological study thus provided strong evidence that \textit{A. nidulans} was actively associated with the child's illness, the presence of precipitating antibodies being consistent with a disseminated infection. Furthermore, \textit{A. nidulans} had been isolated on two separate occasions during life, whereas \textit{A. fumigatus} had been obtained only at necropsy, and then only in small numbers. Tubercle bacilli and actinomycetes were not isolated at any time.

It was concluded that \textit{A. nidulans} was the primary infective agent and that the hyphae observed in lung and paravertebral biopsy material could be referred to this species.

DISCUSSION

Aspergillus nidulans, a common and ubiquitous mould associated with soil and decaying vegetation, is rarely pathogenic for man. Only 15 reports of its occurrence as a human pathogen have been found in the literature, viz., nail infections (Weil and Gaudin, 1919; Ota, 1923; Bereston and Waring, 1945, 1946; Bereston, 1950),
otomycosis (Siebenmann, 1889; Neveu-Lemaire, 1921; Lurie and Brookfield, 1949; Fabricant, 1955; Stuart and Blank, 1955) and pulmonary aspergilloma (Segretain and Vieu, 1957). It has also been isolated from 3 cases of mycetoma (Nicolle and Pinoy, 1905; Pinoy and Masson, 1915; Lacaz and Netto, 1954) and from an inflamed appendix and ileum (Staib, 1959).

It has been suggested (Finegold, Will and Murray, 1959) that antibiotic and cortisone therapy may be an important factor in establishing the fungus as a pathogen. It is tempting to postulate, therefore, that the aspergillus gained entrance while the child was receiving such treatment for the salmonella infection during his first year of life. A smouldering subclinical infection would serve to explain his poor physical development throughout childhood, when he was otherwise apparently active and healthy. Furthermore, the extensive pulmonary fibrosis is consistent with a longstanding chronic infection.

It is apparent that the primary infection was pulmonary and that there was subsequent spread to involve vertebrae and ribs. We know of only five previous reports of destruction of bone by *Aspergillus* (Just, 1930-32; Shaw and Warthen, 1936; Cawley, 1947; Tobler and Minder, 1954; Montreuil, 1955); in these cases the agent, when isolated, was *A. fumigatus*. In the case described by Shaw and Warthen, lesions were found in vertebrae, ribs, and metacarpals, a pattern of distribution closely paralleled in our patient. The finding of *A. nidulans* as a causal agent of osteomyelitis with giant-cell granulomata and abscesses would appear to be unique.

**Summary**

This paper reports the clinical, microbiological and pathological features of a unique case, that of a 6-yr-old boy who had infection of the lung, vertebrae and ribs with *Aspergillus nidulans*.

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GROWING "FIELDS" OF SPLENIC GRAFTS

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PLATE CIII

When splenic autografts from rabbits (Manley and Marine, 1917), rats and mice (Perla, 1936; Calder, 1939; Cameron and Rhee, 1959) are grown in parent host tissues, most of the graft dies in the first day or so, but active growth of the surviving peripheral rim of cells leads within a few weeks to the production of a miniature organ. Recently Cameron and Rhee showed that the adult spleen can be divided into many small pieces at random, and each of them will grow into a minute spleen. They suggested that both white and red pulp are needed for the graft to take. The present study puts the latter supposition to test.

METHODS

The abdominal cavity of 51 male Wistar albino rats previously fed on MRC41 rat cubes with unlimited water was opened by a midline incision under open ether, and the spleen was removed.

In 20 rats, body weight 125–150 g., the spleen was perfused with 20 ml. phosphate buffer saline pH under aseptic conditions and then minced with scissors in a sterile dish (Vaughan et al., 1960). The tissue suspension thus obtained was passed through a 10611 stainless steel sieve, and its cells were counted. Smears stained with Giemsa showed a large number of lymphocytes, reticulum cells and red cells. Some plasma cells and polymorphs were also present. The animals were then divided into three groups, the first receiving by intraperitoneal injection approximately 10,000 cells, the second 100,000 cells and the third 400,000 cells suspended in 1 ml. phosphate buffer saline. Four rats died within 2 days of operation; the remaining animals were killed with ether at the end of 1, 3 and 12 wk.

In 21 rats, body weight 125–200 g., small cross-sections of the spleen measuring 0.5 x 0.5 x 0.3 cm. were cut into frozen sections. A representative section was stained with Ehrlich's haematoxylin and eosin. Sections 10, 25 and 50 μ thick were each grafted into the gastric and testicular omentum of groups of 7 rats. Four animals died within 2 days of operation; the remaining animals were killed with ether at the end of 1, 2 and 13 wk.

In the last experiment one group of 8 rats, body weight 30 g., and another of 2 rats, body weight 125 g., were used; the spleen was crushed and squeezed through a 500 μ stainless steel sieve. Thus granules of about 500 μ in diameter were obtained. A certain number of these were fixed in formol-saline, embedded in paraffin, cut serially and stained with haematoxylin and eosin. Each contained a little red and

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