Suspected primary immunodeficiency syndrome in three related Irish wolfhounds

Three related Irish wolfhound dogs less than one year old presented with a history of chronic nasal discharge and signs of lower respiratory tract disease. These responded well to treatment initially but were chronically recurring. Cursory evaluation of the immune system (full blood counts, globulin determination and fractionation, electrophoresis and lymphocyte blastogenesis) seemed to indicate a cell-mediated immunodeficiency which, because of the age of the patients, is strongly suspected to be primary.

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INTRODUCTION

A syndrome of chronic, recurrent respiratory infections in young Irish wolfhounds was first reported by Wilkinson (1969). The condition was described as being fairly well distributed throughout the breed. This initial description, and Wilkinson's interpretation, has remained relatively unchanged (Bedford 1995). 'Irish wolfhound rhinitis', as described by Wilkinson, occurred in Irish wolfhound puppies which had presented initially with a serous nasal discharge that had progressed to become catarrhal, then purulent and even haemorrhagic, with turbinate ulceration and destruction. Severe cases resulted in a condition resembling atrophic rhinitis in pigs. Many progressed to severe purulent bilateral pneumonia and death. Although several puppies in a litter could be affected, others in the same litter remained unaffected, despite close contact.

A viral aetiology was considered the most likely primary cause, followed by secondary bacterial infection. A 'cytopathic agent' was isolated on a stable monkey kidney cell line from nasal flush material. The virus was thought to be transmitted in utero, or contracted from uterine fluids in the birth canal during birth. An underlying immunodeficiency syndrome was discounted on the basis of normal globulin levels in an unreported number of dogs. No structural or numerical defect could be found in the chromosomes of one dog.

This report describes three related Irish wolfhounds that were presented to the Onderstepoort Veterinary Academic Hospital independently of one another with a syndrome closely resembling the condition described by Wilkinson (1969). Preliminary investigations of immune function, combined with the close familial relationship between the dogs, suggest that a primary immunodeficiency syndrome may be responsible for chronic, recurrent respiratory infections in young Irish wolfhounds.

CASE HISTORIES

Case 1

A five-month-old, 22·5 kg male Irish wolfhound was presented with the chief complaints being hindlimb weakness, mucous nasal discharge, anorexia and depression. The dog had been seen three times over the previous two months for similar problems and had responded well to antibiotic treatment each time. On physical examination, the dog had a moist productive cough, pyrexia and polypnoea. Pneumonia was confirmed radiographically and responded well to treatment.

The dog presented again three months later with a severe mucopurulent nasal discharge. Habitus and appetite were good. Radiographically, pneumonia was once again diagnosed. A diagnostic nasal flush and transtracheal aspiration (TTA) both yielded a heavy purulent exudate. A precipitin test on the dog's serum was negative for *Aspergillus* species. Response to antibiotic treatment based on culture and antibiogram was good.

Three months later, the dog presented again with similar complaints. The nasal discharge was copious and unilateral thoracic auscultation was indicative of a severe pneumonia. A moist productive cough was present with polypnoea. Rectal temperature was elevated (41°C), and there was a generalised lymphadenopathy. A severe left shift neutrophilia was found on haematology. *Streptococcus faecalis* was cultured from the nasal exudate.

At this time, an immunodeficiency

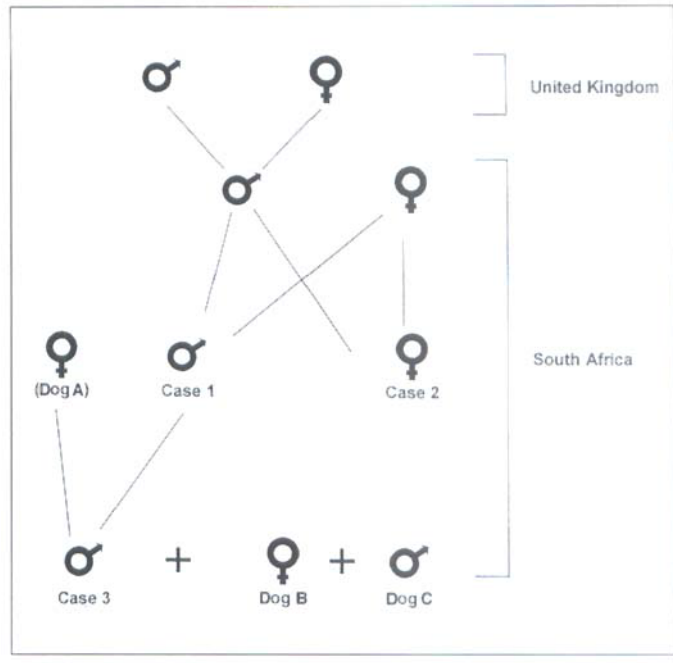

FIG 1. Genetic relationship between the three Irish wolfhounds

syndrome was suspected and electrophoresis, globulin fractionation and lymphocyte transformation testing were undertaken. Response to treatment was slow but favourable as evidenced by an improved appetite and habitus. However, the nasal discharge persisted. The signs recurred several months later and, due to the likelihood of a primary immunodeficiency and poor long-term prognosis, the owner requested euthanasia. The dog was three years old at the time of euthanasia.

Case 2

An eight-month-old, 24·1 kg Irish wolfhound bitch was referred to the hospital for chronically recurring purulent rhinitis and pneumonia. The history indicated chronically recurring weakness, dyspnoea and a purulent nasal discharge. The dog was thin and depressed and had increased lung sounds on chest auscultation. A mild normocytic normochromic non-regenerative anaemia with a left shift neutrophilia and monocytosis was found on haematological evaluation. Bilateral bacterial pneumonia was confirmed radiographically and the dog's condition deteriorated rapidly. *Escherichia coli* was cultured from TTA and nasal flush material.

An immunodeficiency syndrome was suspected, and total and fractionated globulins and lymphoblast transformation testing were requested. With intensive management, the dog made a slow recovery and was discharged on long-term antibiotics, as well as levamisole and cimetidine as immunostimulants. The dog was lost to follow-up.

Case 3

A 10-week-old, 13·6 kg male Irish wolfhound was admitted for a diagnostic workup of a purulent unilateral nasal discharge of four weeks' duration. The dog was alert and in good condition. Nasal exudate cytology was neutrophil-rich with many free and phagocytosed cocci. Culture yielded *Klebsiella pneumoniae* and *Corynebacterium pyogenes*. Fungal culture was negative. Bronchial brush cytology was of very low cellularity. Radiographs of the nasal passages showed increased density with opacification of the frontal sinus on the left side. Nasal biopsy showed an acute catarrhal to purulent rhinitis. There was considerable involvement of the turbinate bone with remodelling characterised by the overwhelming presence of fibrous connective tissue. The reaction was characteristic of osteodystrophia fibrosa. Thoracic radiographs showed an early alveolar pattern.

Bronchial biopsies were taken for electron microscopy to rule out ciliary dyskinesia. Preputial scrapings were taken for viral isolation which proved negative. No cytological evidence of viral inclusions could be found on these samples either.

Antibiotic treatment based on bacterial culture and antibiogram was instituted and the dog was discharged after 16 days, having improved significantly. Because of financial constraints, the dog was given away at discharge and was lost to follow-up.

Pedigree analysis

The pedigrees of all three dogs were obtained and scrutinised, to establish the relationship between the animals. The owners and breeders of the dogs were questioned about any other affected dogs they might have encountered.

A close familial relationship was found between the three dogs (Fig 1). Despite this, however, there is insufficient evidence to report on a mode of inheritance.

Postmortem examination (case 1)

A full gross postmortem examination carried out on case 1 revealed lymph node hyperplasia, mild hydropericardium and bilateral, focally disseminated pneumonia.

Haematoxylin and eosin sections were made from formalinised samples of tissues of interest, including the nasal turbinates, lungs, superficial lymph nodes and bone marrow. Histopathological evaluation of the superficial lymph nodes showed prominent cortical hyperplasia due to marked follicular hyperplasia. There were large numbers of active and developing follicules, but the T-cell parafollicular areas were less prominent and showed moderate numbers of histiocytic cells. The sinuses were filled with histiocytes and abundant eosinophils and neutrophils. The medullary cords contained moderate numbers of plasma cells and pigment-laden macrophages. Histopathology of the lungs confirmed the presence of a moderately severe chronic active bronchointerstitial pneumonia. Rhinitis was confirmed histologically. The bone marrow was mildly hyperplastic with an increased myeloid:erythroid ratio.

Evaluation of immune function

Total white cell counts were within normal range for all the cases on all the occasions they were measured. Differential counts did not show any remarkable inflammatory changes. Lymphocyte counts of all three cases were within the normal reference range in all samples submitted.

Case 1 had markedly elevated total globulins (85·1 g/litre, normal range 20 to 37) at initial presentation. This was characterised electrophoretically as a polyclonal gammopathy. The globulin level had dropped to within normal range on the two subsequent occasions that it was measured over the next 24 months in this dog. Both total globulins and electrophoretograms were within the normal

range for cases 2 and 3 at presentation.

Lymphoblast transformation tests were performed as previously described (Spencer 1993). The mitogens used were the pan-T-cell stimulator, phytohaemagglutinin (PHA; Sigma), the B-cell stimulator, pokeweed mitogen (PWM; Sigma) and/or the T-cell stimulator, concanavalin A (ConA; Sigma). All tests were performed in triplicate. Results were expressed as mean counts per minute. Lymphocytes from two healthy dogs were used as controls and processed in the same way as the patients' lymphocytes. In addition, three related dogs – the mother of case 3 (dog A) and two siblings from the same litter as case 3 (dogs B and C) – were evaluated immunologically.

The results of lymphoblast transformation tests for case 1 are shown in Table 1, and for cases 2 and 3 in Table 2. Results are reported separately because case 1 was evaluated earlier, while cases 2 and 3 were hospitalised at the same time and tested simultaneously using the same control.

Optimal mitogen and cell concentrations were established for the laboratory using the control dogs prior to running the assays on the patients. On comparing the results for the mean counts per minute. it can be seen that cases 1 and 2 and dogs A and C all show a marked decrease in their response to both mitogens used, whereas dog B showed a marked decrease only in its response to pokeweed mitogen. Case 1 showed a marked decrease in lymphocyte blastogenic responses to all three mitogens used. From these lymphoblast transformation studies it would appear that cases 1, 2 and 3 and the closely related dogs A and C have both T- and B-cell deficiencies. Dog B, however, appeared to have an intact T-cell response as evidenced by its blastogenic response to phytohaemagglutinin whereas its B-cell response was diminished.

Table 1. Lymphoblastogenesis expressed as mean counts per minute in case 1

Dog	PHA (SI)	PWM (SI)	ConA (SI)	Medium
Case 1	375 (2·1)	550 (3·1)	210 (1·2)	175
Control	4250 (21·2)	1000 (5·0)	2060 (10·3)	200

SI Stimulation index, PHA Phytohaemagglutinin, PWM Pokeweed mitogen, ConA Concanavalin A

Table 2. Lymphoblastogenesis expressed as mean counts per minute in cases 2 and 3

Dog	PHA (SI) [SD]	PWM (SI) [SD]	Medium [SD]
Case 2	243 (2·3) [19]	414 (4·0) [38]	102 [3]
Case 3	2443 (14·5) [547]	1026 (6·1) [179]	168] [37
Dog A*	2027 (15·3) [282]	868 (6·5) [112]	132 [20]
Dog B*	15,958 (26·5) [1586]	1998 (3·3) [290]	600] [26
Dog C*	1761 (7·7) [319]	1209 (5·2) [193]	229 [13]
Control	9833 (44·9) [242]	4576 (20·9) [687]	219 [42]

SI Stimulation index, PHA Phytohaemagglutinin, PWM Pokeweed mitogen
*Dogs B and C are siblings and from the same litter as case 3; Dog A is the mother of case 3

DISCUSSION

The classic presentation of patients with immune deficiencies includes increased frequency of infection, increased severity of infection, chronic prolonged infection, incomplete clearing between episodes of infection, incomplete or no response to treatment, and infection with organisms of low pathogenicity (Felsburg 1986, 1992). The cases presented here clearly fall into this clinical presentation. Immunodeficiencies may be primary (inborn or congenital) or secondary (acquired) and are further classified according to the immune component of the immune system that is compromised (Degen and Breitschwerdt 1986a). Several primary immunodeficiencies of dogs have been described (Degen and Breitschwerdt 1986b, Felsburg 1986, 1992, Guilford 1987).

The four basic components of the immune system are the antibody-mediated (humoral) immune response, the cell-mediated response, the complement system and the phagocytic system (Degen and Breitschwerdt 1986a). Evaluation for immunodeficiency in the dog and cat has been described and includes evaluation of each of these components (Schultz 1982, Castoldi and others 1988, Gershwin 1992). However, few quantitative assays have been developed for investigating immune function in dogs in vitro, despite the many spontaneous autoimmune and immunodeficiency syndromes seen in this species (Miller 1991, Degen and Breitschwerdt 1986a, 1986b, Felsburg 1986, 1992, Guilford 1987). These tests are specialised and most are not readily available.

Although the evaluation of the immune system in this small study was limited, it allowed an initial assessment of humoral immunity (total globulins, protein electrophoresis, histopathology of lymph nodes and bone marrow in case 1), phagocytes (mature and immature neutrophil counts, monocyte count) and cell-mediated immunity (lymphocyte count, lymphocyte transformation test).

The polyclonal gammopathy in case 1 at

initial presentation was considered appropriate in the light of the severe, chronic pneumonia, and would tend to argue in favour of a functional humoral immune response. The subsequent normal globulin levels in this dog, and the normal levels in case 2 and 3, despite the presence of respiratory infections, may have been due to the milder nature and/or the predominantly upper respiratory localisation of these infections. From these results, it would appear that the humoral immunity in these cases was intact. This concurs with the earlier findings of normal globulins in Irish wolfhound rhinitis (Wilkinson 1969).

Immunoglobulin assay of these cases and the controls was attempted, but technical problems were experienced and the results are not reportable. Unfortunately, insufficient serum remains stored to repeat the assays retrospectively. It is therefore not possible to confirm or exclude the possibility of a specific defect in any immunoglobulin fraction. The histological examination of the superficial lymph nodes of case 1 demonstrated an abundance of plasma cells and B-cell dependent areas, while the T-cell areas appeared to be slightly depleted. The significance of these findings is difficult to interpret in the light of the in vitro blastogenic response. Phagocyte numbers may perhaps be regarded as inappropriate in these cases considering the severity of the pathology present. The possibility of a functional defect in the phagocytic response was not evaluated and remains a possibility.

No firm conclusions can be drawn from the normal lymphocyte counts seen in these cases, although they may argue against a defect in cell-mediated immunity. The lymphoblast transformation test is an in vitro test very suitable as a model for the investigation of cell-mediated immune reactions (cellular immunocompetence) (Janossy and others 1971, Gershwin 1992) and has been used more than any other cellular assay in domestic species (Schultz 1982). The poor response to the mitogens that these cases showed is interpreted as a failure in cellular immunity. Traditionally, phytohaemagglutinin and concanavalin A have been seen as T-cell stimulators and pokeweed mitogen as a B-cell stimulator (Schultz 1982). In the dog, however, the predominant cell responding to all three of these mitogens is the T-cell (Schultz 1982). Although the blastogenic response was measured at only one cell concentration and one mitogen concentration, these concentrations were standardised for the laboratory used. Day-to-day variation, and variation within a day, in mitogenic response does occur (Ross and others 1986) and this cannot be completely excluded in these cases, although samples (from patients and controls) were always drawn at the same time of day (between 08.30 and 09.00). All testing was performed by the same person in the same laboratory.

The available data, though by no means complete, appear to indicate that the 'Irish wolfhound rhinitis' reported here is caused by a primary immunodeficiency syndrome. The dogs were all under one year of age at presentation and were closely related genetically. An abnormal cellular immune response was present in all the dogs, as well as in two clinically normal, closely related animals, which in itself is a highly suspicious finding. A primary immunodeficiency should be considered in young Irish wolfhounds that are presented with recurrent respiratory infections.

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