Dane particles and tubules by sera containing anti-e with immune electron microscopy¹³ might also be a significant finding in this respect. The determination of DNA polymerase as a marker for the Dane particle provides further support for the suggested association between the e antigen and the Dane particle. In a series of patients from an institution for the mentally retarded a close correlation was found between the presence of serum e antigen and the presence of the Dane particle associated DNA polymerase. Conversely, a correlation was found between lack of DNA polymerase and presence of anti-e.¹⁴

If the e antigen turns out to be a constituent of the Dane particle it would not represent the core antigen since patients with e antigen have high serum levels of core antibodies.¹⁵ Furthermore, free core antigens have not been found in the serum of patients with acute or chronic hepatitis-B infection. The e antigen, however, may represent the DNA polymerase protein for which so far no immunological specificity has been determined. Another possibility is that the e antigen represents a matrix protein that links the core antigen to the HBs Ag of the Dane particle. Nevertheless, as suggested previously,^{6 12} it is not

excluded that the e antigen is produced as a specific host response to the replication of the hepatitis B virus.

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Neutrophil pyruvate kinase deficiency with recurrent staphylococcal infections: first reported case

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Summary

A woman with an intracellular killing defect in the neutrophils had neutrophil pyruvate kinase deficiency. She had had recurrent staphylococcal infections throughout her life. The enzyme present was unstable and its kinetics were abnormal.

Introduction

Defective bacterial killing by neutrophils is increasingly becoming recognised as a cause of recurrent bacterial infections. X-linked chronic granulomatous disease is the best known of these non-specific primary immunodeficiencies.^{1 2} Neutrophils from such patients do not increase their oxygen consumption or glucose metabolism after phagocytosis, do not produce hydrogen peroxide or superoxide, and fail to reduce nitroblue tetrazolium (NBT).³ Most patients with chronic granulomatous disease have recurrent abscesses affecting the skin, lymph nodes, lung, bone, and liver, and many die in childhood.^{1 2} Their infections are predominantly with coagulase-positive staphylococci and fungi. There are several variants of chronic granulomatous disease, some of which occur in female patients. They are summarised below.

Glucose-6-phosphate dehydrogenase (G6PD) deficiency	Male patients ⁴
G6PD instability	Female patients ⁵
	Male patients ⁶

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Reduced nicotinamide-adenine dinucleotide (NADH) oxidase deficiency	Male patients ⁷
Glutathione peroxidase deficiency	Female patients ⁷
Job's syndrome	Female patients ⁸
Lipochrome histiocytosis	Female patients ^{9 10}
	Female patients ¹¹

Patients with these disorders fail to reduce NBT normally; however, those with Job's syndrome (red-haired, fair-skinned girls with cold abscesses) are a mixed group, some having normal NBT reduction,¹² and some failure of reduction.¹⁰

One mechanism for the intracellular killing of bacteria seems to depend on the interaction of hydrogen peroxide with the enzyme myeloperoxidase and a halide.¹³ Myeloperoxidase deficiency has been described.¹⁴ These patients reduce NBT normally and only occasionally have a measurable defect in bacterial killing.

We report here a deficiency of neutrophil pyruvate kinase associated with an intracellular killing defect in the neutrophils. This has not been described before. The patient had recurrent staphylococcal infections throughout her 31 years of life and reduced NBT normally.

Case history

The patient, born in February 1944, was the first child of previously unrelated parents. She was of above average intelligence and worked as a needlework instructress. In her first week of life she had a septic finger. Infected eczema developed at 3 weeks and lasted until she was four years of age. She then had a series of staphylococcal boils and abscesses occurring about once a month; some episodes were severe, with infection in the right knee, left mastoid, and perineum. One abscess in the neck discharged for six months. She had staphylococcal pneumonia when 6, 8, and 12 years old. The last attack was followed by a staphylococcal empyema that required rib resection and discharged for about nine months. At the age of 13 she developed staphylococcal osteomyelitis of the lumbar spine with a paravertebral abscess. A sinus in her left loin subsequently discharged for three years. During childhood she had had measles, rubella, chickenpox, and whooping cough, and had recovered normally from each of these.

She continued to have staphylococcal abscesses about once a month until 29 years of age, when she started taking flucloxacillin 250 mg twice a day prophylactically. In the next 18 months she had only two further abscesses.

Chronic nail infection with *Candida albicans* had started at the age of 25 and persisted despite candida-positive transfer factor. She had candida stomatitis terminally. She became progressively breathless with cough and haemoptysis due to large pneumatoceles. When aged 30 she developed cor pulmonale. At that time she had finger-clubbing and severe pulmonary hypertension. Before her death at the age of 31 she had had about 45 hospital admissions. Necropsy showed pneumonia, pulmonary embolism, and deep vein thrombosis. There were no granulomata.

All her serious infections had been caused by *Staphylococcus aureus*. *Candida albicans* infections of the nails and mouth were late events. There was no systemic fungal infection.

Methods and results

The following investigations were carried out during the last five years of the patient's life.

Enzyme assays and kinetic studies were carried out on neutrophils collected by the method of Baehner and Nathan.¹⁵ After performing white cell and granulocyte counts the cells were disrupted either by sonication or by freezing and thawing three times. Other tests carried out are listed in tables I-III.

TABLE I—Results of neutrophil-function tests

Test	Patient's cells	Control cells	Method
Quantitative NBT reduction	0.23	0.20	Baehner and Nathan ¹⁵
Neutrophil chemotaxis to casein	40 cells/high-power field	>20 cells/high-power field	Keller <i>et al</i> ¹⁴
Chemotaxis to endotoxin	3318 cpm	2329 cpm	Gallin <i>et al</i> ¹⁵
Phagocytosis of baker's yeast in autologous serum	6 organisms/cell	6 organisms/cell	Miller <i>et al</i> ¹⁶
Bactericidal test (<i>Staph aureus</i>)	0.49	0.27 (SD ± 0.1)	Chandra <i>et al</i> ¹⁷
Nephelometric method (OD* 140 min/20 min)			
Bactericidal test (<i>Staph aureus</i>)	0.27	0.075 (SD ± 0.05)	Quie <i>et al</i> ¹⁸
Colony count method (OD* 140 min/20 min)			
Metabolic burst ¹⁴ CO ₂ production			Holmes <i>et al</i> ¹⁴
Unstimulated	Normal		
Stimulated	Normal		

*OD = Optical density

Neutrophil responses—The patient's neutrophils killed *Staph aureus* less well than did control cells in both nephelometric and colony count bactericidal tests (table I). Neutrophil chemotaxis in response to casein or endotoxin stimuli and phagocytosis of yeasts were normal, as was NBT reduction and CO₂ production after phagocytosis.

Neutrophil enzymes—Pyruvate kinase (PK) activity was 16% of the control value after 24 hours. All other enzymes measured were normal (table II). Because of the low PK activity we looked for the presence of an abnormal enzyme. The patient's neutrophil PK showed decreased activity above 37°C compared with that of the control (fig 1). The enzyme was also unstable when stored at 0-4°C, its activity decaying rapidly as compared with the control, having only 10% of its original activity after 30 hours (fig 2). The optimum pH was the same for the patient as for the control, though at a lower level (fig 3). Neutrophil and erythrocyte PK electrophoretic patterns were normal in the patient and her family on starch gel at pH 7.5. Owing to the rapid spontaneous decay we were unable to obtain binding constants for other substrates.

Lymphocyte function—The patient's lymphocytes responded normally to in-vitro stimulation by specific and non-specific stimuli. Delayed hypersensitivity skin tests gave negative results (table III).

Other tests—Total white cell count was normal or raised (9×10^9 - 16×10^9 /l; 9000 - $16\,000$ /mm³), with a normal or raised neutrophil count (5×10^9 - 14×10^9 /l; 5000 - $14\,000$ /mm³). Immunoglobulins were

TABLE III—Results of tests of antibody function and cell-mediated immunity

Test	Result	Method
Mantoux 1/1000	Before transfer factor, negative	Ng <i>et al</i> ¹⁹
Candida skin test 1/1000 (Hollister-Stier)	After transfer factor, negative	
Migration inhibition factor (tuberculin antigen)	0.64 (positive <0.73)	Ng <i>et al</i> ¹⁹
Phytohaemagglutinin stimulation	Stimulation index 80 (positive)	
Antistaphylococcal α -haemolysin	2×10^8 IU/l	Ng and Alexopoulos ²⁴
Leucocidin	1/256	
Isohaemagglutinins		
Anti-A ₁	1/32	Espamol <i>et al</i> ²⁵
Anti-A ₂	1/16	
Anti-B	1/64	

TABLE IV—Neutrophil PK ($U \text{ min}^{-1}/10^{-9}$ cells) in patient and her family

Patient	..	..	..	..	..	2.8
Father	..	..	..	..	..	46.7
Mother	..	..	..	..	..	45.0
First younger sister	..	..	..	..	..	39.7
Second younger sister	..	..	..	..	..	40.6

FIG 1—Temperature dependence of neutrophil PK activity. ○ = Patient. ● = Normal control. OD = Optical density.

raised (IgG 369×10^3 IU, IgA 158×10^3 IU, and IgM 223×10^3 IU/l). Anti-staphylococcal antibody was found (table III). Total haemolytic complement was normal (45×10^3 Kabat units/l), as was C3 (76%).

FAMILY STUDIES

The blood counts and neutrophil PK activity of the parents and two younger sisters of the patient were normal. There was no significant variation in activity between any of the samples after storage at 4°C for 24 hours.

TABLE II—Results of intracellular enzyme studies

Cell	Test	Patient's cells	Control cells	Method
Neutrophil	G6PD	$9.6 U \text{ min}^{-1} 10^{-9}$ cells	$10.2 U \text{ min}^{-1} 10^{-9}$ cells	Motulsky and Yoshida ²⁹ Paglia and Valentine ³⁰ Beutler ³¹ (pp 40-1) Beutler ³¹ (pp 51-2) Beutler ³¹ (pp 56-7) Kaplow ³²
	Glutathione peroxidase	$156 U \text{ min}^{-1} 10^{-9}$ cells	$286 U \text{ min}^{-1} 10^{-9}$ cells	
	Phosphohexose isomerase	$56.5 U \text{ min}^{-1} 10^{-9}$ cells	$78.8 U \text{ min}^{-1} 10^{-9}$ cells	
	Phosphoglycerate kinase	$22.0 U \text{ min}^{-1} 10^{-9}$ cells	$32.0 U \text{ min}^{-1} 10^{-9}$ cells	
	PK after 24 hours at 0-4°C	$2.8 U \text{ min}^{-1} 10^{-9}$ cells	$26.6 U \text{ min}^{-1} 10^{-9}$ cells	
	Myeloperoxidase (slide test)	Normal		
Erythrocyte	PK	$7.5 U \text{ min}^{-1} 10^{-9}$ cells	$3.5 U \text{ min}^{-1} 10^{-9}$ cells	Beutler ³¹ (pp 56-7)
Lymphocyte	PK	Normal	Normal	Beutler ³¹ (pp 56-7)

FIG 2—Effect of storage at 0–4°C on patient's neutrophil PK activity.

FIG 3—Variation of neutrophil PK with pH. ○ = Patient. ● = Normal control.

Discussion

This patient's history suggested a defect in the antibody-complement-phagocyte pathway. Normal antibody and complement and normal neutrophil counts pointed to a neutrophil defect, which was confirmed by bactericidal tests with *Staph aureus*. Neutrophils from patients with classic chronic granulomatous disease fail to reduce NBT and fail to increase carbon dioxide production during phagocytosis.¹⁶ Our patient's neutrophils were normal for these tests, indicating that hydrogen peroxide production and hexose monophosphate activity were normal. This pointed to a defect in the latter part of the glycolytic pathway. Pyruvate kinase activity was deficient in her neutrophils. The enzyme present was unstable at 0–4°C and unstable above 37°C as compared with the control.

During phagocytosis glucose consumption and lactate production are usually increased.¹⁷ Since PK deficiency blocks the conversion of phosphoenol pyruvate to pyruvate this could

be responsible for the lack of intracellular energy production in our patient (fig 4). The pH in normal phagocytic vacuoles is lower than in the cytoplasm, and may fall to 3.0.¹⁸ This acidity is at least partially due to lactate formation,¹⁹ which would be expected to be impaired with PK deficiency. The acid medium in the phagosome kills some bacteria—for example, pneumococci—and favours the myeloperoxidase-mediated system as well as the digestion of killed organisms by the lysosomal acid hydrolases.³ This might explain the defect in our patient.

FIG 4—Pathway of neutrophil metabolism. (From Klebanoff.³⁶)

There are three main isoenzymes of PK: type 1 (L) is present in the liver and erythrocytes; type 2 (LM) is present in the kidney; and type 3 (M) is present in the neutrophils, platelets, brain, and muscle. PK deficiency is most clearly recognised in the red cells.²⁰ In most patients no abnormal enzyme has been found,²¹ but PK with abnormal kinetics²² and abnormal electrophoretic patterns^{22–23} has been described. No patient with erythrocyte PK deficiency has been shown to have a leucocyte deficiency. Our patient had normal erythrocyte PK. Type 3 PK (found in neutrophils) is thus the most widespread isoenzyme. There was, however, no clinical evidence of a functional defect in other organs in our patient.

We were unable to show any defect in the patient's parents or two sisters. There was no previous family history of severe infections. We are therefore unable to specify the inheritance in this case.

We wish to thank the many people who helped with this study, particularly the patient's family for their co-operation; Sue Povey for performing the PK electrophoresis; Margaret North, Betty Harvey, and David Webster for the neutrophil function test; Ron Ng for the lymphocyte tests and transfer factor; and Dr F C Harris for performing the necropsy.

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Coeliac disease with farmers' lung

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Summary

Two patients with allergic alveolitis due to mouldy hay antigens (farmer's lung) were shown to have malabsorption due to coeliac disease. As similar associations have been found with other alveolar diseases, this association is probably not fortuitous and further population screening should be done.

Introduction

Several recent case reports have shown an association between coeliac disease and diffuse interstitial pulmonary disease. Villous atrophy due to a gluten-sensitive enteropathy has recently been described with extrinsic allergic alveolitis due to avian exposure (bird fancier's lung)¹ and there are reports of sarcoidosis² and fibrosing alveolitis³ occurring with coeliac disease. Apparently patients with coeliac disease have an increased incidence of other autoimmune disturbances, although the meaning of these changes is not clear. Abnormalities of immunoglobulins in both serum and jejunal secretions have been recorded in coeliac disease,⁴⁻⁸ as has an association with Addison's disease,⁹ Sjögren's syndrome,^{10 11} thyroid disease,¹⁰ and ulcerative colitis.¹²

Fibrosing alveolitis is associated with hyperglobulinaemia¹³ and is found in associations with conditions of possible autoimmune aetiology such as ulcerative colitis,¹⁴ Hashimoto's thyroiditis,¹⁵ rheumatoid arthritis,¹⁵ and Sjögren's syndrome.¹⁴⁻¹⁶ The lungs are, of course, often affected in connective tissue disorders, as in rheumatoid arthritis, systemic sclerosis, polyarteritis nodosa, and systemic lupus erythematosus.

I report here two cases of extrinsic allergic alveolitis due to mouldy hay (farmer's lung) occurring in patients with coeliac disease.

Case 1

A hill farmer aged 40 was admitted with a two-month history of progressively increasing shortness of breath, weight loss, and general

malaise. He had close contact with hay and straw as he carried bales on his back each day to feed the cattle in the fields.

He was dyspnoeic at rest and there were widespread fine crepitations heard throughout both lung fields. X-ray examination of the chest and gel immunodiffusion against *Mycoplasma faeni* confirmed farmer's lung. The symptoms quickly improved in hospital without any specific treatment. His chest improved clinically, although it was some months before his chest x-ray appearances reverted to normal.

Four years earlier he had attended a surgical outpatient clinic with mouth ulcers and diarrhoea. Mouth biopsy had shown chronic non-specific ulceration. His haemoglobin had been 10 g/dl and the white cell count $4.6 \times 10^9/l$ ($4600/mm^3$). The red cells had shown a dimorphic picture. No treatment had been given.

Investigation on the second admission showed: haemoglobin 13.6 g/dl, red cell count $5.4 \times 10^{12}/l$ ($5.4 \times 10^6/mm^3$), packed cell volume 49.9%, mean cell volume 76 fl ($76 \mu m^3$). Thyroid function was normal. Serum carotene was 0.80 $\mu mol/l$ ($43 \mu g/100 ml$) (normal 1.1-2.2 $\mu mol/l$ ($60-120 \mu g/100 ml$)), serum calcium 2.1 mmol/l ($8.4 mg/100 ml$) (normal 2.1-2.6 mmol ($8.4-10.4 mg/100 ml$)), folate $<0.5 \mu g/l$ (normal 3.5-24.0 $\mu g/l$), serum lactic dehydrogenase 624 U/l (normal 100-225 U/L), total protein concentration 81 g/l (normal 60-80 g/l), albumin 46 g/l (normal 35-50 g/l), and globulin 35 g/l (normal 25-30 g/l). He weighed 53 kg. Jejunal biopsy showed subtotal villous atrophy. He was started on a gluten-free diet with considerable improvement in his general health. At the last review, six months after his admission, he had put on 8 kg in weight, and his chest was clear both clinically and on x-ray examination. Haemoglobin was 15 g/dl and calcium levels were normal.

This man had classical extrinsic alveolitis due to mouldy hay but had a concomitant coeliac syndrome, which had been producing symptoms for at least four years.

Case 2

A farmer's wife aged 58 attended another hospital in 1968 complaining of shortness of breath, weight loss, and epigastric soreness. She was recorded as having bilateral basal pleural rubs and being dyspnoeic at rest but not cyanosed. One chest x-ray picture was reported as being normal. Another several weeks later showed fine miliary mottling. Haemoglobin was 14.6 g/dl with a normal white cell count and differential count. Total proteins were increased, with the albumin globulin ratio at 0.9. Serum electrophoresis showed an increase in α_1 - and γ -globulin.

She was readmitted in 1975, when aged 65, and was found to be acutely dyspnoeic. There were crepitations in both lung fields. She was apyrexial. Sputum culture gave a heavy growth of *Klebsiella pneumoniae* and she was treated with antibiotics. Serial sputum cultures for tuberculosis were negative. Haemoglobin was 12.7 g/dl and white cell count $8.1 \times 10^9/l$ ($8100/mm^3$) with an eosinophil count of 6%. Chest x-ray examination showed widespread chronic inflammatory changes. Tests for extrinsic alveolitis showed a positive gel immunodiffusion against *M faeni*. Farmer's lung was diagnosed clinically and on discharge from hospital she was feeling better.

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