CHRONIC GRANULOMATOUS DISEASE – A REPORT IN TWO MALAY FAMILIES

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ABSTRACT

Chronic granulomatous disease (CGD) is a very rare disease whose defect lies in an abnormal intracellular killing resulting in recurrent abscesses, lymphadenitis and granuloma formation. We describe 2 Malay male infants with CGD whom we believe to be the first report of this disorder in Malays. Both children presented with recurrent abscesses, pneumoniae and hepatosplenomegaly; lymphadenopathy was also present in one of the patients. The organisms isolated were catalase positive bacteria. Both neutrophil chemiluminescence (against fungal and bacterial antigens, phorbol myristate acetate) and intracellular killing assays were severely depressed. Recognition of CGD is important as great strides have been made in the treatment of this disease which include gamma interferon therapy besides the conventional prophylactic antibacterial therapy.

Keywords: Chronic granulomatous disease, recurrent abscesses, defective chemiluminescence, intracellular killing.

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INTRODUCTION

The term chronic granulomatous disease is applied to a syndrome of recurrent infections involving skin, reticuloendothelial system, and lungs with an inability of the phagocytes to kill bacteria as a result of a deficiency in converting oxygen to its metabolites[1]. The pattern of inheritance in the majority is sex-linked; however, in 15% of the reported cases, autosomal recessive inheritance had been observed[2]. Up to 1987 slightly more than 300 cases had been reported worldwide[2]. Its incidence is estimated to be 1 in one million in Caucasians[3] but the actual figures in Orientals is not known. While CGD has been reported in Japanese and Chinese[4], there had been no reports in the Malays.

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PATIENTS AND METHODS

Case 1

NJ, an 11-month-old Malay boy was referred to General Hospital Kuala Lumpur (GHKL) in October 1991 for recurrent infections including cutaneous abscesses. From the age of 1 week, the child had frequent boils. He had numerous admissions at the referring hospital (General Hospital, Alor Star) for various infections which included septicaemia and recurrent urinary tract infections. Hepatosplenomegaly had been noted as early as 3 months of age. One week prior to admission to our unit, he had been admitted to the referring hospital for pneumonia.

He had an uneventful birth with a weight of 4.0 kg. There was no consanguinity between the parents. He has one live sibling, a sister, while all his 4 male siblings died in early infancy.

Physical examination revealed a miserable looking child who was afebrile and mildly anaemic. His weight was 6.0 kg (–3 SD below the median on the NCHS chart). Axillary and cervical lymphadenopathy was noted. He had hepatomegaly at 3.0cm and mild splenomegaly. The cardiovascular, respiratory and neurological examinations were essentially normal.

The laboratory examinations were as follows: haemoglobin 7.9 g/L, with a leukocytosis of 24.7 x 10^9/L, neutrophil 36%, lymphocyte 61%, platelets 619 x 10^9/L; serum protein 85 g/L, alkaline phosphate 260 u/L, alanine transaminase 21 u/L; serum iron 6.6 micro mol /L, TIBC 52 micro mol/L. Chest X-ray showed opacities in right upper and mid zones.

Immune function assays

A brief account of the methods of immune function assays to detect the immunological abnormalities will be described here.

Serum immunoglobulins

Immunoglobulin G, A, and M were assayed by single radial immunodiffusion using commercially available plates (Behringe-HG Marburke).

Cellular immune function studies

T and B cell enumeration

Each of the monoclonal antibodies, CD19, CD3, CD4, CD8 (Becton & Dickinson) were added to respective whole blood samples. After incubation, Faclyse solution (Becton & Dickinson) was added to lyse the red blood cells and subsequently centrifuged. The supernatant was discarded, the cells washed twice and finally resuspended in PBS (Phosphate buffered saline). The lymphocyte subsets were identified using the Flowcytometer (Becton & Dickinson).

Lymphocyte proliferation to mitogens

The proliferation of lymphocytes to phytohemagglutinin (PHA) and concanavalin A (Con A) was determined after culturing the PBMC with varying concentrations of mitogens (PHA, ConA), pulsed with H^3 thymidine on day 3, harvested and placed in a scintillation counter 18 hours later. The radioactive count of stimulated lymphocytes was compared to unstimulated lymphocytes (stimulation index, SI) to indicate the degree of proliferation.

Phagocytic function studies

The measurement of phagocytic function by luminol-dependent chemiluminescence was done following the method of Easmon et al[5]. Briefly polymorphonuclear leucocytes (PMN) isolated from heparinised blood was mixed with luminol, opsonised particles (*zymosan, Staphylococcus aureus*) or phorbol myristate acetate (PMA) in the reaction vials which was then placed in the light proof chamber of the luminometer (LKB BioOrbit). The reaction constituents were prewarmed at 37°C prior to use. The resulting output in millivolts (mV) was recorded by a digital printout for every 10 seconds.

The method of addressing killing activity was adapted from Lehrer & Cline[6] with modifications. PMN obtained were incubated with live *Candida* organisms for a period of 60 minutes. The PMNs were then fixed, smeared and finally stained with Wright stain. The percentage of dead to live cells were computed to be the percentage of killing activity.

Serum immunoglobulin (mg/dL) IgG 2190 (n 310-1100), IgA 126 (n 25-75), IgM>483 (45-200). T and B cell enumeration: CD19(B cell) 22%, CD3 (T cell) 65%, CD4:CD8, 1.0; cellular proliferation to mitogens (PHA and ConA) were normal with a stimulation index (SI) of >20.

Phagocytic function assay

Chemiluminescence assay of patient's neutrophils against opsonised *zymosan* (PTZ1), opsonised *staphylococcus aureus* (PTS1) and PMA (PTPM1) compared to normal controls (NZ1, NS1 and NPM1 respectively) is shown in Fig 1. The chemiluminescence response of patient's neutrophil was observed to be severely depressed to the 3 stimuli compared to controls by as much as 94%-97%.

Intracellular killing to *candida* was similarly depressed in patient (PTC1) when compared to control (NC1), 5% and 95% respectively (Fig 2). Severely depressed neutrophil chemiluminescence and intracellular killing in this patient is consistent with CGD. Chemotaxis was normal.

Complement proteins C3, C4 and G6PD levels were found to be within the normal range.

Progress

The patient developed cutaneous abscesses during his first week of hospital stay for which *Chromobacterium violaceum* was isolated. He developed pneumonia subsequently but responded to antibacterial therapy.

Fig 1 – Chemiluminescene assay using opsonised *zymosan* (Z), *Staphylococcus aureus* (S) and PMA (PM) on normal (N) and patient's (PT) neutrophils for Case 1.

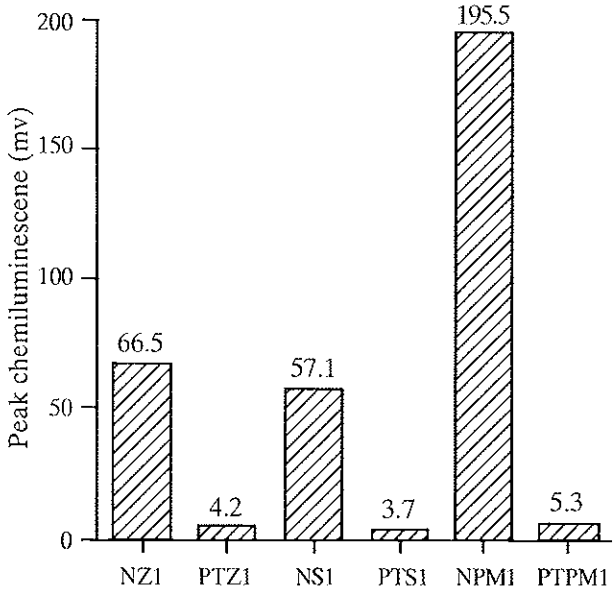
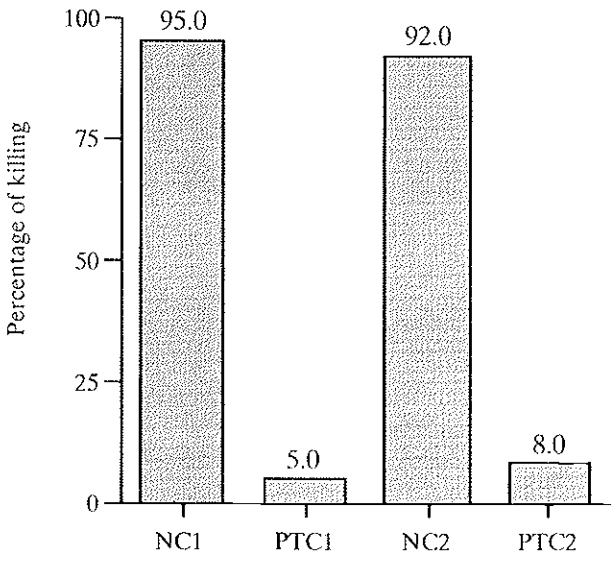


Fig 2 – Candidacidal assay using normal (N) and patient (PT) neutrophils from both Cases 1 and 2.



Case 2

MF, a 5-month-old boy was admitted in April 1992. He had been referred from another hospital (Johore Bahru) for recurrent fever, jaundice and progressive abdominal distension since the age of 2 months. During the period of hospitalisation in Johore Bahru Hospital he was noted to have septicaemia with gross hepatosplenomegaly. Conjugated hyperbilirubinaemia was also observed.

He was born at home with birth weight of 3.5kg. His parents are not consanguineous. His elder brother died at the age of 3 months with similar clinical features – a prolonged fever, hepatosplenomegaly and perianal abscesses. His elder sister is still alive.

He had been immunised with BCG, first dose of hepatitis B and triple/oral polio antigen with no untoward reactions.

On admission to the GHKL he was noted to be severely

jaundiced, anaemic, and febrile. His weight was 5.5 kg (–3 SD below median on the NCHS chart). No dysmorphic features nor lymphadenopathy was noted but he had gross hepatomegaly of 8 cm and splenomegaly of 5 cm. The rest of the system examination did not reveal any abnormality.

Laboratory data were as follows: Hb 8.7 g/L, leukocyte count 6.1 x 10^9/L, differential of neutrophils 65%, lymphocytes 27%, platelets 100 x 10^9/L. Liver function test: total protein 47 g/L, albumin 22 g/L, total bilirubin 334 mmol/L with a conjugated bilirubin of 144 mmol/L, alkaline phosphate 140 u/L, alanine transaminase 62 u/L; hepatitis B antigen negative.

Ultrasound studies did not show liver abscess, choledocal cyst nor a dilated biliary tree. HIDA scan showed poor liver uptake of the isotopes. Bone marrow examination did not reveal any storage changes while urine examination for amino acid chromatogram was normal.

Progress

One week after admission he developed another episode of septicaemia with disseminated intravascular coagulation defect (DIVC); he collapsed requiring subsequent ventilator support. He came out of ventilator support but was noted to have cutaneous abscess and subsequently persistent perianal abscess. His earlier blood culture grew *Staphylococcus epidermidis* but a subsequent blood culture done a week later grew methicillin-resistant *Staphylococcus aureus*. Two months after admission he developed meningitis which was complicated by subdural effusion soon after (no organism was isolated). Appropriate antibacterial therapy was instituted. The subdural effusion was drained and *E. coli* was subsequently isolated from the blood culture. At this juncture the diagnosis of chronic granulomatous disease was entertained.

Immunological investigations were as follows: serum immunoglobulin in mg/dL, IgG 1190(n 240-890), IgA 77.7(n 10-60), IgM 47.9(n 20-90). T and B cell enumeration: CD19-2%, CD3-88%, CD4:CD8 - 2.1:1, cellular proliferation to mitogen (PHA and Con A) were normal.

Phagocytic function assay

Chemiluminescence assay of patient's neutrophil against opsonised *zymosan*, opsonised *Staphylococcus aureus* and PMA (PTZ2, PTS2 and PTPM2 respectively) were similarly depressed compared to normal controls (NZ2, NS2 and NPM2 respectively) by more than 87% as depicted in Fig 3. The intracellular killing of *candida* by patients neutrophils (PTC2) was similarly depressed compared to normal control (NC2), 8% and 94% respectively (Fig 2).

The G6PD and the complement levels C3 and C4 were normal.

Severe depression of stimulated neutrophil chemiluminescence of patient to opsonised *zymosan*, *Staphylococcus aureus* and PMA (89%, 87%, 90%) but intermediate in the mother (60%, 46%, 25%) was observed while chemotaxis was normal (Table I). The diagnosis of this child was consistent with CGD and antibacterial therapy was instituted. However, the child finally succumbed after a hospital stay of 4 months. The chemiluminescenec of his mother's PMN is consistent with that of a carrier.

A piece of the liver taken during the postmortem was the only tissue available for histopathological examination as his parent refused an autopsy. It showed a normal liver architecture, with the single portal tract containing mainly lymphocytes. The hepatic parenchyma showed prominent

Fig 3 – Chemiluminescence assay using opsonised *zymosan* (Z), opsonised *Staphylococcus aureus* (S) and PMA (PM) on normal (N) and patient (PT) neutrophils for Case 2.

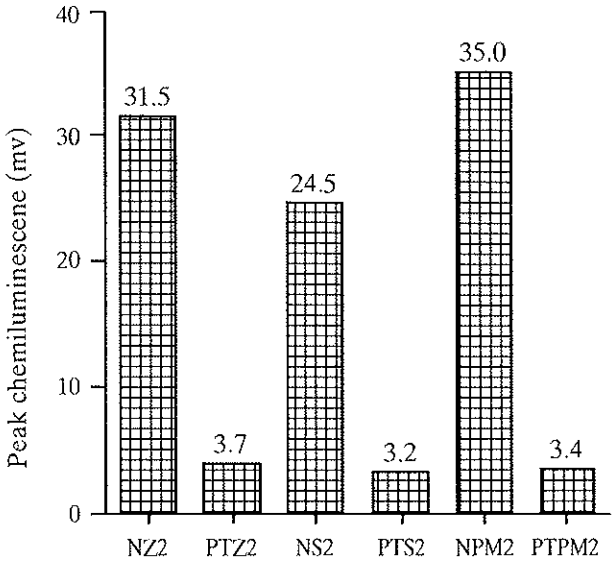


Table I – Chemiluminescence assay of neutrophils from the mother of patient 2.

Subjects	Peak chemiluminescence (mV) using opsonised *zymosan*	opsonised *staphylococcus aureus*	PMA
Mother	12.2	9.8	66.8
Control	31.0	18.0	89.6
% depression	60.0	46.0	25.0

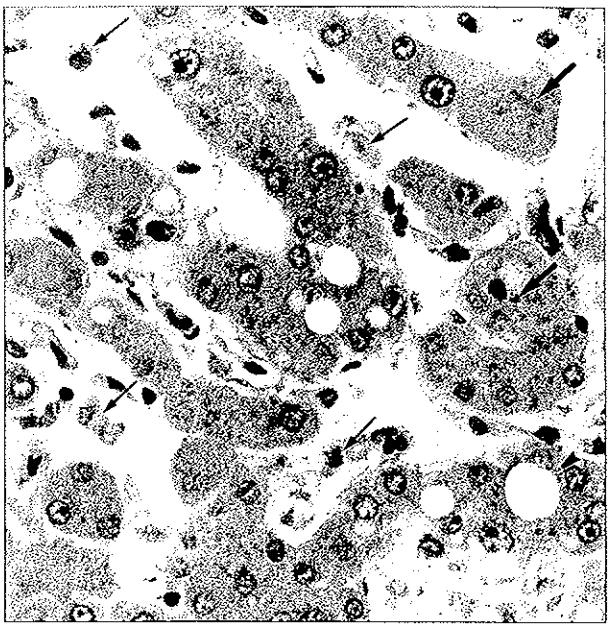
sinusoids with variable Kupfer cell hyperplasia. Scattered foci of microvesicular steatosis were also noted. In addition, the hepatocytes and to some extent the Kupfer cells showed granular brownish pigmentation. Some stained positive for hemosiderosis (Perl's stain) while others were positive for lipofuscin. Bile pigments were absent (Fig 4). Although no characteristic granuloma or microabscess were seen, the presence of pigmented lipid materials in the histiocyte were suggestive of CGD[1,7]; the severe septicaemia, shock that ensued and the various drugs and multiple blood transfusion given could have modified some of the features.

DISCUSSION

The phagocytic system is the earliest non-specific defence mechanism against microbes with the neutrophils, monocytes and eosinophils functioning as effectors and are responsible for ingestion and killing of pyogenic bacteria and fungi. In X-linked type chronic granulomatous disease, the phagocytic abnormality lies in the inability of the neutrophils to generate toxic oxygen intermediates (hydrogen peroxide, hydroxyl radical, superoxides) which are required for the killing process during phagocytosis[8]. This defect has been shown to be related to the absence of cytochrome b 588 and thus impairing the cell's NADH oxidase activity[2].

The defect in chronic granulomatous disease predisposes patients to infection by catalase positive organisms (*Staphylococcus aureus, Aspergillus, Chromobacterium*

Fig 4 – Histopathology of liver section (x 400). Brownish pigments that stained mainly for haemosiderin with some lipofuschin were present within hepatocytes (large arrows) and to a lesser extent in Kupfer cells (small arrows) within dilated hepatic sinusoids. Foci of steatosis (arrow head) were also seen.



violaceum;). The catalase destroys hydrogen peroxide (produced by the organism) which could otherwise be used by the host neutrophil to kill the same bacteria. In catalase negative organism on the other hand, the endogenous hydrogen peroxide not destroyed provides the host with toxic oxygen derivative for bactericidal activity against that same organism[9].

The clinical features of both the above patients are consistent with the diagnosis of chronic granulomatous disease. Both patients were males. There had been death in male siblings of both in early infancy, sparing the female siblings. The symptoms started in early infancy with formation of recurrent abcesses in the skin and perianal areas; reticuloendothelial involvement was manifested by enlarged livers and spleens and lymphadenopathy. Pneumonia was seen in both. However jaundice as in Case 2 is an atypical presentation. Interestingly, the organisms isolated from both patients were catalase positive bacteria viz *Chromobacterium violaceum* and methicillin resistant *Staphylococcus aureus. Staphylococcus epidermidis and E. coli* were also isolated from blood of Case 2.

Our diagnosis is further corroborated with the markedly reduced chemiluminescence of neutrophils from both patients when stimulated with *zymosan, Staphylococcus aureus* and PMA in comparison to that of controls – viz a reduction of almost 90% of controls. Microbicidal activity of the neutrophils against *Candida* was also depressed. The mother of Case 2 showed a depression of stimulated neutrophil chemiluminescence that was intermediate between normal and diseased individuals indicating a carrier status (Table I). However Case 2 had a typical presentation of jaundice with liver histopathological features suggestive of CGD. However, we do not discount the possibility of it being a variant of chronic granulomatous disease[1]. Heterogeneous group of variants of CGD has been described[7].

The impaired respiratory burst of CGD phagocytes have been detected by both chemiluminescence assay and nitroblue tetrazolium dye test. We have preferred the chemiluminescence assay as it is the easiest, most rapid and most reproducible[1,10] of all the assays to detect respiratory burst. Furthermore, the assay based on NBT reduction is not considered specific for the detection of superoxide or hydroxl radicals[11]. In chronic granulomatous disease marked reduced chemiluminescence is observed[12].

Prophylactic antibiotics especially with the use of trimethoprim/sulfamethoxazole once a day have been shown to be effective in reducing the frequency of infections in CGD patients[9]. Successful bone marrow transplant has been reported in one case[12,13]. Graft failure and loss of graft after temporary engraftment have been a problem in allogeneic bone marrow transplant. Recent evidence supports that therapy with gamma interferon in CGD is effective in reducing the frequency of severe infections as well as being well tolerated[14].

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