Chronic Granulomatous Disease with Neutrophil Membrane Cytochrome b Deficiency : Demonstration by Immunochemical Staining with Monoclonal Antibody

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Cytochrome b deficiency in the peripheral granulocytes of two male patients with
chronic granulomatous disease was demonstrated by an immunocytochemical assay
using a monoclonal antibody, 7D5, against human neutrophil cytochrome b. A
mosaic of cytochrome b positive and negative neutrophils, indicating a carrier state
in an X-linked trait, was found in the mother of patient 1 but not in the mother
of patient 2. ——— chronic granulomatous disease ; cytochrome b ; leukocyte

Chronic granulomatous disease (CGD) is an inherited syndrome characterized by a recurrence of bacterial and fungal infections that is due to the absence of phagocytic killing of microorganisms (Tauber et al. 1983 ; Seger 1984). Cytochrome b, a component of the NADPH-dependent superoxide (O_2^-)-generating oxidase system, has been demonstrated to be absent in the neutrophils of X-linked CGD patients (Segal et al. 1983 ; Bohler et al. 1986). Although cytochrome b is usually determined spectroscopically on dithionite reduced minus oxidized differential spectra (Segal et al. 1983). A recently developed immunocytochemical assay using a monoclonal antibody against human neutrophil cytochrome b, designated 7D5, has been demonstrated to detect the lack of the antigen (cytochrome b) in neutrophils from X-linked CGD patients (Nakamura et al. 1987a, b). This immunocytochemical method provides a new tool for the diagnosis and genetic study of CGD.

In this communication two CGD patients are described. Their peripheral phagocytes were demonstrated immunocytochemically as well as spectroscopically to be deficient in cytochrome b. An unusual feature is that the mother of one of the patients is not a carrier.

CASE REPORTS

Patient 1 (K.S.)

A 12-year-old boy, 138 cm in height and 28 kg in weight, the only child of healthy parents. He has had a history of frequent infections since his early infancy including perianal abscess, pyoderma, otitis media, lymphadenitis, pneumonia and aspergillosis. Since the diagnosis of CGD was made at the National Children's Hospital in Tokyo at the age of 1 year and 6 months he has undergone treatment with sulfamethoxazole/trimethoprim. In the last 3 years, the pulmonary aspergillosis has remained little changed despite treatment with antifungal drugs including amphotericin B. The diagnosis of CGD was confirmed by the absence of nitroblue tetrazolium (NBT) reduction and luminol dependent chemiluminescence (CL) in neutrophils stimulated by phorbol myristate acetate (PMA), opsonized zymosan (OZ), and a chemotactic factor, *N*-formyl-methionyl-leucyl-phenylalanine (FMLP). Severely impaired superoxide production of PMA stimulated granulocytes was also found (Table 1).

Patient 2 (H.S.)

A 6-year-old boy, 105 cm in height and 17.8 kg in weight. A sister and parents are in good health. Since his early infancy he has had recurrent infections such as pyoderma, liver abscess, pneumonia complicated with pyothorax, pulmonary tuberculosis, lymphadenitis and periodontitis. At age 5 he was first diagnosed as having CGD; this was based on the findings that his neutrophils were deficient in NBT reduction and luminol dependent CL after stimulation by PMA, OZ and FMLP, and bactericidal activities. Superoxide production of stimulated granulocytes was severely impaired (Table 1).

MATERIALS AND METHODS

A neutrophil-rich fraction was prepared from acid citrate-dextrose treated whole blood by polyvinyl-pyrrolidone sedimentation as previously reported (Wakayama et al. 1982), followed by hypotonic lysis of residual red blood cells. The cell concentration of the

TABLE 1. *Granulocyte functions of CGD patients*

	Patient 1 (K.S.)	Patient 2 (H.S.)	Control
NBT* (%)	0	0	95
Chemiluminescence (% of control's)†			
Opsonized zymosan	<0.01	0.1	100
FMLP	0.2	2.5	100
PMA	0.01	0.1	100
O ₂ -production* (n mole/min/10 ⁶ cells)	0.03	0.07	7.1

* PMA stimulation.

† Different instruments were used for estimation in each patient.

fraction was adjusted to $0.3\text{--}1.0 \times 10^7$ per ml. The cytochrome b content of the neutrophils was measured spectroscopically by reduced minus oxidized difference spectra using sodium dithionite as a reducing agent in conjunction with carbon monoxide treatment as previously reported (Pasquier et al. 1985). Immunocytochemical studies were based on the method using the monoclonal antibody, 7D5, as previously described (Nakamura et al. 1987a). Cells (mainly neutrophils) adhering to a slide glass were incubated with 7D5, which was followed by immunocytochemical staining with an avidin-biotin-peroxidase system.

RESULTS

The cytochrome b content of the neutrophils as determined spectroscopically was undetectable in patient 1 and markedly decreased in patient 2 (Table 2). While the mother of patient 1 showed a cytochrome b content intermediate between the patient and controls, the mother of patient 2 showed a value compatible with that in the controls. Immunocytochemistry using the MAb 7D5 showed few antigens detectable in the neutrophils from patients 1 and 2 (Fig. 1). Neutrophils from the mother of patient 1 were disclosed to have two populations, one (18%) positive for 7D5, and another negative for 7D5. In contrast, neutrophils from the mother of patient 2 were almost all 7D5-positive (Table 2). His father also was positive for 7D5.

DISCUSSION

In both patients described in the present paper the lack of cytochrome b in leukocytes was demonstrated by immunocytochemical studies as well as by spectroscopic assays. This confirms the previous report that the immunocytochemical staining with the monoclonal antibody, 7D5, is of practical use in demonstrating the lack of cytochrome b in male patients with CGD (Nakamura et al. 1987a, b). It was, however, unexpected that in the mother of patient 2, who appeared to be an obligatory heterozygote, all granulocytes examined were positive for cytochrome b by immunocytochemical staining.

Although the heterogeneity of CGD is known to be more extensive than just X-linked, cyt b negative and autosomal, cyt b positive forms (Borregaard et al.

TABLE 2. *Cytochrome b measurements of granulocytes from CGD patients and their mothers by immunocytochemical and spectrophotometric methods*

	7D5-positive cells (%)	cyt b content (p mole/ 10^7 cells)
Patient 1 (K.S.)	0	<2.6
Mother	18	18
Patient 2 (H.S.)	0	9-17*
Mother	99	50-60
Controls	99	48-98: mean 74

* Results of repeated tests.

Fig 1. Immunocytochemical detection of the antigen reacting with 7D5 in peripheral granulocytes from patients (a, patient 1; b, patient 2) and their mothers (c, patient 1's; d, patient 2's).

1983; Seger 1984; Segal 1985; Weening et al. 1985; Bohler et al. 1986), the findings observed in the mother of patient 2 remain inexplicable. Further family studies are awaited for the clarification of genetic variation in the patient.

Recently, Royer-Pokora et al. (1986) cloned a gene on the X-chromosome of which transcript was found to be absent or structurally abnormal in X-linked CGD. The predicted protein deduced from the cDNA, designated as the X-CGD protein, has not been shown to correspond to cytochrome b. On the other hand, two subunits consisting of cytochrome b have been demonstrated to be deficient in phagocytes with X-linked CGD (Segal 1987). The primary lesion in X-linked CGD, in particular in relation to the cytochrome b deficiency, remains to be elucidated.

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