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## CASE REPORT

### NORMAL MICROBICIDAL FUNCTION OF MONOCYTES IN A GIRL WITH CHRONIC GRANULOMATOUS DISEASE

C. WEEMAES,<sup>1</sup> P. LEIJH,<sup>2</sup> D. BLUSSÉ VAN OUD ALBLAS,<sup>2</sup>  
J. VAN DER MEER<sup>2</sup> and R. VAN FURTH<sup>2</sup>

From the <sup>1</sup>Department of Paediatrics, University Hospital, Nijmegen  
and the <sup>2</sup>Department of Infectious Diseases, University Hospital,  
Leiden, The Netherlands

**ABSTRACT.** Weemaes, C., (Department of Paediatrics, University Hospital, Nijmegen, The Netherlands), Leijh, P., Blussé van Oud Alblas, D., van der Meer, J. and van Furth, R. (Department of Infectious Diseases, University Hospital, Leiden, The Netherlands). Normal microbicidal function of monocytes in a girl with chronic granulomatous disease. *Acta Paediatr Scand*, 70: 421, 1981.—The history of a 13-year-old girl with a syndrome resembling Chronic Granulomatous Disease (C.G.D.) is described. Metabolic studies in granulocytes and monocytes classified the patient as having C.G.D. The granulocytes failed to kill *Staphylococcus aureus* and *Candida Albicans*; however, the killing of these microorganisms by the patient's monocytes was nearly normal. Family studies revealed no abnormalities in the phagocytic cells of the parents and the siblings.

**KEY WORDS:** Chronic Granulomatous Disease

Chronic granulomatous disease (C.G.D.) is a disorder in which the inability of phagocytic cells to kill certain ingested bacteria or fungi leads to recurrent severe bacterial and fungal infections (1, 2). *Staphylococcus aureus* and other catalase-positive microorganisms are the organisms that cannot be killed normally by the granulocytes of children with C.G.D. and are the usual source of infection (3). In affected patients the granulocytes fail to show the normal stimulation of respiratory activity, increased O<sub>2</sub> consumption, oxidation of glucose through hexosemonophosphate shunt and generation of hydrogen-peroxide (H<sub>2</sub>O<sub>2</sub>), events associated with bactericidal action (4, 5). The monocytes of patients with C.G.D., have a microbicidal defect similar to that of granulocytes: intracellular killing of both *Staphylococcus aureus* (6, 7) and *Candida albicans* (8, 9) is impaired.

C.G.D. has been originally described in

males as a syndrome with an X-linked mode of transmission (10). A similar syndrome has been described in females (11, 12) and the functional and metabolic defects of granulocytes from these patients are indistinguishable from those of male patients. To our knowledge, no studies on monocyte function in female C.G.D. patients have been reported. This case report concerns a girl with a mild C.G.D.-syndrome and abnormal killing by granulocytes, in whom the monocyte functions were practically unaffected. An X-linked mode of transmission could not be demonstrated.

## CASE REPORT

A 13-year-old girl had an uneventful medical history until the age of nine, when she developed scaling inflammatory lesions of the upper lip and nose and intermittent fever. Further physical examination was normal, but roentgenograms showed bilateral pulmonary infiltrates. The only abnormal laboratory findings were leucocytosis and an



Table 1. Leucocyte function

Origin of serum or cells	Phagocytosis of <i>S. aureus</i>				Opsonic activity for <i>S. aureus</i>	
	Granulocytes		Monocytes		60 min (%)	120 min (%)
	60 min (%)	120 min (%)	60 min (%)	120 min (%)		
Patient	97.0	97.8	90.0	96.8	94.0	99.1
Father	99.8	99.8	98.0	99.6	97.0	99.5
Mother	99.7	99.8	98.3	99.1	97.0	99.6
Sister 1	N.D.	99.8	99.8	99.8	93.2	98.0
Sister 2	99.8	99.8	99.3	99.7	99.1	99.8
Normal-controls	96.3±2.8	99.0±1.1	94.8±6.7	98.2±4.2	96.3±2.8	99.0±1.1
C.G.D.-controls	96.8±4.3	98.1±2.1	91.3±7.8	N.D.		

elevated erythrocyte sedimentation rate. The causative agent of the pulmonary infiltrates could not be cultured and antimicrobial therapy was unsuccessful. On glucocorticosteroid treatment the patient recovered, however, rapidly. A recurrence of the pulmonary infiltrates after some months was treated successfully again with glucocorticosteroids. At the age of 10 years she was admitted to the pediatric ward of the University Hospital Nijmegen for an evaluation of her host defence mechanisms. At that time, she was a thin, but healthy appearing girl with scaling inflammatory lesions of the upper lip and nose. She had a conjunctivitis and keratitis. The remainder of the physical examination was normal. Her height was according to age. X-rays of the chest revealed no abnormalities. The serum immunoglobulins were normal: IgG 13 g/l, IgA 2 g/l, IgM 2 g/l, IgE was slightly increased (885 U/ml). The patient's lymphocytes responded normally in vitro to phytohaemagglutinin and poke-weed mitogen and positive delayed hypersensitivity skin reactions were obtained with mumps, trichophyton and *Candida albicans*

as antigens. At the age of 11 years she developed Raynaud's phenomenon. Tests for rheumatoid factor and antinuclear antibody were negative. No antibodies to thyroid, stomach, muscle or mitochondria were detected. The last two years she had some episodes of recurrent ulcerative stomatitis and skin lesions of the upper lip and nose. Her parents and two sisters are healthy.

## MATERIALS AND METHODS

Phagocytosis and intracellular killing of *Staphylococcus aureus* and of *Candida albicans* by granulocytes and monocytes were measured as described elsewhere (9, 13). In patient's serum the opsonic activity for staphylococci was determined with the same assay as for the phagocytosis (13), however, instead of donor serum patient's serum was used. The metabolic burst during phagocytosis was measured as O<sub>2</sub> consumption (14) and H<sub>2</sub>O<sub>2</sub> production (15) by Dr D. Roos of the Central Laboratory of the Netherlands, Red Cross Blood Transfusion Service, Amsterdam, and as nitroblue tetrazolium (NBT) reduction (16). Chemiluminescence was tested in the presence of Luminol (17) by Dr E. Mills at the Laboratory of Micro-

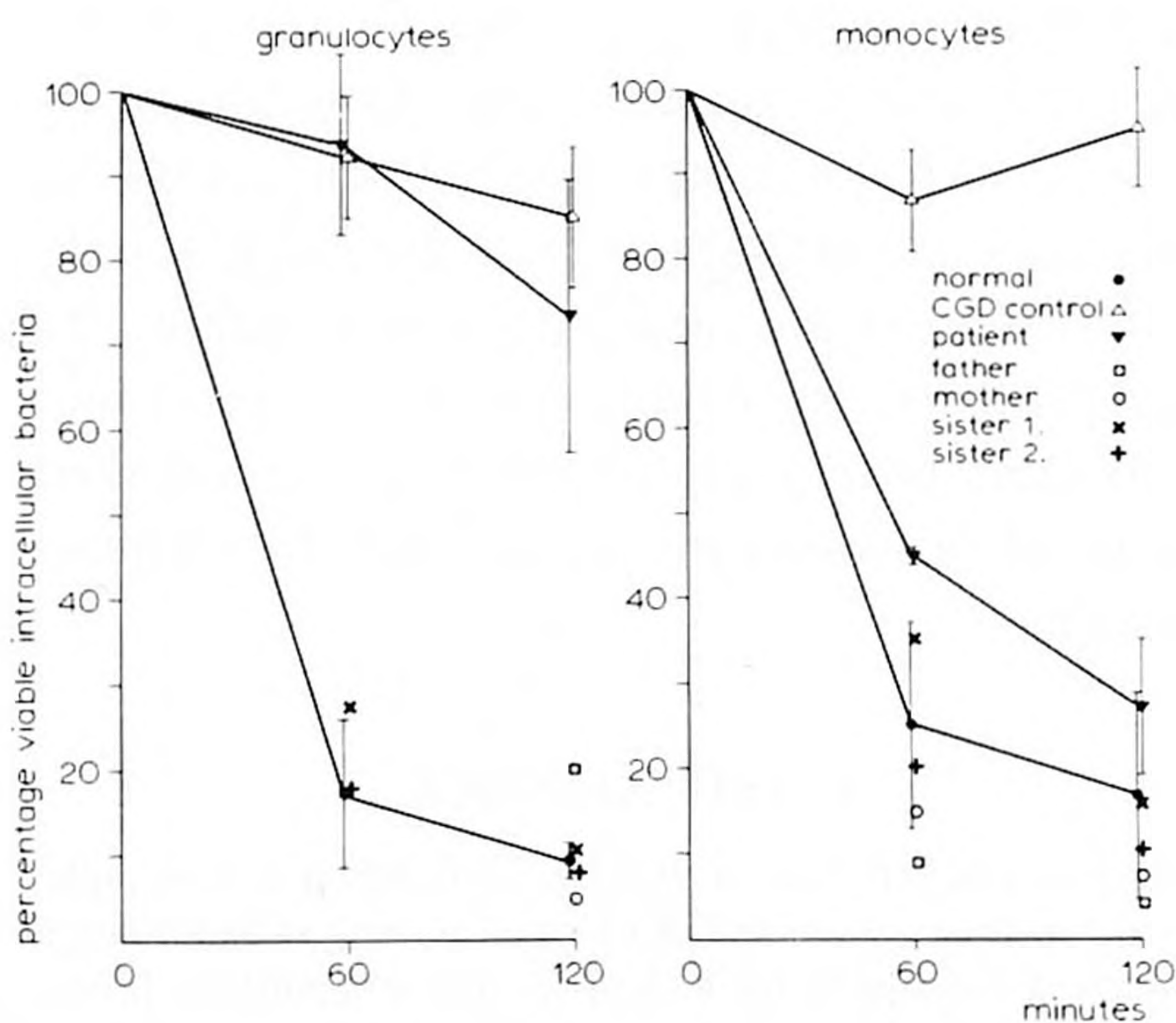


Fig. 1. Rate of intracellular killing of *Staphylococcus aureus*.

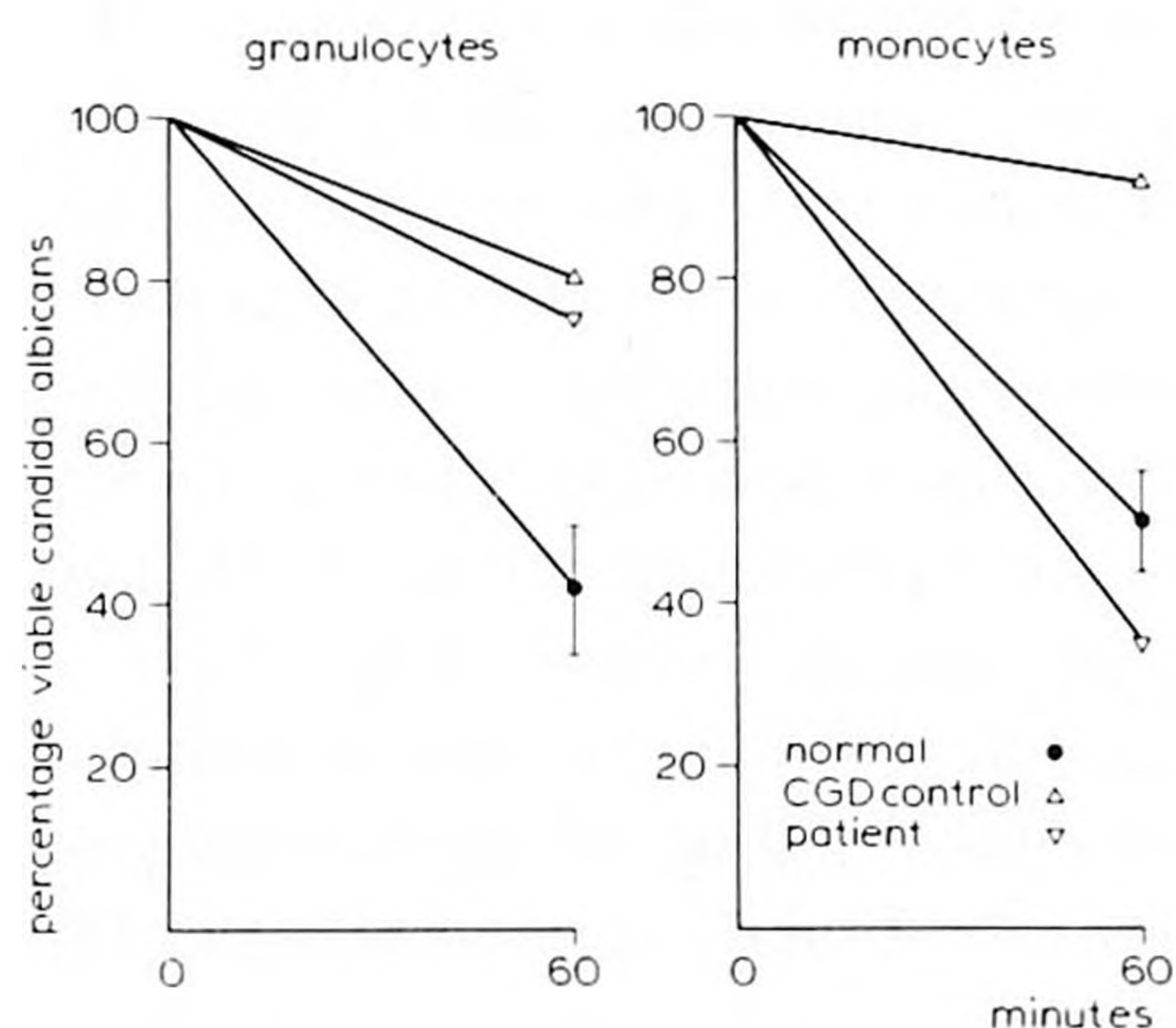


Fig. 2. Rate of intracellular killing of *Candida albicans*.



Table 2. Metabolic activity

	O <sub>2</sub> consumption (nmol O <sub>2</sub> / 10 <sup>6</sup> cells/min)	H <sub>2</sub> O <sub>2</sub> production (nmol/ 10 <sup>6</sup> cells/min)	Chemiluminescence (mean peak response to Zymosan particles (5 mg), luminol augmented cpn × 10 <sup>3</sup> )
<b>Granulocytes</b>			
Normal controls	4.2–9.6	3.0–5.0	426
Patient	0.6	0.0	6
Mother	6.5	4.5	402
Father	5.6	3.0	564
Sister <sub>1</sub>	7.6	3.9	
Sister <sub>2</sub>	4.7	3.1	
<b>Monocytes</b>			
Normal controls		8 ± 3	1 140
Patient		0	7

biology, University of Utrecht. The following enzymes were tested: glutathion reductase (18), glutathion peroxidase (19) and myeloperoxidase (20). As controls granulocytes and monocytes of normal subjects and of two boys with classical X-linked inherited C.G.D. (9) were used. Random migration and chemotaxis of granulocytes and monocytes was measured by a modified Boyden chamber assay (21), using casein (1 mg/ml) and endotoxin-activated plasma.

## RESULTS

The total and differential white blood cell counts of the patient were normal at the time when the leucocyte functions were studied. The phagocytosis of *Staphylococcus aureus* (Table 1) and *Candida albicans* by granulocytes and monocytes was normal. The op-

sonic activity of patient's serum was normal (Table 1).

The intracellular killing of *Staphylococcus aureus* and *Candida albicans* by granulocytes was impaired equally as found for other patients with C.G.D. (Fig. 1–2). The intracellular killing by monocytes, however, was almost normal (tested at three occasions over a period of eighteen months) (Fig. 1–2), whereas for the C.G.D. controls an impairment of the bactericidal activity of monocytes was found (Fig. 1–2).

No reduction of NBT was found in the granulocytes of the patient. The metabolic burst was not stimulated in granulocytes and monocytes of the patient (Table 2), where both pa-

Table 3. Chemotaxis assay

Cells	Distance travelled towards attractant <sup>a</sup>			
	Random migration <sup>b</sup>	Casein <sup>c</sup>	Endotoxin activated plasma <sup>d</sup>	
			Patient	Control
<b>Granulocytes</b>				
Patient	26.6 ± 2.6	66.3 ± 8.6	55.8 ± 6.8	54.4 ± 3.6
Control	26.9 ± 3.0	66.7 ± 7.5	57.0 ± 6.9	60.5 ± 7.1
<b>Monocytes</b>				
Patient	41.7 ± 4.1	71.7 ± 4.8	65.4 ± 5.4	62.3 ± 6.7
Control	45.6 ± 3.4	75.0 ± 5.5	65.7 ± 5.5	65.9 ± 7.2

<sup>a</sup> Distance in  $\mu \pm 1$  S.D. travelled into a filter with 3  $\mu$  poresize for granulocytes and 8  $\mu$  poresize for monocytes.

<sup>b</sup> Gey's solution on both sides of the filter.

<sup>c</sup> Casein 1 mg/ml in Gey's solution.

<sup>d</sup> 10% solution in Gey's of fresh heparinised plasma, incubated for 30' with *E. coli* endotoxin (15  $\mu$ g/ml) at 37°C.



tient's parents and the two sibs showed a normal metabolic activity. Chemiluminescence studies revealed an abnormal response in the patient's granulocytes and monocytes (Table 2). The granulocytes of the parents showed a normal chemiluminescence pattern (Table 2). The glutathion reductase, glutathion peroxidase and myeloperoxidase were normal in the patient's granulocytes. Random migration and chemotaxis of granulocytes and monocytes were within normal limits (Table 3).

### DISCUSSION

The granulocytes of the patient described in this paper show abnormal microbicidal function and abnormal oxidative metabolism manifested by deficient  $O_2$  consumption,  $H_2O_2$  production, NBT reduction and chemiluminescence. These findings with the clinical syndrome (though rather mild) classify her as having C.G.D. The similarly disturbed oxidative metabolism of the monocytes fits the C.G.D.-picture, but the nearly normal microbicidal function of the monocytes is an unexpected finding. Thus the microbicidal function of monocytes is not solely dependent on hydrogen peroxide production. In this respect a parallel may be drawn with the microbicidal capacities of murine peritoneal macrophages, which produce very little  $H_2O_2$  (22), but are nevertheless capable to kill ingested micro-organisms (13).

In female patients with C.G.D. it has been suggested that there is an autosomal mode of transmission (23). However, chemiluminescence studies performed on granulocytes of female C.G.D. patients and their relatives provide evidence for an X-linked mode of inheritance (23).

This observation is in accordance with the Lyon hypothesis (24) and the female C.G.D. patients are supposed to be carriers with a large proportion of functionally defective leukocytes as a consequence of random X-inactivation. Although a number of signs in our patient—episodes of ulcerative stomatitis,

Raynauds phenomenon, pulmonary infiltrates responding to corticosteroids—resembles the illness described in carriers of C.G.D. (25), the results of the chemiluminescence and the metabolic studies, performed on leucocytes of the mother and the sisters do not show an X-linked mode of transmission. The genetic defect can still be autosomal recessive. A spontaneous mutation, even on the X-chromosome, can not be excluded, either. However, this does not explain the different microbicidal capacity of the patient's granulocytes and monocytes.

A need for the study of monocyte function in other female C.G.D. patients is clear. This might contribute to further insight into the microbicidal mechanisms of granulocytes and monocytes and the wide clinical spectrum of the C.G.D. syndrome.

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(C. W.) Department of Paediatrics  
 University of Nijmegen  
 St. Radboud Hospital  
 Geert Grooteplein Z. 20  
 6500 HG Nijmegen  
 The Netherlands