

## Severe Infectious Complications in a Girl Suffering from Atopic Dermatitis Were Found to Be Due to Chronic Granulomatous Disease

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Recently, the diagnosis of a variant form of chronic granulomatous disease (CGD) could be established in an 11-year-old girl who had been treated for atopic dermatitis for many years. In addition to severe superinfections of lesions of the skin, the following symptoms were found currently or in her history: an episode of chronic diarrhoea (suspected as lactose intolerance), an endomyocarditis, a paranephritic abscess and recurrent lymph node abscesses. This case is demonstrated to underline the importance of extensive immunologic diagnostics in situations of recurrent severe infections of the skin, especially if other organs are involved. Diagnosis and type of CGD were strongly indicated by flow cytometrical measurement of H<sub>2</sub>O<sub>2</sub> and cytochrome b<sub>558</sub> – expression by neutrophils and confirmed by a Western blot test. No immunoreactive p47<sup>phox</sup> could be found in the patient's cells. In this autosomal recessive variant of CGD some retained ability of phagocytes to produce reactive oxygen intermediates was present. Special management of patients with CGD is necessary to prevent serious infectious complications. Genetic counselling is another important consequence of the correct diagnosis. **Key words:** Skin infections; Microcytofluorometry; Dihydrorhodamine 123; NADPH-oxidase complex.

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Atopic dermatitis is a chronic eczematous skin disease of which the main symptoms are severe itching, dry skin and eczema localized mainly in the face, neck and on the flexor sides of the extremities (1). The previous two decades have produced an enormous amount of information on immune changes in atopic patients (2, compare overview Ref. 3). Infections of the skin and mucosa are very common complications in these patients.

Immunodeficiency syndromes can be associated with clinical symptoms of atopic dermatitis. In chronic granulomatous disease (CGD), observations of an apparent association with atopic dermatitis have been reported (4). CGD is a heterogeneous group of uncommon X-linked or autosomal inherited disorders characterized by recurrent pyogenic infections. They are due to an impaired production of microbicidal reactive oxygen intermediates (ROI) by phagocytes. The infections usually, but not always, begin early in life and may lead to death in childhood (5–7). Suppurative infections are mainly caused by catalase-producing bacteria and fungal organisms – often by microorganisms normally considered to have low-grade pathogenicity. The main symptoms of CGD are infections of the surface areas (skin, mucosa, e.g. lung, gut) as

well as of the system of mononuclear phagocytes like lymph nodes, liver and bone marrow. Abscesses in the liver are considered to be an almost pathognomonic sign. However, infections can occur anywhere and may take many forms.

Investigations have confirmed that defects in the NADPH-oxidase of phagocytes, the enzyme complex responsible for superoxide-anion formation, cause CGD (8–10). Up to now, in all cases of this disease one of two membrane-bound and two cytosolic components of this NADPH-oxidase complex could be found to be defective due to mutations in the respective encoding genes (reviewed in Ref. 10).

### CASE REPORT

#### Case history

Clinical symptoms, present from the first day of life, consisted of chronic severe dermatologic manifestations – eczema infantum with severe infections of the skin and chronic diarrhoea suspected as lactose intolerance. The patient was treated using milk-free diet for 5 years. The girl spent the first year of her life in a pediatric hospital because of her serious nutritive condition. At the age of 2 years, a paranephritic abscess on the right kidney had to be drained. Wound healing complications followed and necessitated clinical treatment of about 2 months. Furthermore, an abdominal fistula occurred 6 months later. At the age of 5 years, a severe impetigo contagiosa following chronic impetigenized eczema (hospitalization of 3 months) of both ears led to a bacterial endomyocarditis. Treatment of this endomyocarditis necessitated another 3 months of hospitalization.

From the 5th year of life, recurrent cervical and submandibular lymph node abscesses (2–7/year) appeared, which almost always necessitated surgical treatment. At the age of 10 years, a sanguineous-purulent vulvovaginitis was established. Symptoms like infra- and retroauricular rhagades, nummular eczema of the face and eczema of the big flexures, angulus infectiosus, recurrent purulent rhinitis, recurrent conjunctivitis, axillary rash, white dermographism, dry skin and abolished pharyngeal reflex could be observed continuously during the course of her life.

#### Family history

Mother and sister presented minor signs of atopy (1). Serum IgE levels were 141 IU/ml and 204 IU/ml, respectively. The genetic father could not be studied.

#### Laboratory results

ESR 12/38 mm, routine blood values, proteinelectrophoresis, serum-creatinine and transaminases were in normal ranges. Culture of vulvar smear: *Staph. aureus*. Culture of cutaneous smear: *Staph. aureus*, *E. coli*, *Pseudomonas aeruginosa*.

#### Humoral immunity

Serum-IgG, -IgM and -IgA levels were in normal ranges, as determined by immunodiffusion (Behring, Marburg, Germany).

Serum-IgE level (MAST-Diagnostica, Reinfeld, Germany) was increased in the acute phase (1430 IU/ml). After oral treatment with cotrimoxacol it was 241 IU/ml. Specific IgE antibodies against birch



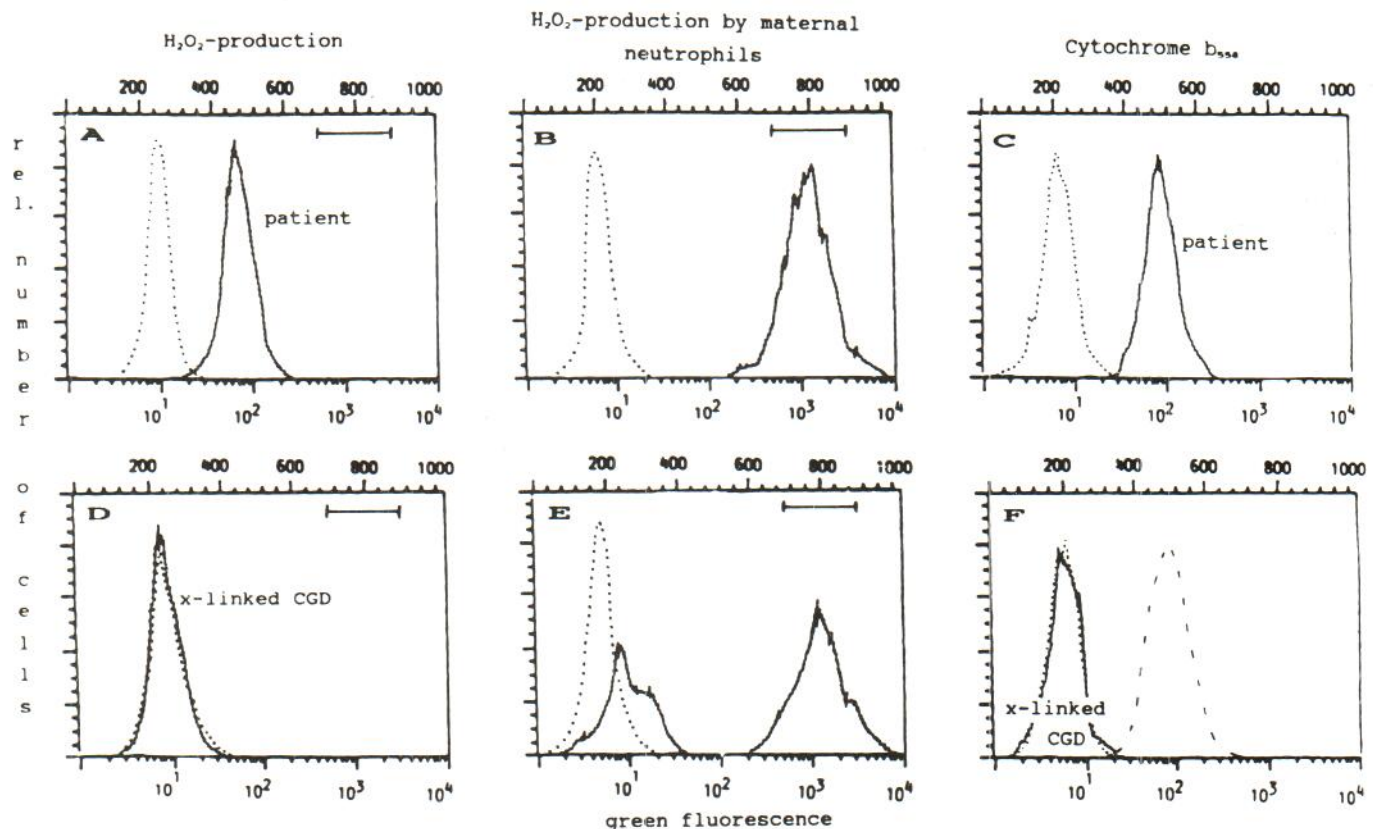


Fig. 1.  $H_2O_2$ -production of neutrophils from the patient (A) and from her mother (B) before (dotted line) and after (continuous line) activation with PMA, and cytochrome  $b_{558}$  expression (C, continuous line) of the patient's neutrophils (C, dotted line: staining with goat-anti-mouse-antibody alone as negative control). For comparison, the respective results of neutrophils from a boy suffering from "classic" X-linked CGD (D, F; approx. 65% of all CGD cases) and from his mother (E) are shown. Bars in A, B, D, E indicate the normal range of peak channel fluorescence (3 to 97% of results are in this range,  $n=50$  children). Interrupted line in F: For comparison, cytochrome  $b_{558}$  expression of neutrophils from healthy donor. The strength of relative green fluorescence reflects the strength of  $H_2O_2$  production or of cytochrome  $b_{558}$  expression, respectively. For methodological details of measurement of  $H_2O_2$  see Ref. 12 and of cytochrome  $b_{558}$  see Ref. 16.

pollen, grass pollen, mugwort pollen, *Dermatophagoides pteronyssinus* and *farinae*, epithelia of cat and dog, proteins of milk and egg, or *Candida albicans* could not be detected (MAST-Diagnostica).

#### Cellular immunity

Leukocyte subsets in the peripheral blood were determined by using two-colour immunofluorescence carried out on an Epics-Coulter flow cytometer (Krefeld, Germany). Fluorescein (FITC) or phycoerythrin (PE) conjugated monoclonal antibodies were purchased from Coulter and Dako-Diagnostika (Hamburg, Germany). A slightly increased number of activated T cells (CD2+/HLA-DR+ lymphocytes = 10.7%; in controls = 0–8%) could be observed, but no pathological changes for other cell markers were found. Phytohemagglutinin (PHA) induced lymphocyte transformation showed a slightly increased stimulation index (104,1). Delayed type hypersensitivity of the skin, determined by using the Multitest Merieux, Leimen, Germany, was in the normal range.

#### Phagocyte functions

1. *Measurement of  $H_2O_2$  production by dihydrorhodamine (DHR) 123 and microcytofluorometry.* To test the functional abilities of neutrophils from our patient, we used a simple method which was introduced recently (11) and adapted for diagnostic purposes (12, 13). The cells of our patient demonstrated a very decreased but not totally absent phorbol-myristat-acetate (PMA) induced shift in green fluorescence (Fig. 1A). This indicated a residual  $H_2O_2$  of approx. 4–8% as compared to neutrophils from her healthy mother (Fig. 1B). The mother

(and the sister, not shown) had one normal homogenous population of neutrophils with respect to  $H_2O_2$  production. In contrast, X-linked carriers of CGD (10) demonstrate two such phagocyte populations, as shown in Fig. 1E (compare overview Ref. 6). The sex of the patient and the result from the testing of her mother (Fig. 1B) already indicated an autosomal trait of inheritance of the patient's CGD.

2. *Lucigenine-enhanced chemiluminescence (14) and cytochrome  $c$ -reduction.* To further confirm the diagnosis, production of  $O_2^-$  was measured by these two methods. As compared to cells from different healthy donors, the patient's PMA-activated neutrophils produced only 1–4% of counts per 20 min and of maximal nmol  $O_2^-$ -production per min, respectively. A quantitative nitro blue tetrazolium (NBT) test (15), using extraction of the reduced NBT with dimethylformamide and measurement of the optical density at 540 nm against dimethylformamide blank in a spectrophotometer, was also in a pathological range: 10% in comparison to control values of 27–40%.

3. *Staining of neutrophils with the monoclonal antibody 7D5.* The expression of cytochrome  $b_{558}$  by the patient's neutrophils was determined by staining with the monoclonal antibody 7D5 (which binds to the small alpha chain subunit) and subsequent flow cytometry as described (16). In contrast to the patient with the X-linked CGD (Fig. 1F), the neutrophils from our patient expressed normal amounts of the cytochrome (Fig. 1C).

4. *Determination of the cytosolic factor  $p47^{phox}$ .* Rabbit polyclonal anti- $p47^{phox}$ -immunoglobulin was a kind gift from B. M. Babior (La Jolla, USA). After separation of the cytosolic proteins (and for com-



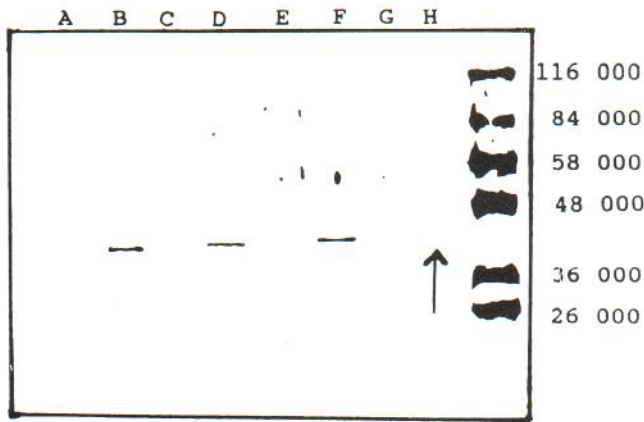


Fig. 2. Content of p47<sup>phox</sup> in the cytosol of neutrophils from the patient (H, arrow) and, for comparison, from two healthy donors (D, F) and from the boy (B) with X-linked CGD (same patient as demonstrated in Fig. 1 D-F) as evaluated by a Western blot technique. Protein fractions of cytosol (B, D, F, H) and of membranes (A, C, E, G) have been isolated and analyzed. Figures on the right indicate the respective molecular weights.

parison of the membrane fractions) from neutrophils in a SDS-PAGE-gel, immunoblotting was performed according to standard techniques. No immunoreactive p47<sup>phox</sup>-peptide could be found in the cytosol from the patient's neutrophils (Fig. 2 H, arrow), whereas it was normal in the cytosol of neutrophils from healthy donors (Fig. 2 D, F) and from the patient with x-linked CGD (Fig. 2 B).

5. *Other findings.* The neutrophils from the patient normally expressed FC RII, and III, LFA1 (CD11a/CD18) and CR3 (CD11b/CD18). Furthermore, phagocytosis of yeast cells of the species *Candida albicans* and chemotaxis under soft agar (17) in a gradient of FMLP were also found to be normal. However, neutrophils from the patient were insufficient in cytoplasmic acidification in response to PMA, as measured by using SNARF-1 in flow cytometry (18). This is another finding typical for phagocytes from CGD patients.

Taken as a whole, the results clearly demonstrate that the patient suffers from an autosomal inherited form of CGD, caused by strongly diminished or defective cytosolic factor p47<sup>phox</sup>, with some retained ability to produce ROI.

#### Therapy

For one year, the patient has been treated prophylactically with cotrimoxazol 4 mg/kg body weight p.o. every day and with IFN gamma 50 mg/m<sup>2</sup> surface area s.c. three times a week (19). During this period no purulent infections of the skin, of other organs or abscesses of the lymph nodes have been observed. The eczema has been improved as well. The patient is doing well and the quality of her life is much improved. The production of oxygen intermediates by phagocytes and the presence of p47<sup>phox</sup> were measured before, immediately after and every 3 months after introduction of IFN gamma. However, no alterations of the initial results could be observed (compare with Ref. 19).

The patient is examined routinely every 3 months.

#### DISCUSSION

Even though the patient clearly suffered from atopic dermatitis, several symptoms could not be explained easily by this disease alone and indicated an additional defect in defence mechanisms. A Job syndrome was suspected, but the relatively low level of IgE was not characteristic of this disorder. However, some of the actual symptoms and some of those in the case history are altogether very typical of CGD (7). Moreover, the prompt clinical improvement after application of

antibiotics like ciprofloxacin or anti-TBC-drugs is typical of CGD. This effect is due to the ability of these drugs to penetrate membranes of host cells, thereby killing intracellular agents. Obviously, in the case presented the disturbed defence is not mainly caused by the atopy. On the contrary, an aggravation of the dermatitis by infections, e.g. with *Staph. aureus*, due to CGD is much more plausible. This view is supported by the success of the therapy of the CGD. Only minimal symptoms of the atopic dermatitis, if any, could be observed during one year of therapy with co-trimoxazol and IFN gamma.

CGD comprises several biochemical defects that today can be mapped. In general, the diagnosis should be made as early as possible, the typical opportunistic agents of the disorder should be kept in mind and the patients kept under close observation. The prognosis of CGD was very poor before appropriate therapy was introduced (19). Good surveillance offers the chance to stop infections before damage of organs can occur and to greatly improve the prognosis.

In addition to CGD patients like the girl presented, special dermatologic manifestations such as aphthous stomatitis or discoid lupus can also occur in girls and women who are heterozygous for X-linked CGD (e.g. 20, 21).

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