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Analysis of the gene and gene product in patients with X linked chronic granulomatous disease (XL-CGD)

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Several congenital imnunodeficiency diseases including Wiskott Aldrich syndrome, agammaglobulinemia, severe combined immunodeficiency disease (SCID) and X-linked lymphoproliferative syndrome are inherited in an X-linked manner and have been more ore less precisely mapped on the X-Chromosome, mainly by the method of linkage analysis using polymorphic DNA markers [1].

The gene, which is mutated in XL--CGD is situated in the middle of the short arm of the X-chromosome proximal to the locus for Duchenne muscular dystrophy (DMD) [2].

Two patients with cytogenetically detectable deletions in Xp21 manifesting two ore more X-linked diseases were of great importance for the precise localisation and cloning of the CGD gene, the first human gene to be cloned solely on the basis of it's chromosomal localisation [3]. KC, a female heterocygous for CGD, OTC deficiency, Duchenne muscular dystrophy and retinitis pigmentosa [4] and BB, a male with CGD, DMD and retinitis pigmentosa [5]. BB's DNA was an important starting point to detect a 5 kb mRNA-by a method called subtractive hybridisation which proved to be the transcript of the CGD-gene. This transcript is translated into a glycosylated transmembrane protein identical to the large 91 kD subunit of the cytochrom b complex of the phagocyte membrane [6].

Cytochrome b 558 is a component of the respiratory burst oxidase of the phagocyte membrane responsible for the O_2- generation and thus for bacterial killing, a process which, in most of CGD cases is completely and in some partially deficient [7].

B cytochrome is composed of a large 91 kD subunit, encoded by the CGD gene on the X-chromosome and a small subunit of 22 kD encoded on a autosome (Fig. 1). The small subunit may be carrying the heme group, the spectrum of which is absent in practically all forms of X linked CGD [8].

Materials and Methods

We have analysed 16 patients with XL-CGD on the DNA level by means of hybridisation with the CGD cDNA and on the protein level using antibodies raised against the 91 kD and the 22 kD subunit. (All reagents were generous gifts by Stuart Orkin).

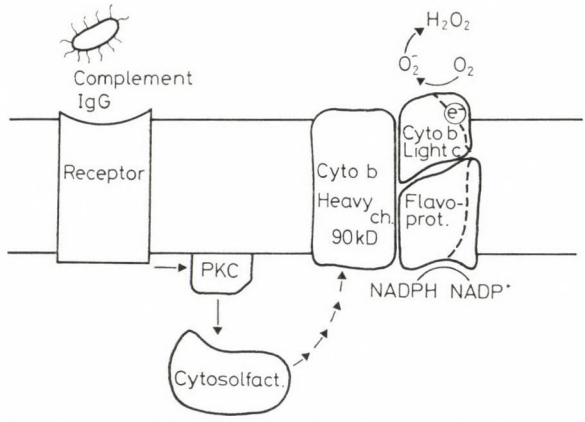

FIG. 1. Bacterial Killing and Cytochrome b Complex. The respiratory burst oxidase composed of the cytochrome b complex with its two subunits of 91 and 22 kD and a flavoprotein catalyzis the one electron reduction of oxygen to O_2-using NADPH as electron donor. This system is activated by a cytosolic factor which is phosphorylated by protein kinase C (PKC)

The results of these analyses are presented with special reference to a patient affected both with XL-CGD and the X-linked McLeod syndrome. This patient allowed the fine mapping of the McLeod gene, which encodes for the red cell Kell antigen precursor substance Kx, that is absent in the McLeod phenotype [9].

The patient, now 13 years old, suffers from recurrent bacterial and fungal infections. In addition he presents with a microcytic, hypochromic anemia with acanthocytosis.

Laboratory evaluation: NBT-test was negativ, O_2-production and cytochrome b content of his neutrophils were O. He had weak expression of Kell antigen and no expression of Kx. The karyotyp was 46 XY, without indication of a deletion. The patients mother and sister had heterozygous values for NBT, O_2-, cytochrome b, and the Kell- and Kx-antigens.

Southern blot analysis: DNA was extracted from whole blood, digested and transferred to nitrocellulose filters by Southern blotting. The probe used for hybridisation was a radioactivly labelled cDNA probe of 3,5 kb containing almost the entire CGD cDNA [3].

Western blot analysis: Neutrophil membrane extracts were separated on SDS--polyacrylamid-gradient gels (7—12%) and transferred to nitrocellulose filters, which were the incubated with the antisera raised against the 91 and 22 kD subunits of cytochrome b.

RESULTS AND DISCUSSION

The resulting fragment pattern of Msp 1 digested DNA of the CGD/ /McLeod patient, his family and a healthy female control was identical for all family members except for the patient himself (lane 2 in Fig. 2), whose DNA yielded no hybridisation signal, thus indicating a deletion of the entire CGD gene. The deletion in the patient is not de novo, but inherited from the mother. Signal intensity of the mother and sister (lane 3 and 4), both carriers for CGD and McLeod, was identical to that of a healthy uncle(lane 7) but only 50% of a healthy

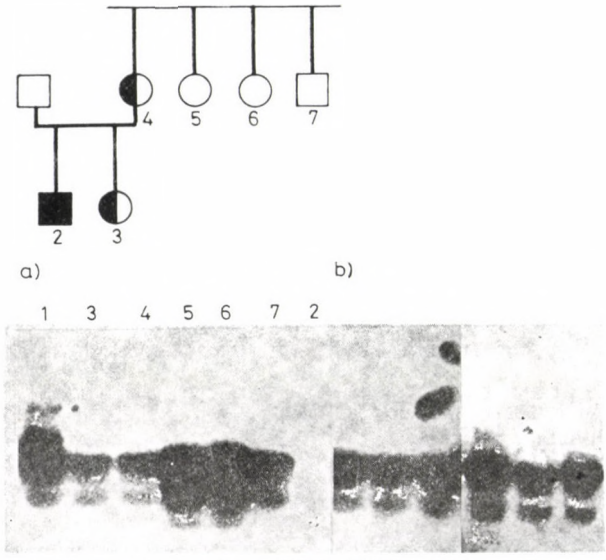

FIG. 2. (A): Southern blot analysis with a CGD cDNA of Msp 1 digested DNA of the patient with CGD and McLeod and his family. Lanes 1,5 and 6, female control and two healthy aunts show 100% signal intensity; lane 2, patient (no signal); lanes 3 and 4, hemizygous mother and sister (50% signal intensity), lane 7, healthy uncle. (B) Identical fragment pattern of Msp I digested DNA of six X-CGD patients without McLeod syndrome

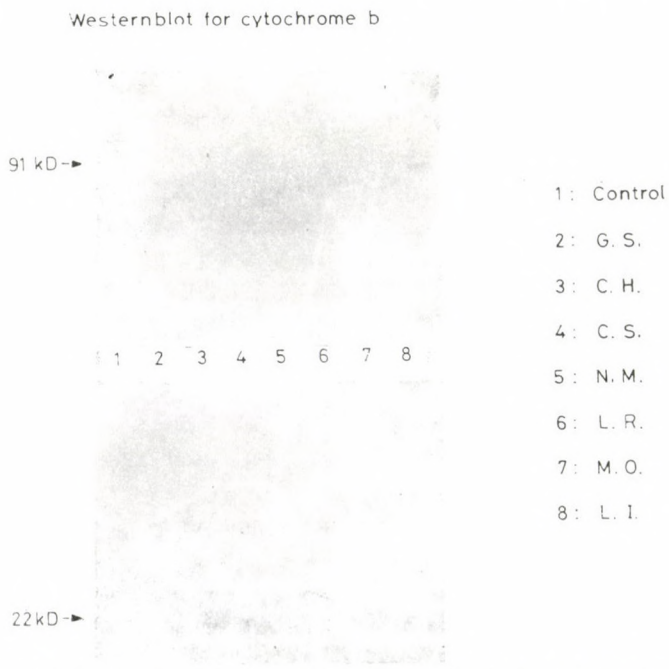

FIG. 3. Westernblot for both subunits of Cytochrome b. In lane 1 (control) and lane 8 (female with autosomal form of CGD) positiv reaction with antisera against the 91 and 22 kD protein. In lane 2, 3, 4, 5 (X-CGD patients without McLeod) and lane 7, CGD/McLeod patient no reaction with antibodies against the Cytochrome b subunits

female control(lane 1), indicating that mother and sister are hemizygous for the deletion. 15 patients with XL--CGD but without McLeod syndrome were analysed in the same way. Six of these patients' samples are shown in the figure. Their DNA, digested with Msp 1, yielded a normal nonpolymorphic fragment pattern, without any indication of a deletion or a gene rearrangement.

In families such as reported here, *prenatal diagnosis of CGD* by DNA--analysis can be accomplished readily by detecting or excluding the deletion in a male fetus. In the majority of X-linked CGD families prenatal diagnosis by either functional analysis of fetal neutrophils aspirated by fetoscopy [10] or analysis with closely linked DNA markers from Xp21 can be made available [11].

We determined the extent of the deletion found in the CGD/McLeod patient by hybridizing his DNA with three flanking probes, OTC, CX5.7 and 754 [9] (Results not shown). The presence of normal fragment patterns indicated, that these loci were not deleted. The deletion breakpoints therefore lie between the OTC-locus and DXS148 (detected by CX5.7) and span a region of probably not more than 1000 kb in Xp21. The McLeod locus can therefore be mapped very closely to the CGD locus.

Figure 3 shows the results of the *Western blot analysis*. The control in lane 1 and the female with an autosomal recessive form of CGD in lane 8 react with both antisera, revealing the presence of the 91 and the 22 kD subunits of cytochrome b. The CGD/ /McLeod patient (lane 7) and the other X-CGD patients (lane 2–6) did not react. They lack both subunits of the cytochrome b complex. Therefore genetic deficiency of only one component (the 91 kD subunit) encoded by the CGD gene seems to be critical for the heme spectrum but also for the stability of the 22 kD subunit.

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