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Dysfonctionnement de la NADPH oxydase des phagocytes dans la  
granulomatose septique chronique de type X<sup>+</sup>  
Modèle d'étude : les cellules PLB-985 CGD X

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–<sup>191</sup>TSSTKTIRRS<sup>200</sup> and <sup>484</sup>DESQANHFAVHHDEEKD<sup>500</sup>

–on NADPH oxidase activation

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## List of the abbreviations

bp	base pair
cDNA	complementary deoxyribonucleic acid
CGD	chronic granulomatous disease
CSF	cell-free system
DFP	diisopropylfluorophosphate
DMF	dimethylformamide
DNA	deoxyribonucleic acid
EDTA	ethylenediaminetetraacetic acid
FACS	flow cytometry
FAD	flavin adenine dinucleotide
fMLP	formyl-methionyl-leucyl-phenylalanine
FNR	ferredoxin-NADP <sup>+</sup> reductase
GST	glutathione S-transferase
GTP $\gamma$ S	guanosine 5'-3- <i>O</i> -(thio) triphosphate
H <sub>2</sub> O <sub>2</sub>	hydrogen peroxide
HRPO	horse-radish peroxidase
INT	iodonitrotetrazolium
K <sub>m</sub>	constant of Michaélis-Menten
mAb	monoclonal antibody
MPO	myeloperoxidase
mRNA	messenger ribonucleic acid
MW	molecular weight
NAD(P)H	Reduced form of nicotinamide-adenine dinucleotide (phosphate)
O <sub>2</sub> <sup>-</sup>	superoxide anion
PBS	phosphate-buffered saline
PCR	polymerase chain reaction
Phox	phagocyte oxidase
PKC	protein kinase C
PMA	phorbol 12-myristate 13-acetate
PMN	polymorphonuclear neutrophils
PMSF	phenylmethyl sulfonyl fluoride
GDI	GDP dissociation inhibitor
RLU	relative luminescence unit
ROS	reactive oxygen species
RP	recombinant protein
SDS	sodium dodecyl sulfate
SDS-PAGE	SDS-polyacrylamide gel electrophoresis
SOD	superoxide dismutase
STZ	serum-treated zymosan
TLCK	tosyl-L-lysine chloromethyl ketone
WT	wild-type

## Summary

Chronic granulomatous disease (CGD) is a rare immuno-deficiency syndrome in which phagocytes lack NADPH oxidase activity. The most common form resulted from mutations in the *CYBB* gene encoding gp91<sup>phox</sup> or Nox2, which is the redox center of the oxidase complex. Very few mutations, referred as X<sup>+</sup>-CGD, led to a normal expression of gp91<sup>phox</sup> with a defective oxidase activity. X<sup>-</sup>-CGD is another rare variant, characterized by a diminished gp91<sup>phox</sup> expression with a weak or absent oxidase activity. The study of these rare variants is useful to establish relationships between a sequence of gp91<sup>phox</sup> and a specific function. The X-CGD PLB-985 cellular model, in which the *CYBB* gene encoding gp91<sup>phox</sup> was interrupted, exhibits an X<sup>0</sup>-CGD phenotype (Zhen et al. 1993).

Mutagenesis approach and stable transfection in X-CGD PLB-985 cells were used to study the defective molecular mechanisms of three X91<sup>+</sup>-CGD mutations, one X91<sup>-</sup>-CGD mutation, and the structure-function analysis of two essential domains of gp91<sup>phox</sup>. WT-Nox2 transfected PLB-985 cells exhibited the same oxidase activity as the original WT PLB-985 cells. Phenotypes of all the studied X<sup>+</sup> or X<sup>-</sup>-CGD were the same as those of the neutrophils from the patients.

The impact of an X<sup>+</sup>-CGD double mutation **His303Asn/Pro304Arg** and of its each mutation on NADPH oxidase functions was carefully dissected in this cellular model. Although the His303Asn mutation has a more severe inhibitory effect on NADPH oxidase assembly and activity than the Pro304Arg mutation, neither mutation can be considered as a polymorphism. **Leu505Arg** mutation originated from a second X<sup>+</sup>-CGD was supposed to be located in the potential adenine-binding site (<sup>504</sup>**GLKQ**<sup>507</sup>). According to the Taylor's 3D model of the C-terminus of Nox2 (Taylor et al. 1996), this mutation is localized at the end of an  $\alpha$ -helical loop, a potential cytosolic factors binding site which could regulate the NADPH access to its binding site. In a simplified cell-free system using purified mutated cytochrome *b*<sub>558</sub>, this mutation diminished the affinity for NADPH and NADH ( $K_m$ -mutant=3 $\times$  $K_m$ -WT). In optimal conditions, this system needs much more p67<sup>phox</sup> to reach the maximal turn over of the oxidase. Leu505Arg mutation seems to alter the structure of  $\alpha$ -helical loop affecting the p67<sup>phox</sup> binding and the NADPH access to its binding site. However no evidence was found for a direct binding of Leu<sup>505</sup> to NADPH. The study of a third X<sup>+</sup>-CGD case due to an **Asp500Gly** mutation in the same  $\alpha$ -helical loop and reproduced in the X-CGD PLB-985 cell model, pointed out the importance of this region on assembly process and electron transfer from NADPH to FAD.

The crucial role of two potential cytosolic domains of gp91<sup>phox</sup> <sup>191</sup>**TSSTKTIRRS**<sup>200</sup> (D-loop) and <sup>484</sup>**DESQANHFVHHDEEKD**<sup>500</sup> ( $\alpha$ -helical loop) were investigated using the same cellular model as above. The RR9192EE-gp91<sup>phox</sup> mutation, known to inhibit oxidase activity and oxidase assembly, was used to validate the methodology (Biberstine-Kinkade et al. 1999). We found that the charged residues in the D-loop (Lys<sup>195</sup>, Arg<sup>198</sup>, Arg<sup>199</sup>) are essential to maintain oxidase activity, but are not involved in oxidase assembly process nor in electron transfer from NADPH to FAD. The same region of Nox1/3/4 is totally functional for oxidase activity, suggesting the existence of a common mechanism underlying the activation of Nox family. Two super-mutants (R199Q, D-loop<sub>Nox4</sub>) were observed to produce more H<sub>2</sub>O<sub>2</sub> activated by PMA or fMLP. The charged residues (Asp<sup>484</sup>, His<sup>490</sup>, Asp<sup>500</sup>) in the C-terminal region (residues 484-500) are essential to support oxidase activity, the oxidase complex assembly, and electron transfer from NADPH to FAD.

Finally an X<sup>-</sup>-CGD case (Ser193Phe) reproduced in the X-CGD PLB-985 cellular model demonstrated that the low cytochrome *b*<sub>558</sub> synthesis was related to a defect in glycosylation process of gp91<sup>phox</sup>.

**Key words:** X<sup>+/-</sup>-CGD, gp91<sup>phox</sup> or Nox2, cytochrome *b*<sub>558</sub>, NADPH oxidase, X-CGD PLB-985 cells