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The Release of Superoxide by Human B cells is Down-Regulated at the Gene Expression Level[1]

Microchimerism after renal transplantation and blood transfusion ouse dust mites in the city of Salvador-BA

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Abstract

Objective: *We investigated the NADPH oxidase activity, cytochrome b_{558} content, and gene expression of gp91-phox and p47-phox in normal EBV-transfor-med B lymphocytes, as compared to EBV-transfor-med B lymphocytes from patients with X-linked chro-nic granulomatous disease (CGD), normal peripheral blood neutrophils or mononuclear cells, and the A301 or C8166 lymphoblastoid cell lines.*

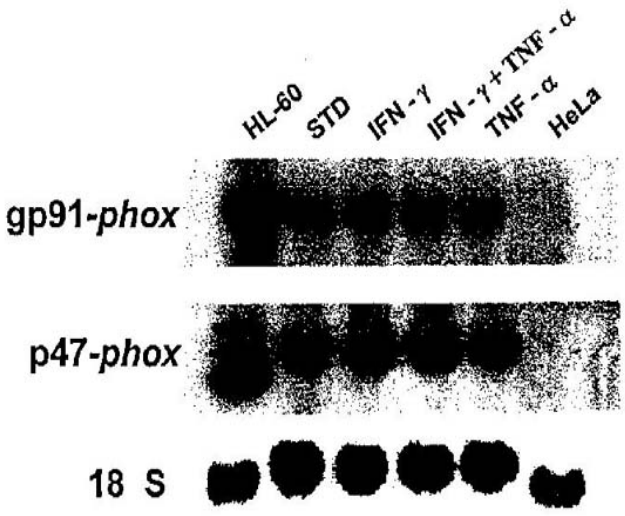
Methods: *CGD phenotypes included both "classic" disease with no detectable gp91-phox protein (termed $X91^0$) and "variant" phenotype with reduced but de-tectable gp9l-phox protein ($X91^-$).*

Results: *Normal EBV-transformed B lymphocytes show a dose dependent PMA-induced superoxide release. Culturing these cells with IFN-g (100 U/ml), TNF-a (1000 U/ml), alone or in combination for se-ven days, caused a modest increase in their NADPH oxidase activity (p>0.05 in all situations). Normal EBV-transformed B lymphocytes have lower NADPH oxidase activity and cytochrome b_{558} content than peripheral blood neutrophils or mononuclear cells (p<0.05 in all situations). In contrast they have higher NADPH oxidase activity and cytochrome b_{558} content than $X91^-$ CGD EBV-transformed B lymphocytes (p<0.05 in all situations). A301 or C8166 lymphoblas-toid cell lines, and $X91^0$ CGD EBV-transformed B lymphocytes have barely detectable NADPH oxidase activity or cytochrome b_{558} content (p<0.05 in all si-tuations). Gene expression studies also show a modest increase in expression and transcription rates of gp91-phox and p47-phox genes in normal EBV-transformed B cells cultured with IFN-g (100 U/ml), TNF-a (1000 U/ml), alone or in combination during seven days.*

Conclusion: *The NADPH oxidase activity and cy-tochrome b_{558} content of EBV-transformed B lympho-cytes are limited at the trancriptional level of genes encoding components of the NADPH oxidase system.*

We further investigated the transcription rates of the genes encoding gp91-*phox* and p47-*phox* in nuclei obtained from EBV-transformed B lym-phocytes, as assessed by nuclear run-on assays[47]. As shown in figure 5, culturing B lymphocytes with IFN-g (100 U/ml) ant TNF-a (1000 U/ml) for seven days caused a respective 1.3- and 1.2-fold increase in the transcription rates of the ge-nes encoding gp91-*phox* and p47-*phox*, in para-llel with NADPH oxidase activity, cytochrome b_{558} content, and steady state mRNA levels.

Fig. 4 – Gp91-*phox* and p47-*phox* gene expression in EBV-transformed B lymphocytes: Each lane contains 10µg total RNA from normal EBV-transformed B lymphocytes, cultured in standard (STD) conditions or with IFN-g (100 U/ml), TNF-a (1000 U/ml), alone or in combination du-ring seven days; HL-60 cells differentiated with IFN-g (100 U/ml) during two days; or HeLa cells cultured in STD conditions, as indicated. The figure shows a representative northern blot probed with ^{32}P-labeled cDNAs for the indi-cated genes; 18S probing was used as a normalization pa-rameter. Culturing normal EBV-transformed B lympho-cytes with IFN-g (100 U/ml), TNF-a (1000 U/ml), alone or in combination during seven days, caused a modest increa-se on the transcripts of gp91-*phox* and p47-*phox* genes (p>0.05 in all situations, n=3).



Rev. bras. alerg. imunopatol. 1999; 22(1):34-43 respiratory burst; superoxide; free radicals; inflam-mation; phagocytes.

Resumo

Objetivo: *Comparamos a atividade NADPH oxida-se, conteúdo do citocromo b_{558} e expressão dos genes gp9l-phox e p47-phox entre linfócitos B normais imortalizados com vírus Epstein-Barr (linfócitos B EBV); linfócitos B de pacientes com doença granulo-matosa crônica (DGC) imortalizados com vírus Eps-tein-Barr; neutrófilos e células mononucleares do sangue periférico normal; e as linhagens linfoblas-tóides A301 e C8166.*

Métodos: *Os fenótipos de DGC incluíram a doença "clássica", caracterizada pela ausência da proteína gp91-phox (denominada $X91^0$) e seu fenótipo "varian-te", caracterizado pela quantidade reduzida, porém detectável da proteína gp9l-phox ($X91^-$).*

Resultados: *Linfócitos B transformados pelo EBV liberam superóxido de maneira dose-dependente quando estimulados pelo PMA. A cultura destas célu-las com IFN-g (100 U/ml) e/ou TNF-a (1000 U/ml), durante sete dias, resulta em modesto aumento de sua atividade NADPH oxidase (p>0.05 em todas situa-ções). Linfócitos B EBV têm melhor atividade NADPH oxidase e conteúdo de citocromo b_{558} do que neu-trófilos e células mononucleares do sangue periférico normal (p<0,05 em todas situações). Em contrapar-tida, linfócitos B transformados pelo EBV têm maior atividade NADPH oxidase e conteúdo de citocromo b_{558} do que linfócitos B EBV $X91^-$ (p<0,05 em todas situações). As linhagens de células linfoblastóides A301, C8166 e linfócitos B EBV $X91^0$ têm atividade NADPH oxidase e conteúdo de citocromo b_{558} quase indetectáveis (p<0,05 em todas situações). Nossos es-tudos de expressão gênica revelam um modesto aumento na expressão e atividade transcripcional dos genes gp9l-phox e p47-phox genes em linfócitos B EBV cultivados com IFN-g (100 U/ml) e/ou TNF-a (1000 U/ml) durante sete dias.*

Conclusão: *A limitação da atividade NADPH oxi-dase e conteúdo do citocromo b_{558} em linfócitos B transformados pelo EBV, ocorre ao nível transcrip-cional dos genes que codificam componentes do sis-tema NADPH oxidase.*

Rev. bras. alerg. imunopatol. 1999; 22(1):34-43 explosão respiratória; superóxido; radicais livres; inflamação; fagócitos.

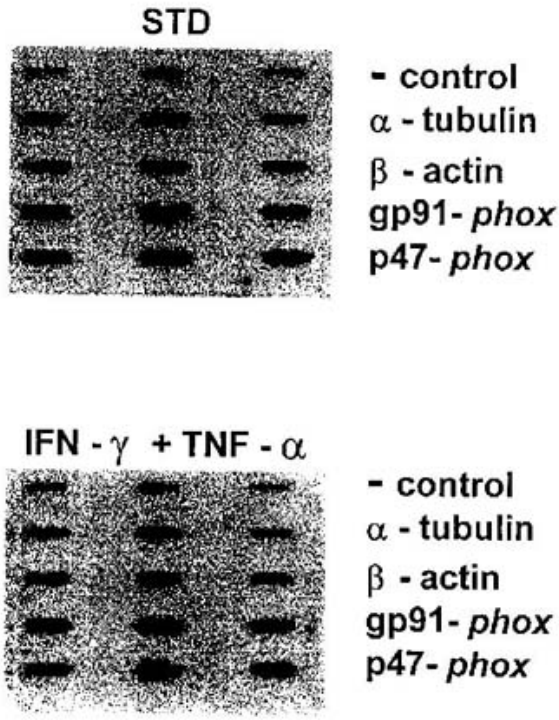
Introduction

Phagocytes, such as macrophages and granulo-cytes, contain a membrane associated nicotinami-de adenine dinucleotide phosphate (NADPH)[2] o-xidase that produces superoxide and other reacti-ve oxygen intermediates responsible for microbi-cidal, tumoricidal, and inflammatory activities[1,2]. Defects in oxidase activity in chronic granuloma-tous disease (CGD) lead to severe, life-threate-ning infections that demonstrate the prime impor-tance of the oxygen-dependent microbicidal sys-tem in host defense[3,4]. The enzyme system res-ponsible for superoxide generation forms a small trans-membrane

Discussion

Our results show that PMA induces dose-de-pendent NADPH oxidase activity in normal EBV-transformed B lymphocytes. This activity was approximately 10% of that observed in fresh peripheral blood neutrophils or mononuclear cells and significantly higher than that observed in X-linked CGD B lymphocytes or other lym-phoblastoid cell lines. Furthermore, culturing the EBV-transformed cells with IFN-g or TNF-a , alone or in combination, did not significantly affect the capacity of these cells to release supe-roxide. In parallel, the cytochrome b_{558} content in these cells correlated with their NADPH oxidase activity.

Fig. 5 – Transcription rates of genes encoding gp91-*phox* and p47-*phox* in EBV-transformed B lymphocytes: Repre-sentative nuclear run-on assay showing the transcription rates of the indicated genes in nuclei from normal EBV-transformed B lymphocytes. Culturing B lymphocytes with IFN-g (100 U/ml) and TNF-a (1000 U/ml) during seven days caused a modest increase on the transcription rates of genes encoding gp91-*phox* and p47-*phox* (p>0.05, n=2). The "housekeeping genes" a -tubulin and b -actin were included as constitutive controls.



Our gene expression studies have shown that EBV-transformed B lymphocytes transcribe the genes encoding the oxidase components gp91-*phox* and p47-*phox*, but to a much lower extent than phagocytic cells lines[22, 23, 51]. In addition, IFN-g , TNF-a , alone or in combination did not significantly affect transcription or steady-state mRNA levels of the oxidase genes, although the-se cytokines have well characterized receptors and transduction mechanisms in B lymphocy-tes[52, 53]. This low level of expression is in proba-

electron transport system that re-sults in the oxidation of NADPH on the cytoplas-mic surface and the generation of superoxide on the outer surface of the membrane. Individual protein constituents and their genes have been identified and cloned[5-9]. The terminal electron do-nor to oxygen is a unique low-midpoint-potential flavocytochrome, termed cytochrome b_{558} [10,11], located primarily in the plasma membrane[12]. Cytochrome b_{558} is a heterodimer composed of a 91 kDa glycoprotein (termed *gp9I-phox,* for glyco-protein, 91 kDa, *of phagocyte* oxidase) and a 22 kDa nonglycosylated polypeptide (p22-*phox*)[13]. Activation of the NADPH oxidase complex from a resting state to full superoxide-generating acti-vity requires the chemical modification and trans-location of additional subunits from the cytosol to the oxidase complex on the cell membrane[14-16]. Two such polypeptides with M_r 47 kDa and 67 kDa (p47-*phox* and p67-*phox*) have been iden-tified and their genes cloned[8,17]. Low molecular weight G proteins associated with the oxidase in-clude rac2, which translocates with the cytosolic oxidase proteins, and rap1, which closely associa-tes with the p22-*phox* component in the membra-ne[18]. A newly-identified and cloned cytosolic component of the oxidase p40-*phox,* associates with p67-*phox*[19,20]; but its role in oxidase activity remains unknown.

In studies examining tissue specificity of ex-pression for the genes encoding the two chains of the cytochrome b_{558} heterodimer, the gp9I-*phox* gene was expressed mainly in differentiated pha-gocytes, yet that for the p22-*phox* was constitu-tively expressed in a variety of cell lineages[5,21]. However, the two genes undergo parallel induc-tion by various cytokines, including interferon-gamma (IFN-g), in monocyte-derived macropha-ges and granulocytes[22,23]. Despite being non-pha-gocytic cells, B lymphocytes also express the cy-tochrome b_{558}, [24] and possess NADPH oxidase activity, they accurately reproduce the bioche-mical and molecular defects of CGD in patient- -derived lymphoblastoid cell lines[25]. 4b -phorbol 12-myristate 13-acetate (PMA) has been routinely used to induce superoxide release in either B lym-phocytes isolated from tonsils[26] or Epstein-Barr-virus-transformed B lymphocytes[25,27]. Additional stimuli, such as lipopolysaccharide (LPS), alumi-nium fluoride, ionomycin, arachidonic acid tumor necrosis factor-alpha (TNF-a), interleukin (IL)-I, IL-6, and cross-linking of surface antigens such as IgM, IgD, IgG, HLA-DR, and CD 19 also sti-mulate B lymphocytes to release superoxide[27-30].

A major drawback in the use of B lymphocytes as a model system for the study of the NADPH oxidase system is their low oxidase activity. Our aims were to investigate whether oxidase activity and the expression of its components could be up-regulated by inflammatory cytokines in B cell lines, and to determine the biochemical and mo-lecular basis of this low NADPH oxidase activi-ty, previously attributed to a post-transcriptional block in cytochrome b_{558} expression[31].

Materials and Method

bly not related to the EBV-transformation pro-cess54, since normal B lymphocytes obtained from tonsils also release small amounts of supe-roxide after stimulation by PMA or cross-linking of immunoglobulin reports[26]. Thus, B lympho-cyte's low NADPH oxidase activity appears to be regulated primarily at the transcriptional level in contrast to previous observations, proposing a post-transcriptional block in expression of cyto-chrome b_{558}[31]. This discrepancy can be explained by the fact that those investigators[31] studied only two EBV-transformed B lymphocyte cell lines and did not perform any nuclear run-on assay in order to assess transcription rates of genes enco-ding components of the NADPH oxidase system.

It is noteworthy that IFN-g and TNF-a did not synergise to stimulate the NADPH oxidase acti-vity or gene expression in EBV-transformed B lymphocytes, as usually occurs in phagocytic cell lines[22, 23, 51]. The lack of significant stimulation of the transcription rates for the genes encoding gp91-*phox* and p47-*phox* by IFN-g and TNF-a in EBV-transformed B lymphocytes, in parallel with the biochemical and gene expression stu-dies, suggests that the NADPH oxidase system is constitutively expressed in B lymphocytes. Actu-al enzyme activity is proportional to stimulus-de-pendent activation involving downstream events.

The teological basis for constitutive expression of a superoxide generation system in the B cell lineage remains a subject for speculation. Consi-dering that B lymphocytes are not phagocytic cells, it is unnecessary to produce sufficient su-peroxide for microbial killing. Thus, another ex-planation for the NADPH oxidase activity in B lymphocytes is a possible involvement of supero-xide in antigen processing or presentation. Supe-roxide perhaps can be involved in antigen pro-cessing. In fact, serum immunoglobulin levels in CGD patients is generally very high, and attri-buted to chronic polyclonal activation in CGD patients[55, 56]. However, superoxide might have a direct modulatory effect on immunoglobulin pro-duction by B lymphocytes. Considering that B lymphocytes live longer than phagocytes[57], per-haps a constitutive NADPH oxidase activity and not a strong respiratory burst as observed in pha-gocytes reflects an evolutionary defense mecha-nism avoiding additional tissue damage during inflammation. Alternatively, superoxide genera-tion could be involved in antigen processing or antibody production, but serve as one of several redundant pathways, such that other mechanisms promote this process in CGD cells.

We conclude that expression of the genes en-coding gp91-*phox* and p47-*phox* in EBV-trans-formed B lymphocytes correlates with their NADPH oxidase activity and cytochrome b^{558} content. However, despite the parallels we have established between B cells and phagocytes in terms of reproducibility of molecular defects in CGD patients, inflammatory cytokines do not up--regulate oxidase gene expression in the B cell li-nes. Thus, results from investigations about the NADPH oxidase using EBV-transformed B lym-phocytes require careful interpretation

Cell culture. EBV- transformed B lymphocy-tes were developed from peripheral blood mono-nuclear cells of healthy individuals and were compared with EBV-transformed B lymphocytes from previously characterized CGD patientes, normal fresh isolated peripheral blood mononu-clear cells or neutrophils, and the A301 and C8166 lymphoblastoid cell lines[32].

The CGD EBV-transformed B lymphocytes were developed from two patients with X-linked CGD. The first patient has a splice site mutation in the first intron of CYBB gene encoding gp91-*phox*[33,34] and the phenotype of variant X-linked CGD ($X91^-$). In this nomenclature for CGD phe-notype, "X" represents the X-linked mode of inheritance, "91" indicates that the gp91-*phox* component of the phagocyte oxidase is affected and the subscript symbols indicate an undetecta-ble (0), diminished (-), or normal (+) level of gp91-*phox* protein[3]. This patient's phagocytes show NADPH oxidase activity in a range of 10% normal, contain equivalent levels of cytochrome b_{558} [35], and show an unusually dramatic response to IFN-g both *in vitro* and *in vivo*[36,37]. The second patient has a deletion mutation between exons 11-13 of gp91-*phox*[34] and the phenotype of "clas-sic" X-linked CGD with no oxidase activity or detectable gp91-*phox* protein ($X91^0$). His phago-cytes have no NADPH oxidase activity or cyto-chrome b_{558}. Normal fresh peripheral blood cells were obtained from laboratory personnel. Proce-dures and consent forms were approved by the Committee on Protection of Human Subjects in Research of State University of Campinas Medi-cal School.

To initiate B lynphocyte cultures, peripheral blood was fractionated by Ficoll-Hypaque cen-trifugation[38] and the mononuclear cells cultured with supernatants from B95-8, an EBV-producer cell line[25-39]. After EBV-transformation, B lym-phocytes from healthy individuals or from CGD patients were cultured in RPMI 1640 medium su-plemented with 10% heat inactivated fetal bovine serum, 2 mM L-glutamine, 100 U/ml penicillin, and 100 µg/ml streptomycin, at 37ºC in a humid atmosphere saturated with 5% CO_2. To examine the effects of cytokines, B lymphocytes were cul-tured for seven days with human recombinant IFN-g (100 U/ml), TNF-a (1000 U/ml), alone or in combination. Normal peripheral blood mono-nuclear cells or neutrophils, and the A301 and C8166 lymphoblastoid cell lines were not expo-sed to cytokines. Cells counts and viability, mo-nitored on a daily basis, were always above 90%.

NADPH oxidase activity. Superoxide release was assessed by a modified superoxide dismuta-se (SOD) inhibitable cytochrome *c* reduction assay[40]. Briefly, cells were transferred to six well polystyrene plates (2×10^6 cells per well); the plates were centrifuged and the supernatant was replaced by Hanks' balanced salt solution (HBSS) without phenol red, containing cytochro-me *c* (50 µM) and the indicated concentration of 4b -phorbol 12-myristate 13-acetate (PMA). Nor-mal EBV-transformed B lymphocytes were incu-bated with PMA in a range of 3-300 nM. The other within the limits of the model system.

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cells received 30 nM PMA. Half of the wells received SOD (60 U/ml) at the beginning of the experiment. After one hour incubation, the plates were placed on ice and the other half of the wells received SOD (60 U/ml). The absor-vance of the supernatants was monitored at 550 nm and the amount of superoxide released was calculated using an extinction coefficient of 0.21 nM^{-1} cm^{-1}. The results were expressed as nmol of superoxide released per 10^6 cells per hour.

Cytochrome b_{558} content. Cytochrome b_{558} was measured by a spectroscopic method designed to avoid the interference of mitochondrial cytochro-mes or hemoglobin[41]. On the day of the experi-ment, 1 x 10^7 cells were harvested, washed 3 ti-mes with PBS and lysed with 2% Triton X-100 in 0.1 M KH_2PO_4 buffer at pH 7.25, for 30 minu-tes on ice. The lysate was contrifuged at 27,000 x g 30 minutes at 4ºC and the supernatant assayed by spectrophotometric scanning (400-600 nm, 750 nm/min). The test sample received 10 µM KCN, 10 µM NaN_3, a few grains of sodium di-thionite and was then aerated by dropwise pipe-tting over 3 minutes. The spectrum of the aerated sample was stored in the spectrophotometer me-mory. The sample was reduced again with a se-cond addition of dithionite and rescanned. The resulting difference spectrum, representing (redu-ced second time)-minus-(aerated after first reduc-tion), was obtained. The amount of cytochrome b_{558} was estimated from the height of the band at 558 nm, using an extinction coefficient of 21.6 cm^{-1} nM^{-1}. The results were expressed as pmol of cytochrome b_{558} per 10^7 cells.

Gene expression studies. Total cell RNA was extracted by guanidine HCl method[42] and analy-zed by Northern blots performed according to standard procedures[43]. Hybridization probes were full-length cDNAs for the human gp91-*phox*, and p47-*phox* genes[5, 17]. Procedures for sequential cy-cles of filter stripping and re-probing were per-formed as described by Gatti *et al*[44]. Equal loa-ding of lanes was demonstrated by examination of gels after ethidium bromide staining and by re-hybridization with a 5.8-kilobase *Hin*dIII res-triction fragment of rat 18S ribosomal cDNA[45]. Positive control RNA was obtained from HL-60 cells differentiated with IFN-g (100 U/ml) and negative control RNA from HeLa cells[22,46]. Mes-sage was measured quantitatively by computer analysis of phosphorimager data.

Transcription rates of genes encoding gp91-*phox*, and p47-*phox* were assessed by nuclear run-on assays with minor modifications of pre-viously published procedures[47]. Briefly, EBV-transformed B lymphocytes nuclei were isolated by cell lysis in 0.05% Nonidet P-40. Freshly pre-pared nuclei were incubated 30 minutes at 30ºC in a reaction mixture containing [^{32}P]UTP (250 µCi, 3000 Ci/mmol) in buffer modified from Greenberg *et al*[47] by addition of 0.8 mM $MnCl_2$. Newly synthesized RNA was prepared by extrac-tion in guanidine thiocianate and ethanol precipi-tation. Equal amounts of incorporated label from each group (1-2 x 10^7

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cpm) were then hybridized to saturating amounts of cDNA probes, immobi-lized on filters by slot blotting. The probes used in these experiments included cDNAs for gp91-*phox* and p47-*phox* genes[5,17], ahybridization ne-gative control (plasmid without insert), and cons-titutively expressed genes (b -actin and a -tubu-lin)[48]. We calculated relative rates of transcrip-tion by computer analysis of phosphorimager data. The calculations of relative transcription rates were normalized to negative control and to rates for the constitutively-expressed genes a -tu-bulin and b -actin.

Statistics. Descriptive statistical calculations were performed and the results were represented either as bar/line charts or box plots showing the minimum, 25th percentile, median, 75th percenti-le, and maximum values[49]. The Mann-Whitney U test was used for comparison between groups; a *p* value <0.05 was considered significant[50].

Results

NADPH oxidase activity of EBV-transformed B lymphocytes. Normal EBV-transformed B lym-phocytes show dose-dependent superoxide relea-se in response to PMA over the range of 3-300 nM (fig. 1, p<0.05 in all situations, n=9). Cultu-ring these cells with IFN-g (100 U/ml) for seven days caused a trend to increased NADPH oxidase activity, but without statistical significance (fig. 1, p>0.05 in all situations, n=9). Further experi-ments assessed the PMA (30 nm)-induced supe-roxide release of normal EBV-transformed B lymphocytes cultured in standard (STD) condi-tions or with IFN-g (100 U/ml), TNF-a (1000 U/ml), alone or in combination for seven days. These results were compared to EBV-transfor-med B lymphocyte lines derived from X91^{-} and X91^{0} CGD patients, normal fresh peripheral blood mononuclear cells and neutrophils, and the A301 or C8166 lymphoblastoid cell lines, which were not stimulated with cytokines. These com-parative experiments (summarized in fig. 2) allo-wed us to distinguish four levels of NADPH oxi-dase activity in the studied cell lines: (respective-ly from highter to lower) 1- Normal peripheral blood mononuclear cells or neutrophils (p<0.05, n=5), 2 - Normal EBV-transformed B lympho-cytes (p<0.05, n=5), 3 – X91^{-} CGD EBV-trans-formed B lymphocytes (p<0.05, n=5), and 4 – X91^{0} CGD EBV-transformed B lymphocytes, A301, and C8166 lymphoblastoid cell lines. Cul-turing EBV-transformed B lymphocytes with IFN-g (100 U/ml), TNF-a (1000 U/ml), alone or in combination during seven days, did not cause a statistically significant increase in the NADPH oxidase activity of these cells (fig. 2, p>0.05 in all situations, n=5).

Fig. 1 - NADPH oxidase activity of EBV-transformed B lymphocytes: Dose dependent induction of superoxide (O_2^-) release by normal EBV-transformed B lymphocytes by 4b -phorbol 12-myristate 13-acetate (PMA), 3-300 nM (*p<0.05 in all situations, n=9). Cells were cultured in standard (STD) conditions or in the presence of interferon-gamma (IFN-g , 100 U/ml) for seven days. IFN-g caused a mild increase on the superoxide release by EBV-transfor-med B lymphocytes, however, this increase was not sta-tistically significant (p>0.05 in all situations, n=9).

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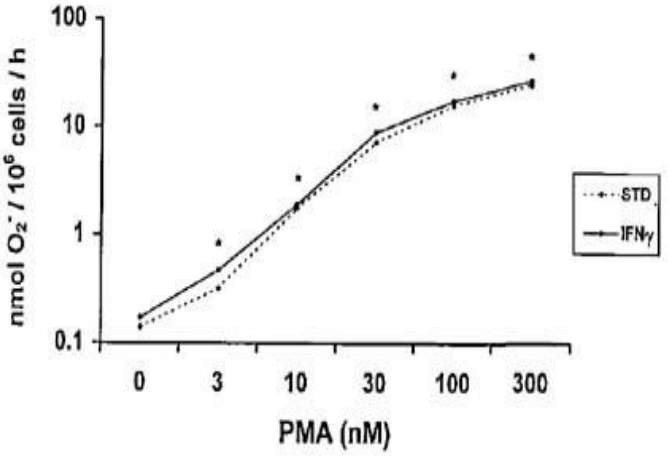
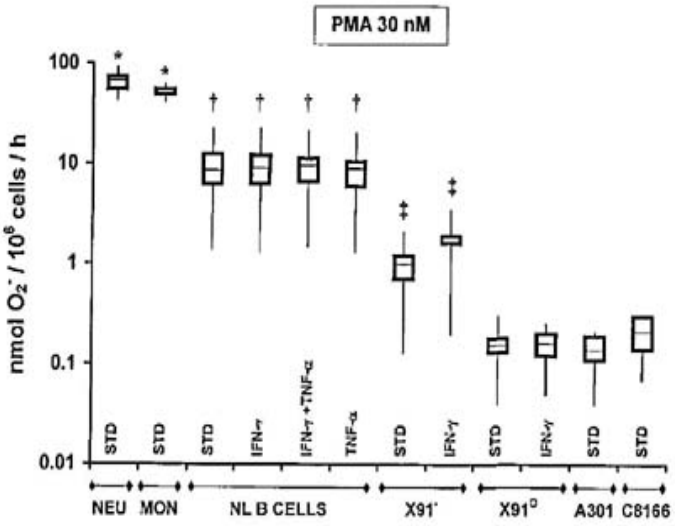


Cytochrome b_{558} content of EBV-transformed B lymphocytes. We further assessed the cytochro-me b_{558} content of normal EBV-transformed B lymphocytes, compared to $X91^-$ or $X91^0$ CGD EBV-transformed B lymphocytes, normal fresh peripheral blood mononuclear cells or neutro-phils, and the A301 or C8166 lymphoblastoid cell lines. In parallel with the NADPH oxidase activity, these experiments (presented in fig. 3) allowed us to distinguish the neutrophils ($p<0.05$, n=5), 2 – Normal EBV-transformed B lymphocy-tes ($p<0.05$, n=5), 3 – $X91^-$ CGD EBV-transfor-med B lymphocytes ($p<0.05$, n=5), and 4 – $X91^0$ CGD EBV-transformed B lymphocytes, A301, and C8166 lymphoblastoid cell lines.

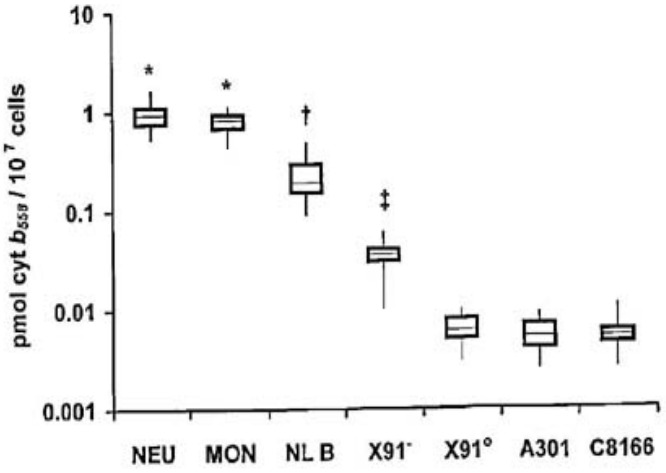
Fig. 2 - NADPH oxidase activity of EBV-transformed B lymphocytes compared to other cell types:4b -phorbol 12-myristate 13 acetate (PMA, 30 nM) induced more supero-xide (O_2^-) release in normal peripheral blood mononuclear cells (MON) or neutrophils (NEU) than in the other cell lines (*$p<0.05$, n=5). Under the same circumstances, nor-mal EBV-transformed B lymphocytes released more supe-roxide than EBV-transformed B lymphocytes derived from a patient with variant X-linked ($X91^-$) chronic granuloma-tous disease (CGD) ($^\dagger p<0.05$, n=5). These in turn, release more superoxide than EBV-transformed B lymphocytes derived from a patient with classic X-linked ($X91^0$) CGD or lymphoblastoid cell lines A301, and C8166, all cultured in standard (STD) conditions ($^\ddagger p<0.05$, n=%). Culturing EBV-transformed B lymphocytes with IFN-g (100 U/ml) or TNF-a (1000 U/ml), alone or in combination, for seven days did not cause a statistically significant increase on the NADPH oxidase activity of these cells (P>0.05 in all situa-tions, n=5).



Expression of genes encoding components of the NADPH oxidase in EBV-transformed B lym-phocytes. Considering the results showing the lo-wer NADPH oxidase activity and cytochrome b_{558} content of EBV-transformed B lymphocytes when compared to peripheral blood mononuclear cells or neutrophils, we extended our investiga-tion to the gene expression level, as assessed by northern blot hybridization[43]. Figure 4 shows that expression of the genes encoding gp91-*phox* and p47-*phox* in EBV-transformed B lymphocytes correlates with the cells' NADPH oxidase activi-ty and cytochrome b_{558} content. Furthermore, culturing EBV-transformed B lymphocytes with IFN-g (100 U/ml) alone for seven days caused a respective median 1.7- and 1.3-fold increase in gp91-*phox* and p47-*phox* gene expression (p> 0.05 in all situations, n=3). TNF-a (1000 U/ml) alone caused a respective median 1.4- and 1.1-fold increase in gp91-*phox* and p47-*phox* gene expression (p>0.05 in all situations, n=3). IFN-g (100 U/m) plus TNF-a (1000 U/ml) caused a respective median 1.8- and 1.2-fold increase in gp91-*phox* and p47-*phox* gene expression (p> 0.05 in all situations, n=3). Figure 4 also shows the strong induction on the expression of gp91-*phox* and p47-*phox* genes in HL-60 cells diffe-rentiated with IFN-g (100 U/ml) for two days. It is noteworthy that no significant synergism bet-ween IFN-g and TNF-a occurred in the induction of these genes in EBV-transformed B lympho-cytes.

Fig. 3 – Cytochrome b_{558} content of EBV-transformed B lymphocytes compared to the other cell types: normal peri-pheral blood mononuclear cells (MON) or neutrophils (NEU) have a higher cytochrome b_{558} content than all the other cell lines (*p<0.05, n=5). Normal EBV-transformed B lymphocytes have a higher cytochrome b_{558} content than EBV-transformed B lymphocytes derived from a patient with variant X-linked ($X91^-$) chronic granulomatous disea-se (CGD) (†p<0.05, n=5). These in turn, have a higher cy-tochrome b_{558} content than EBV-transformed B lympho-cytes derived from a patient with classic X-linked ($X91^0$) CGD, A301, and C8166 lymphoblastoid cell lines (‡p<0.05, n=5).

The Release of Superoxide by Human B cells is



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