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# CHEMILUMINESCENCE RESPONSE OF HUMAN B-CELL LINES

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Epstein-Barr virus (EBV)-transformed human B lymphoblastoid cells share certain properties with monocytes: they are capable of presenting protein antigens to antigen-specific T-lymphocytes and of releasing an Interleukin 1-like factor. It was our interest to study whether transformed B-cells resemble monocytes by generating toxic oxygen radicals. Human B-cell lines were developed from human peripheral blood lymphocytes by EBV-transformation. The induction of the respiratory burst in the B-cells was assessed by chemiluminescence (CL) in the presence of lucigenin. B-cells were stimulated with phorbol-myristate-acetate (PMA), zymosan particles, the chemotactic peptide f-met-phe, the complement split product C5a and with a recently described granulocyte activating cytokine (GRAM). Stimulation with PMA elicited a distinct CL-response in the tested B-cell lines. The CL-signal was significantly reduced by superoxide dismutase, but not by D-mannitol and catalase. No significant response to any of the other stimuli was detected. Furthermore, none of the stimuli induced a luminol-enhanced CL signal, which, in contrast to lucigenin, is dependent on the presence of peroxidase.

Our results indicate that EBV infected B-cells were able to generate significant amounts of reactive oxygen species, particularly superoxide. It appears that virus transformation uncovers genetic information which is usually not expressed in non-transformed B-cells.

## INTRODUCTION

It has been reported recently that human B-lymphoblastoid cells which were developed by transformation with Epstein-Barr-virus (EBV) from human peripheral blood lymphocytes, exhibit certain functions normally related to monocytes. Transformed B-cells are able to present soluble protein antigens to antigen specific T-lymphocytes.[1,2] This ability of B-cells is usually ascribed to monocytes and related phagocytic cells. Furthermore, EBV transformed B-lymphocyte lines produce an interleukin-1 like factor.[3,4] Reportedly, rather all of the tested cell lines were able to serve as antigen presenting cells that enabled purified human T-lymphocytes to proliferate in one-way mixed-lymphocyte reactions and in response to Concanavalin A. These results demonstrated that EBV transformed B-cells which are capable to serve as accessory cells have the capacity to produce an interleukin-1 like factor. In contrast to B-cells, mononuclear phagocytes were enabled to generate significant amounts of toxic-oxygen radicals under stimulation.[5,6] Therefore, we investigated in the present study, if EBV transformation of B-cells also uncovers this property usually ascribed to phagocytic cells. A lucigenin-dependent chemiluminescence was used to assess the induction of the respiratory burst.[7,8]

## MATERIALS AND METHODS

Ficoll solution, culture media and fetal calf serum (FCS, screened for mykoplasma contamination and mitogenic activity were obtained from Biochrom Seromed (Berlin, FRG). Antibiotics were purchased from Gibco (Ohio, U.S.A.). Lucigenin, superoxide dismutase (SOD), phorbol-myristate-acetate (PMA) were from Sigma (Munich, FRG).

### *Preparation of mononuclear cells*

Mononuclear cells were isolated from the defibrinated venous blood from healthy blood donors as described previously.[19] Cells were finally suspended in RPMI 1640 supplemented with 2 mM glutamine, 100 U/ml penicillin, 100 $\mu$g/ml streptomycin and 2.5 $\mu$g/ml fungizone plus 10% heat inactivated FCS.

### *Virus*

Transforming Epstein-Barr virus (EBV) was obtained from the supernatant of the B95-8 marmoset lymphoblastoid line.[9]

Culture conditions: EBV transformation was done as described previously.[10,11] In brief, 15 to 20 $\times$ $10^6$ MNC per 0.5 ml medium and 2.5 ml EBV containing B95-8 supernatant supplemented with 20% FCS were cultured in culture flasks (Becton and Dickinson, Heidelberg, FRG) at 37°C without $CO_2$. Cells were fed with fresh medium after 7 to 10 days. Colony growth was controlled daily by microscopic analysis. When colonies were visuable, the medium was exchanged.

### *Characterization of transformed cell-lines*

After 6 months of continuous growth the B-cell cultures were considered as being established B lymphoblastoid cell lines (B-LCL). Cells were stained for surface immunoglobulin (sIg) as described previously[12–14] by using directly fluorescinated rabbit anti-human antisers (H and L chains IgG, IgA, IgM; IgG/F(ab)$_2$, Behring, Frankfurt, FRG).

### *Immunoglobulin production*

Immunoglobulins, released in the cell free supernatant, were assayed by an enzyme linked immunosorbent assay as described by[15] and modified by.[16]

### *Mycoplasma staining*

The cells were screened for mycoplasma contamination according to[17] by using the fluorescent Hoechst 33258 stain.

### *Chemiluminescence measurements*

B-cells were washed once in Hepes-buffered Hanks' balanced salt solution pH 7.4 (HBSS), containing 1 mg/ml bovine serum albumine (BSA), and adjusted to concentration of 1 $\times$ $10^6$/ml in the presence of 2 mM lucigenin or 10 $\mu$M luminol.

Aliquots (200 $\mu$l containing 1 $\times$ $10^6$ cells each, were placed into unsealed polystyrene luminescence tubes (Lumacuvette/Abimed, Düsseldorf, FRG). Measurements were performed in a 6-channel Biolumat LB 9505 (Berthold, Wildbad, FRG) interfaced via an Apple IIE computer to an Epson RX 80 graphic printer. The thermostat was set at 37°C throughout. The cells were stimulated with phorbol-myristate-acetate (100 ng/ml), the chemotactic peptide f-met-phe ($10^{-4}$ M), zymosan particles (1 mg/ml), the complement split product C5a (prepared as described previously[18], and with a recently described granulocyte-activating cytokine (GRAM,[19]). Usually, integral counts from a 0 to 60 min incubation interval after addition of supernatants to the B-cells, were measured, indicated as counts. For assaying the influence of oxygen radical scavengers superoxide dismutase (SOD, 200 $\mu$g/ml), catalase (200 $\mu$g/ml) and D-mannitol (100 mM) were used.

## RESULTS

EBV transformed B-cell lines were developed from peripheral blood lymphocytes of six normal healthy individuals. In the presence of lucigenin all B-cell lines responded to stimulation with PMA with a distinct CL-signal (Figure 1a). The time course of B-cell activation was different from that of polymorphonuclear leukocytes (PMN) stimulated with PMA (Figure 1b). The peak maximum was reached within 30 min after addition of the stimulus, the enhanced CL-response was maintained for hours before returning to the base-line. However, the peak maximum was 100-fold lower than that induced by PMA in PMN. Superoxide dismutase significantly inhibited the CL-response of the transformed B-cells (Table I, Figure 1a). Neither catalase nor D-mannitol exhibited any significant effect on the CL-response (Table I). Besides PMA the cells were stimulated with the chemotactic peptide f-met-phe, with zymosan-particles, with the complement split product C5a and with a recently described granulocyte-activating mediator (Table II). In contrast to PMA, none of the tested stimuli induced a significant CL-response of the transformed B-cells. Furthermore, we

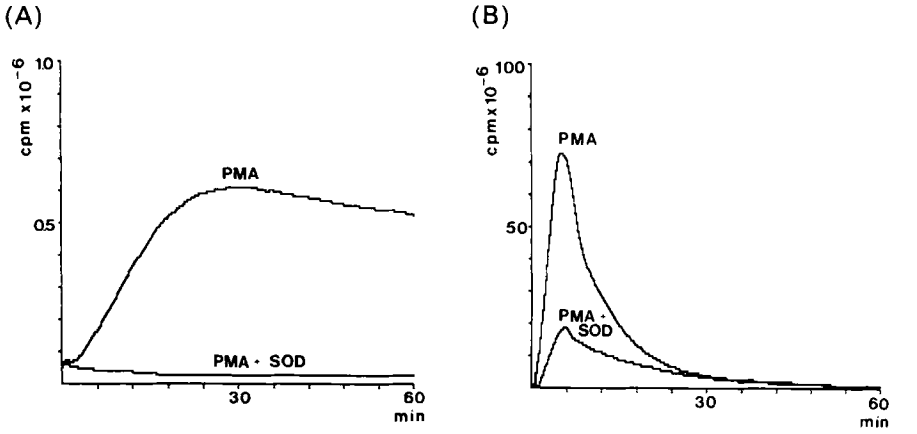


FIGURE 1 Original recording of the time-course of chemiluminescence in B-cells and PMN. (A) human B-cells (1 $\times$ $10^6$/200 $\mu$l) and (B) PMN (1 $\times$ $10^6$/200 $\mu$l) were stimulated with PMA (100 ng/ml) in the presence or absence of SOD (200 $\mu$g/ml, indicated as PMA + SOD). 60 min. integral counts were measured.

TABLE I
Chemiluminescence response of human B-Cell lines – Influence of oxygen radical scavengers
CL-Response (counts × $10^{-3}$)[a]

| | 1[b,e] | 2[e] | 8 | 9[e] | 10[e] | 11 |
|---|---|---|---|---|---|---|
| PMA (100 ng/ml) | 3,650[c] | 4,212 | 14,660 | 15,873 | 8,195 | 19,404 |
| PMA + SOD (200 μg/ml) | n.d.[d] | n.d. | 3,271 | 1,179 | 1,199 | 1,699 |
| PMA + Catalase (200 μg/ml) | n.d. | n.d. | 13,453 | 16,343 | 8,995 | 23,013 |
| PMA + D-Mannitol (100 mM) | n.d. | n.d. | 8,972 | 16,706 | 10,674 | 25,637 |
| Background | 1,178 | 1,872 | 1,523 | 1,201 | 1,813 | 1,725 |

[a]60 min integral counts were measured
[b]No. of proband
[c]mean of duplicate experiments
[d]not determined
[e]concentration of B-cells: 0.5 × $10^6$/200 μl

TABLE II
Chemiluminescence response of human B-Cell lines – Influence of different stimuli
CL- Response (counts × $10^{-3}$)[a]

| Stimulus | 1[b,e] | 2[e] | 8 | 9[e] | 10[e] | 11 |
|---|---|---|---|---|---|---|
| PMA (100 ng/ml) | 3,650[c] | 4,212 | 14,660 | 15,873 | 8,195 | 19,404 |
| f-met-phe ($10^{-4}$ M) | n.d.[d] | n.d. | 2,082 | 1,204 | 1,508 | 1,900 |
| Zymosan (1 mg/ml) | 1,278 | 2,020 | 2,232 | 1,541 | n.d. | 1,413 |
| C5a ($10^{-7}$ M) | 1,193 | 2,112 | n.d. | n.d. | n.d. | n.d. |
| GRAM (1:3) | 1,519 | 2,390 | n.d. | n.d. | n.d. | n.d. |
| Background | 1,178 | 1,872 | 1,523 | 1,201 | 1,813 | 1,725 |

[a]60 min integral counts were measured
[b]No. of proband
[c]mean of duplicate experiments
[d]not determined
[e]concentration of B-cells: 0.5 × $10^6$/200 μl

tested the response of the B-cells to stimulation in the presence of luminol. No CL-response was seen with all stimuli tested (Data not shown).

## DISCUSSION

The present study demonstrates that EBV transformed B-cell lines were able to generate a significant CL response under stimulation with PMA. Since superoxide dismutase in contrast to D-mannitol and catalase significantly inhibited the chemiluminescence signal, it is supposed that mainly superoxide is released under stimulation. Furthermore, stimulation could only be performed in the presence of lucigenin, but not in the presence of luminol, which is dependent on the simultaneous presence of myeloperoxidase.[20,21] The possible influence of mykoplasmas, frequently present in cell cultures, could be ruled out, since only B-cell lines free of mykoplasma contamination were tested. Only PMA was effective in stimulating the oxidative burst. On PMN PMA binds to a specific receptor.[22] After specific receptor-ligand binding induction of the respiratory burst is thought to be the result of a translocation of NAD(P)H oxidase- components such as cytochrome $b_{-245}$ from an intracellular granular pool.[23]

The calculated rate of cytochrome *b* reduction was found to be equal to the measured rate of superoxide production.[24] It has recently been shown that phorbol esters as PMA directly stimulate the NAD(P)H oxidase through activation of proteinkinase C which is known to be the major phorbol ester receptor.[25] Lucigenin-dependent CL represents a sensitive measure for NAD(P)H oxidase activity.[26]

The presence of a NAD(P)H oxidase in normal human B-cells has not been published before. Furthermore, B-cells were reportedly not found to generate significant amounts of oxygen radicals. Therefore, we suggest the EBV transformation uncovers genetic information which leads to te presence of a NAD(P)H oxidase, which is usually not expressed in non-transformed B-cells. Our results support the findings of Volkman *et al.*[27] who demonstrated that B-cell lines reduced nitroblue tetrazolium and generated superoxide as measured by cytochrome c-reduction. However, further evaluation of the subcellular events underlying the described phenomenon is necessary to verify the hypothesis presented.



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