



OPPOSITE EFFECT OF LINEARLY POLARIZED LIGHT ON BIOSYNTHESIS OF INTERLEUKIN-6 IN A HUMAN B LYMPHOID CELL LINE AND PERIPHERAL HUMAN MONOCYTES

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The effects of linearly polarized light (LPL) and diffuse light (DL) on the *in vitro* interleukin-6 (IL-6) production in a human B lymphoma cell line (BMNH) and peripheral monocytes of healthy volunteers were compared. Our data show that there was a significant increase of IL-6 and IgM production in BMNH after exposure to LPL. The increase in IgM secretion was a consequence of its autocrine regulation by IL-6, since in the presence of anti-IL-6 and anti-IL-6 receptor antibodies the LPL-induced IgM secretion was abolished. In contrast to the stimulatory effect on B cells, exposure of human mononuclear phagocytes to LPL markedly reduced the production of IL-6 induced by subsequent stimulation of cells with bacterial endotoxin (LPS). The inhibition as most pronounced when suboptimal doses of LPS were applied. Under identical experimental conditions, DL had no effect on the IL-6 and IgM production of either B cells or monocytes.

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INTRODUCTION

The treatment of wounds and superficial skin ulcers with low power laser light accelerates their healing, as is widely known (Mester and Mester, 1989).

It also known that only the polarized nature of the light source and neither the wavelength nor the coherence is responsible for the biostimulating effect (Mester *et al.*, 1978). The beneficial healing effect of the LPL has been demonstrated on patients with refractory wounds, such as skin ulcers (Fenyő, 1984). The favourable effect of LPL on the healing of skin injuries can be explained by the stimulation of epithelial growth and granular tissue regeneration. Our earlier experiments using primary human embryonal fibroblasts exposed to LPL, but not with DL, demonstrated a marked increase in the binding of lectin and polycationized ferritin to the plasma membrane (Kubasova *et al.*,

1988). Moreover, blast formation and sheep red cell binding also increased following *in vitro* exposure of purified T lymphocytes to LPL, whilst DL had a negligible effect (Kubasova *et al.*, 1995).

The present study provides a different approach to characterizing the effect of LPL on the production of a multifunctional cytokine, IL-6 in two human cellular model systems *in vitro*: a B cell line and peripheral monocytes. Moreover, the stimulation of a B cell line with LPL results in greater IL-6, and hence IgM, production, which also provides an explanation for our earlier observations on the increase of plasma IgM in patients exposed to LPL (Fenyő, 1984).

MATERIALS AND METHODS

Cells and cell cultures

BMNH cells (Epstein-Barr virus bearing, IgM secreting B cell lymphoma) were cultured in the

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presence of RPMI medium supplemented with 5% (v/v) foetal calf serum and antibiotics (5 U/ml penicillin and 5 µg/ml streptomycin) in 24-well plates (10^6 cell/500 µl/well). After exposure to light the BMNH cells were kept for 48 h in 5% CO₂, then the supernatants were saved and kept at -80°C until used for quantitative cytokine and immunoglobulin measurements. In certain experiments BMNH cells were treated with 10 µl of undiluted antisera (rabbit-antihuman IL-6) or normal rabbit sera (Sigma), or 2 µg PM1 (IgG1 monoclonal mouse antibody against 80 kDa chain of IL-6R complex, kindly provided by Dr T. Taga, Tokyo) or with a control IgG1 mouse monoclonal antibody. The required amount of anti-IL-6 and anti-IL-6 receptor antibodies had been previously determined.

Heparinized blood samples were taken from healthy volunteers ($n=6$). The mononuclear cells were enriched by Ficoll-Hipaque gradient centrifugation (Hokland *et al.*, 1994). The monocytes were further purified in sequential adherence steps in 24-well microplates (Greiner) of 500 µl volume each. The purity and viability of monocytes in the monolayer ($5 \times 10^5/\text{cm}^2$) were both higher than 96%, as detected by neutral esterase reaction and crystal violet stainings, respectively. The cells were first cultured in three parallels in the presence of RPMI medium supplemented with 5% (v/v) foetal calf serum, glutamate and antibiotics (5 U/ml penicillin and 5 µg/ml streptomycin) and exposed to light (see below). After being exposed to light (see below), the medium (control) was replaced by a fresh one containing lipopolysaccharide (LPS) in two doses, 1 µg/ml (suboptimal dose) and 5 µg/ml (optimal dose) and cultivated for 48 h. In all experiments the supernatants were saved and kept at -80°C until used for quantitative cytokine measurements.

Treatment of the cell cultures with LPL and DL

Samples of the cell cultures were exposed to LPL as well as to DL from 80 mm distance, for 0, 5, 30, 60, 120 and 300 s. The light source (Bioptron AG Ltd., Switzerland) emits polarized light with a 97% degree of polarization. The light beam of the halogen bulb is polarized by a Brewster mirror in the wavelength range of 400 nm < λ < 2000 nm, and has a power density of 40 mW/cm². The energy density of the light falling onto the samples ranged between 0.2 J/cm²–12 J/cm² depending on the exposure time. The experimental and physical conditions were exactly the same in the case of the DL source with the only difference being that it was

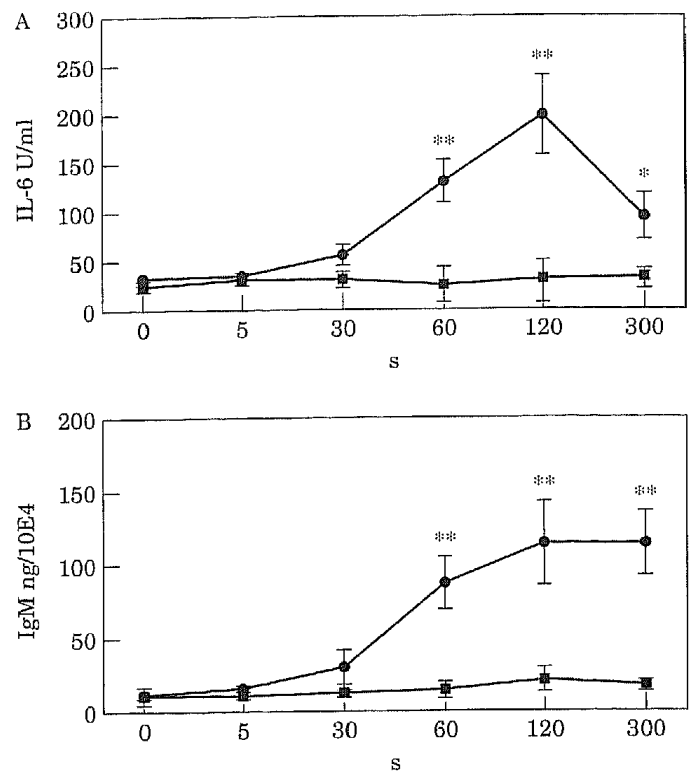


Fig. 1. IL-6 (Fig. A) and IgM (Fig. B) production by BMNH lymphoma cells in 48 h, following exposure to LPL and DL. Mean of five experiments \pm standard error of mean (SEM) values are demonstrated. (Student's *t*-probe, *: $P < 0.05$, **: $P < 0.01$). For details, see Materials and Methods.

not polarized. The depth of the nutrient solution exposed to light was 5 mm in all experiments.

Measurement of IL-6

Quantitative measurements of IL-6 were made using sandwich ELISA kits (Amersham, Biotrak, Braunschweig, Germany). In several cases the IL-6 immunoassays were compared to B9 bioassay (Aarden *et al.*, 1987).

Statistical analysis

Statistics W1.01 (Blackwell) was applied to Student *t*-probe to check the statistical significance between the values of different groups.

RESULTS

Effect of LPL on IL-6 and IL-6-dependent IgM production of the BMNH cell line

IL-6 production by the BMNH cell line is shown in Figure 1A. After exposure to LPL these cells showed a substantial increase of autocrine IL-6

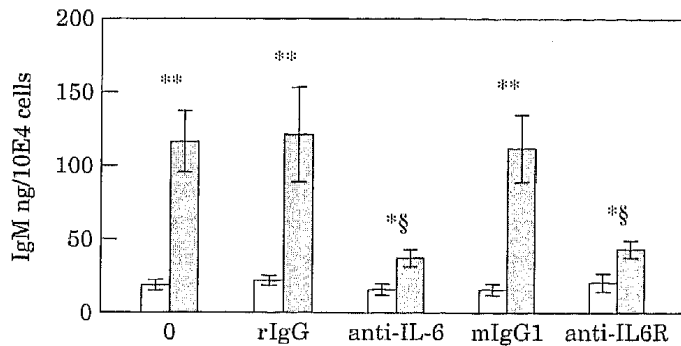


Fig. 2. IgM production of BMNH cells in 48 h, following exposure to LPL and DL for 120 s in the presence of medium (O), IgG from non-immunized rabbit (rIgG), polyclonal anti-human IL-6 IgG (anti-IL-6), control mouse monoclonal IgG1 (mIgG1) and monoclonal anti IL-6 receptor antibody (anti-IL6R). The significance of differences was calculated on the basis of values obtained after treatments with control and test (anti-IL-6 or anti-IL-6 receptor) antibodies. Mean of five experiments \pm standard error of mean (SEM) values are demonstrated. (Student's *t*-probe, *: $P < 0.05$, **: $P < 0.01$, LPL compared to DL, §: compared to the medium control.) For details, see Materials and Methods.

production with the 60 s exposure time, which is further elevated after 120 s of exposure. However there was no significant effect if the exposure time was 30 or 300 s. DL had no effect at all on IL-6 production. These results mean that an energy density lower than 2.4 J/cm^2 had no measurable effect on IL-6 production, nor did an energy density over 12 J/cm^2 .

We also studied IgM production of BMNH cells (Fig. 1B), which followed closely that of IL-6, except that it did not drop at 300 s. To prove that the autocrine action of IL-6 is responsible for the IgM production in BMNH, we set up experiments to support the view that the LPL-induced IL-6 is directly involved in regulating the production of IgM. To get evidence for the role of IL-6, we used polyclonal neutralizing anti-IL-6 and a monoclonal antibody (PM1) directed against the binding site of the 80 kDa element of the IL-6 receptor complex. Figure 2 shows that the neutralization of IL-6 and the blocking of the IL-6 receptor both markedly diminish the effect of LPL (120 s exposure) on IgM production. This proves the essential role of LPL-induced IL-6 in IgM production. Pre-immune (control) rabbit or control mouse IgG1 at the same concentration had no effect on IgM secretion. Similar results were obtained when only a 60 s exposure was used (not shown).

Effect of LPL on the IL-6 production of monocytes

In the monocyte cell culture the effect of LPL in the constitutive secretion of IL-6 and the secretion

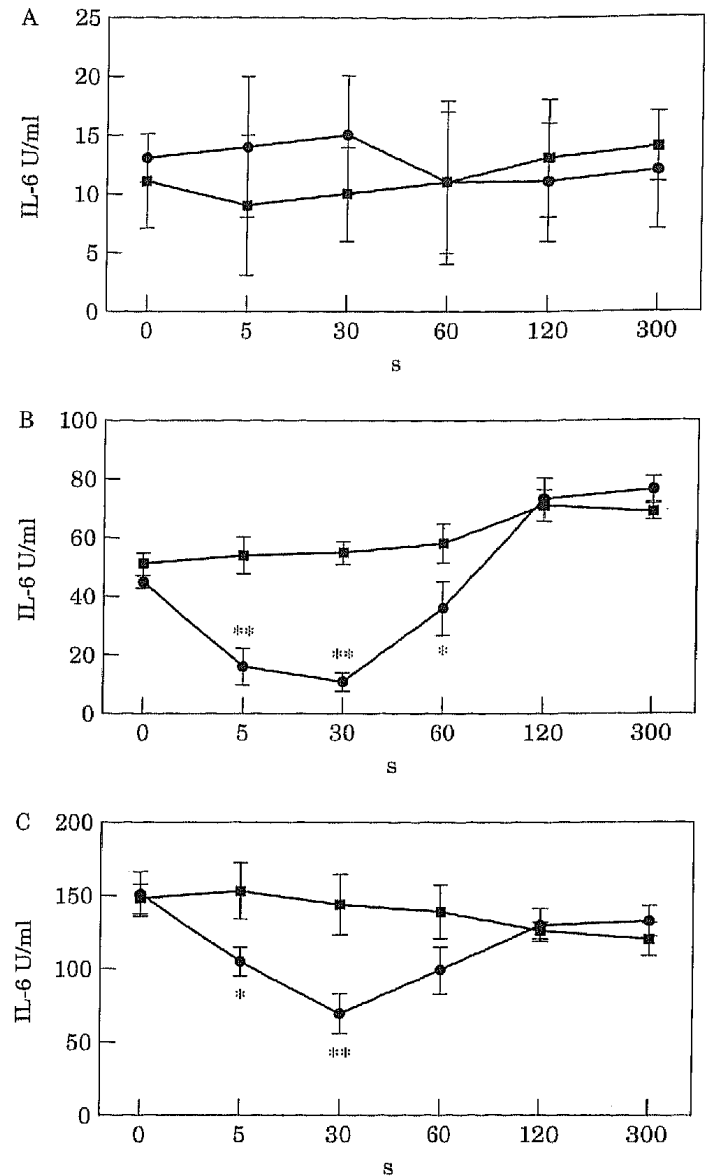


Fig. 3. The effect of medium alone (Fig. A), $1 \mu\text{g/ml}$ LPS (Fig. B) or $5 \mu\text{g/ml}$ LPS (Fig. C) on the production of IL-6 by human peripheral monocytes after 48 h, previously exposed to LPL and DL for 0–300 s. Means of six experiments \pm standard error of mean (SEM) values are demonstrated. (Student's *t*-probe, *: $P < 0.05$, **: $P < 0.01$.) For details, see Materials and Methods.

induced by bacterial endotoxin (LPS) was studied. Without LPS, exposure of a monocyte monolayer to LPL or DL for a duration of between 5 and 300 s induced no significant change in the constitutive IL-6 production (Fig. 3A). However, we observed a very pronounced decrease in the secretion of IL-6 in experiments after stimulation with a sub-optimal dose of LPS ($1 \mu\text{g/ml}$) followed the exposure to LPL for a duration of 5 or 30 s (reductions of 67 and 85%, respectively, see Fig. 3B). No decrease was seen in the case of exposure to DL (Fig. 3B). With exposure times 60 s

(2.4 J/cm²), a slight effect, but with longer times neither LPL nor DL had any effect on the IL-6 secretion. A similar reduction of IL-6 was observed in the case of exposures to LPL for 5 s and 30 s (33% and 60%, respectively) when optimal dose (5 µg/ml) of LPS was applied after exposure. A much smaller (not significant) inhibitory effect was found at 60 s of exposure (Fig. 3C). No significant effect of either LPL or DL on the constitutive or LPS-induced expression of tumour necrosis factor (TNF) α and interleukin-1 β (IL-1 β) was observed (results not shown).

DISCUSSION

Wound healing involves a highly complex set of physiological processes regulated by many different cellular and humoral factors (Podolsky, 1997; Ono *et al.*, 1995a). One possible approach to studying the biological mechanism responsible for accelerated wound healing and scar formation caused by exposure to LPL is to examine its effect on cytokine production in model systems.

Monocytes and lymphocytes react to various inflammatory signals by prompt generation of a variety of inflammatory cytokines, such as IL-6, IL-1 α and β , and TNF α (Gauldie *et al.*, 1992).

Subsequently, these inflammatory cytokines induce multiple effects in the responding cells and tissues. Hepatocytes expressing plasma membrane receptors for IL-6, IL-1 α and β , as well as TNF α respond to these cytokines by increasing the biosynthesis of many acute phase proteins, such as C reactive protein, fibrinogen and haptoglobin. Alternatively they may reduce the biosynthesis of albumin and transferrin (Baumann and Gauldie, 1994; Heinrich *et al.*, 1990). Obviously, various local effects that influence the biosynthesis and secretion of inflammatory cytokines may markedly alter the pattern of local and more systemic elements of inflammatory reactions.

The biological effects of low power visible laser light have been abundantly studied showing the beneficial effect on wound healing and tissue regeneration (Boder *et al.*, 1983; Karu *et al.*, 1987; Kupin *et al.*, 1987). In the case of treatment of wounds and skin ulcers with either low power laser or LPL *in vivo*, the optimal exposure dose generally suggested is about 4 J/cm² energy density (Mester and Mester, 1989; Kubasova *et al.*, 1995; Nemeth, 1993). This value of energy density is based on empirical experience collected from using low power laser sources or LPL for the treatment of skin ulcers. The present experiments provide an *in vitro* confirmation of this empirical experience:

the significant increase in the IL-6 production by BMNH cells was found in the energy density range of 2.4 J/cm² < E_d < 12 J/cm². Currently our experimental evidence provides no explanation as to why LPL acts on IL-6, but not on IgM, production in such a narrow time interval. One might speculate that the prolonged action on IgM production is due to a more extended response of B cells to IL-6. However, the specific blockade of IgM production by antibodies against IL-6 or its receptor is rather more convincing for the mechanism of the LPL action.

The presented *in vitro* findings suggest that LPL influences cytokine production, while exposure to DL of the same energy density has no measurable effect. The stimulatory effect of a low frequency pulsed electromagnetic field on the constitutive and PHA- and TPA-induced production of IL-1 and IL-6 by peripheral blood cells has also been shown (Cossaricca *et al.*, 1993). Our data suggest an opposite, cell-specific effect of LPL on IL-6 production by monocytes and the BMNH cell line. While in our experiments LPS-induced IL-6 secretion was inhibited by LPL, the spontaneous production of IL-6 by a B lymphoma cell line was further increased.

These *in vitro* findings correlate with earlier *in vivo* data indicating a marked increase (85%) of serum IgM in patients suffering from leg ulcers (*ulcus cruris*) treated by non-coherent linearly polarized light. In this study we provide a partial explanation for the cellular basis of this increase. Since albumin is a negative acute phase protein (inhibited by IL-6), and the activated peripheral monocytes (together with endothelial cells and fibroblasts) are responsible for most of the systemic IL-6 production, our data demonstrating the inhibition of IL-6 production by stimulated monocytes may explain earlier findings on the approximately 80% increase of plasma albumin (Fenyő, 1984). Other data show that TGF- β is one of the most important promoting factors in wound healing (Cossaricca *et al.*, 1993; Chegini, 1997) and is markedly antagonistic to IL-6 (Ono *et al.*, 1995b). One may speculate that the systemic inhibition of IL-6 production (e.g. by monocytes) induced by polarized light intensifies the effect of TGF β on wound healing.

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