EFFECT OF COMPACT FLUORESCENT LAMP EXPOSURE ON CYTOKINE RELEASE BY PERIPHERAL BLOOD MONONUCLEAR CELLS

E. Ruello([1])*, G. Acri([1]), F.M. Salmeri([1]), C. Sansotta([1]), B. Testagrossa([1]), L. Denaro([1]).

([1]) Department of BIOMORF - University of Messina - Italy

*eruello@unime.it

Compact fluorescent lamps (CFLs) have replaced the old incandescent lamps to save energy and protect the environment, at the same time.

CFLs are known for issues:

- the generation of electric and magnetic fields,
- the emission of UV radiation,
- the mercury content,
- “dirty electricity”.

Data from literature on the impact of exposure of the immune cells to different light sources are conflicting. Physical stimuli such as light sources may activate peripheral blood mononuclear cells (PBMCs) to secrete cytokines and other soluble factors, which may favor pro-inflammatory responses or trigger anti-inflammatory/reparative phenomena.

The purpose of this work is to study the action of CFL irradiation, at different exposure times, on human peripheral blood mononuclear cells (PBMCs) *in vitro*, by detection of the possible effects on cytokine production.

Materials and Methods

Compact Fluorescent Lamp

- Globe: diameter 12.2 cm,
- Power: 30 W,
- Color temperature: 2700 K (soft white),
- Luminous efficacy: 60 lm/W,
- Luminous flux: 1800 lm.

Materials and Methods

Subjects

The work was carried out at the Department of BIOMORF, University of Messina, Italy, from March to September 2019. Twelve apparently healthy volunteers of our staff were enrolled in the study. They all could understand the study design and objectives. Before participating in our study, they signed an informed consent form allowing us to use their biological fluids and cells.

Inclusion criteria

- ***Age*** 22 to 41 (mean age of 30.4±5.9, M:F = 1:1),
- ***Hematologic parameters*** within the range of physiological values,
- ***Leukocytes*** ranging from 6500/mm^3 to 8300/mm^3,
- ***Lymphocytes*** ranging from 1740/mm^3 to 2530/mm^3,
- ***Monocytes*** ranging from 265/mm^3 to 410/mm^3.

Exclusion criteria

- History of photosensitivity,
- History of UV exposure for at least 6 months before the study,
- History of acute or chronic inflammatory diseases,
- History of auto-immune, metabolic, endocrine, cardiovascular, and oncological diseases,
- Treatment for cancer (radiation, chemotherapy, immunotherapy, biological therapy),
- Oral intake of medications (e.g., photosensitizing agents, steroidal and/or non-steroidal anti-inflammatory agents),
- Treatment with hormone therapy,
- Pregnancy.

Materials and Methods

PBMC Collection and Preparation

Blood was obtained by venipuncture using heparin as an anticoagulant.

Heparinized whole blood

Histopaque 1.077

PBMCs were isolated at room temperature by density gradient centrifugation at 400 *g* of the venous samples on a polysucrose and sodium diatrizoate solution (Histopaque 1.077) for 30 min, according to the technique of Böyum.

The PBMCs were collected at the interface, washed twice in phosphate-buffered saline (PBS), counted, and suspended again at a concentration of $1x10^7$/ml in a culture medium of PBS supplemented with 10% heat-inactivated fetal bovine serum, 2 mMol glutamine, 100 U/ml penicillin–streptomycin, 10 mMol HEPES, and 50 Mol mercaptoethanol.

Plasma
Platelets

PBMCs
Histopaque 1.077

Erythrocytes
Granulocytes

Materials and Methods

PBMC Treatment

- Samples of 100 µl containing $1x10^6$ cells of PBMCs were placed in uncovered polystyrene 24-well plates (diameter 15 mm) and irradiated with the CFL for different exposure times (30, 60, and 90 min, respectively), at a distance of 20 cm.

- The cells, supplemented with culture media to a final volume of 1 ml, were then incubated in a 5% CO_2 incubator at 37 °C, in a humidified atmosphere, for 24 h. Unirradiated PBMCs of the same subjects were incubated in the same conditions and used as controls. No mitogen was used to stimulate PBMCs.
- All treatments were performed in a sterile environment to avoid any accidental contamination and improper stimulation of the cells. At the end of incubation, the viability of PBMCs was >95%, as assessed by the Trypan blue exclusion method.

- Cell suspensions were centrifuged at 400 *g* for 15 min and the supernatants harvested, aliquoted, and stored at -80 °C until being used for cytokine determination by enzyme-linked immunosorbent assay (ELISA).

Materials and Methods

Determination of IL-12 by sandwich-based ELISA technique

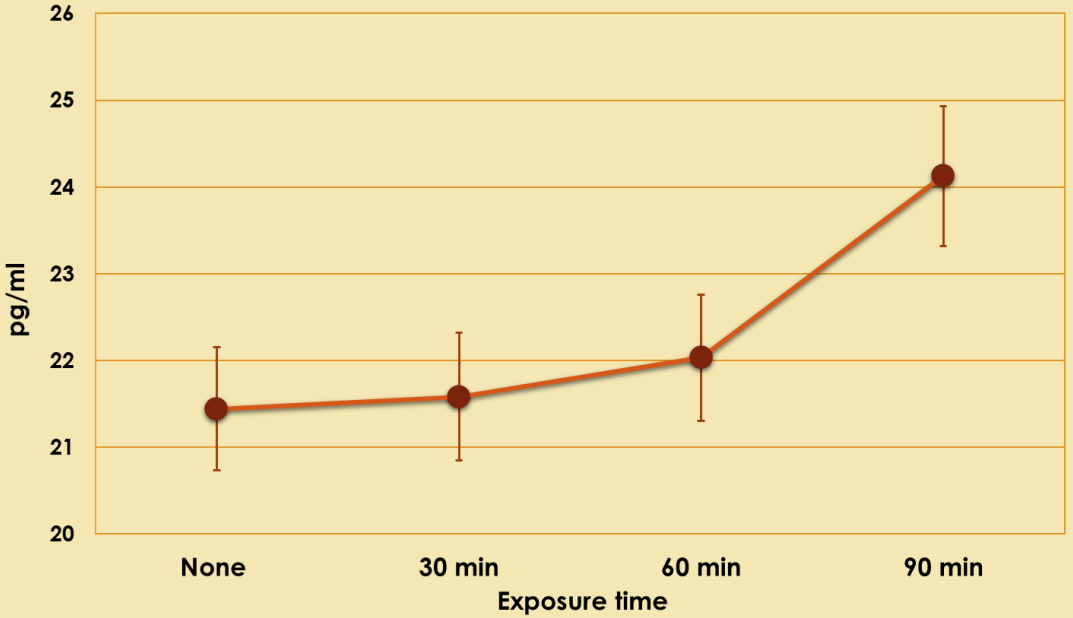
- Reagents, control samples and experimental samples preparation
- Sample addition in each well of 96-anti IL-12-precoated microtiter plate, incubation
- Washing to remove the unbound IL-12 cytokine
- Specific biotinylated detector antibody addiction to capture IL-12 cytokine, incubation
- Washing to remove the unbound reagents
- Avidin-HRP enzyme addition, incubation
- Washing to remove the entire unbound enzyme
- Colorimetric substrate solution addiction, incubation in the dark
- H_2SO_4 stop solution addiction
- Absorbance measurement using 450 nm as a detection wavelength

Results

Table 1. Effects of exposure to a Compact Fluorescent Lamp on the production of the pro-inflammatory cytokine IL-12 (pg/ml) by 24 h-cultured peripheral blood mononuclear cells.

	Exposure Time			
	None	**30 min**	**60 min**	**90 min**
Subject 1	20,7	20,8	21,4	23,6
Subject 2	21,4	21,4	21,9	23,8
Subject 3	21,6	21,9	22,3	24,7
Subject 4	21,9	22,1	22,5	24,2
Subject 5	22,1	22,2	22,6	23,9
Subject 6	20,2	20,3	20,7	22,7
Subject 7	21,5	21,6	21,8	24,5
Subject 8	22,5	22,7	23,2	25,6
Subject 9	21,3	21,3	21,7	23,6
Subject 10	22,4	22,6	23,1	25,3
Subject 11	20,8	21	21,6	23,5
Subject 12	20,9	21,1	21,6	24,1

Figure 1. Effects of exposure to a Compact Fluorescent Lamp on the production of the pro-inflammatory cytokine IL-12 (media±SD) by 24 h-cultured peripheral blood mononuclear cells.

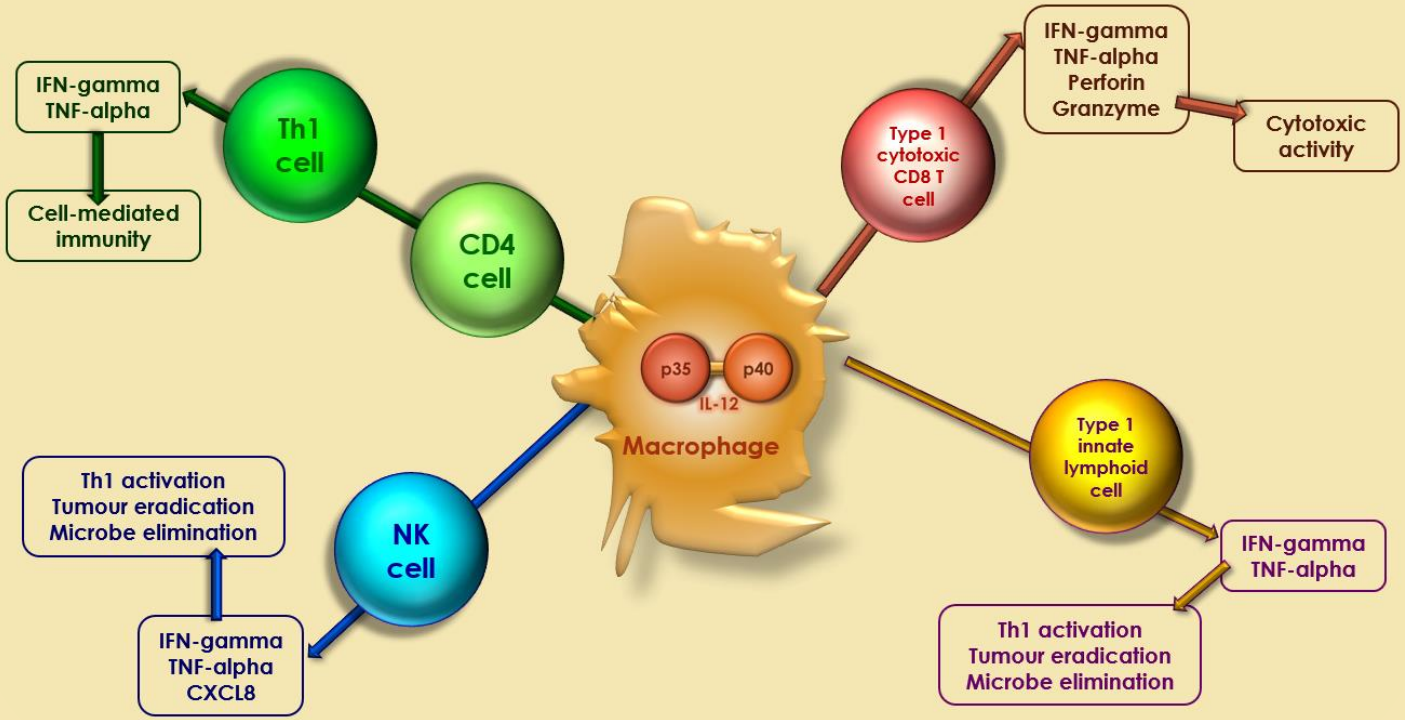


Discussion and Conclusions

This study represents an approach to understand the possible effects of CFLs on the behaviour of the immune system cells.

Limits:

- the small number of samples,
- isolated human PBMC culture time (24 h only)
- the exposure times
- direct irradiation without any cover or skin, which may act as a barrier and modify the actual effect of radiation.



The CFL light source could represent a "sterile" stimulus able to activate and drive the immune cells toward different responses.