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PRELIMINARY STUDY OF THE INFLUENCE OF CUBIC C_{60} ON CULTURED HUMAN MONOCYTES : LACK OF INTERLEUKIN-1 β SECRETION.

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Abstract. Cultured human monocytes have been incubated with powdered cubic [60] fullerene. No modification has been observed with [60] fullerene when compared with negative control (monocytes alone).

Key words: C_{60}, fullerene, interleukins, monocytes.

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Introduction

In a recent report[1], it has been shown that human leucocytes phagocyte cubic [60] fullerene particles.
It is known that interleukin-1 β (IL-1β) secretion reflects monocyte activation. Increased monocyte IL-1β secretion has been reported[2] after phagocytosis of many particles such as urates, silica, asbestos, ... The present work was carried out in order to determine whether [60] fullerene entails the same effect.

Experimental

Patients : 30 ml of heparinized whole blood were obtained by venipuncture from five healthy donors (3 men and 2 women aged from 28 to 44, med. = 35).

Monocyte cultures : Mononuclear cells were isolated by Ficoll-hypaque gradient density. Cytocentrifuged smears of the mononuclear cells were stained with May-Grünwald-Giemsa solution for differential counts. The separated cells were suspended at 2.10^6/ml in RPMI 1640 (Gibco life biotechnologies, France) supplemented with 0.5 % fetal calf serum (Eurobio, France), 1 % L-glutamine (Gibco life biotechnologies, France), 1 % (streptomycin 25 mg-penicillin 50 000 U/ml) mixture (Biomerieux, France), and then incubated in duplicate or triplicate at 37°C in a humidified atmosphere of 95 % air and 5 % CO_2 for 1,5 h.

After incubation cells were washed three times with unsupplemented RPMI 1640 to remove unadherent cells. Then they were cultured with either supplemented RPMI (RPMI control or negative control) or supplemented RPMI added with 3 µg/ml LPS (LPS control used as positive control) or 1 mg/ml [60] fullerene ([60] fullerene control) or 1 mg/ml 1-2 µm synthetic graphite powder (Aldrich, France) (graphite control). After 18 h incubation, supernatants were centrifuged at 2000 g for 10 minutes, and then aliquoted. Part of the aliquots was frozen at -80°C until IL-1β analysis. The remaining aliquots were kept at room temperature for LDH enzymatic activity determination (used as cell lysis index).

Supernatant LDH enzymatic activity were determined as previously described[1].

IL-1β assay : IL-1β supernatant contents were determined using immunoenzymetric assay (Immunotech, France) according to the laboratory procedure.

To visualize whether [60] fullerene or graphite could interfere in IL-1β determination, anti-IL-1β antibodies coated microplate wells were incubated with 1 mg/ml [60] fullerene or graphite in supplemented RPMI for 2 hours at 37 °C. After three washes in pH 7.4 phosphate buffered saline (Sigma, France), LPS control supernatants were tested for IL1-β by the treated antibodies. Results obtained were then compared with

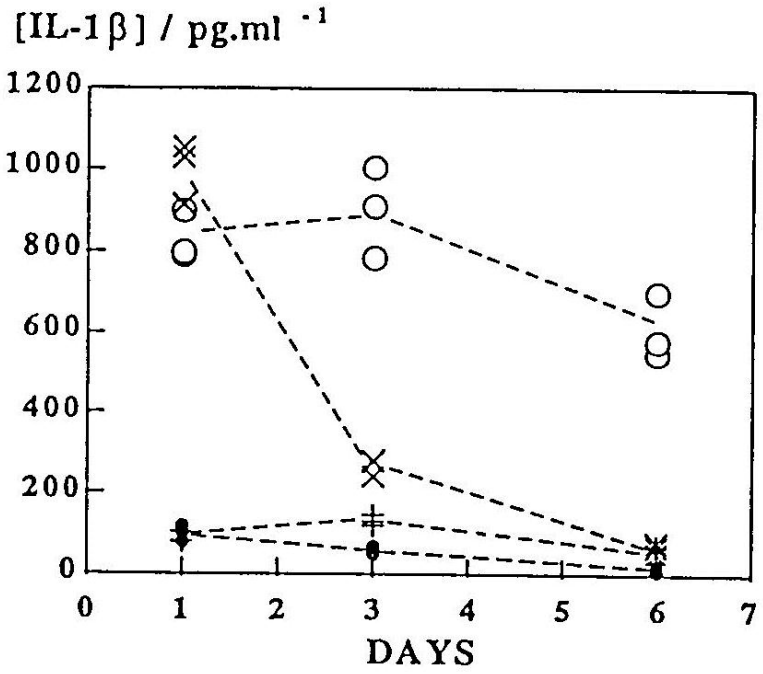

FIG 1 - Monocytes IL-1β secretion in RPMI (5.10^5 monocytes per ml) : (+) Monocytes alone ; Monocytes with: (•) 1 mg/ml of [60] fullerene, (x) 1 mg/ml of graphite, (o) 3 µg/ml of LPS.(Dashed lines have been drawn as guides for the eyes).

those achieved without [60] fullerene or graphite prior incubation. In the same way LPS control supernatants were mixed with 1 mg/ml [60] fullerene or graphite RPMI suspensions for 2 hours at 37 °C and then checked for IL-1β levels.

Results and Discussion.

Figure 1 shows comparative IL-1β levels obtained after 1, 3 and 6 days incubation in RPMI, LPS, graphite and [60] fullerene supernatant controls.

High amounts of IL-1β are detected in LPS control during the whole experiment. With graphite IL-1β secretion is also increased at J = 1 and then decreases quickly. With [60] fullerene the response intensity does not exceed that of the negative control (RPMI control).

To check whether this lack of response with [60] fullerene is due to a possible scatter, the monocyte distribution inside the wells has been determined by measuring supernatant IL1-β levels obtained at J = 1 with 8 negative controls. It comes [IL1-β] = (134 ± 15) pg/ml. This result eliminates the possibility of scatter.

Cell lysis could explain low IL-1β level detected in supernatant [60] fullerene control. So LDH enzymatic activity has been measured in supernatants. Figure 2 shows that LDH excretion with [60] fullerene is significantly lower than with graphite. These results agree with those previously obtained[1].

We have also checked that [60] fullerene does not interfere with IL-1β determination. No difference has been observed in IL-1β levels between LPS supernatant control alone and mixed with [60] fullerene suspension as described in method. In the same way no difference has been observed between normal IL-1β determination and after microplate incubation with [60] fullerene.

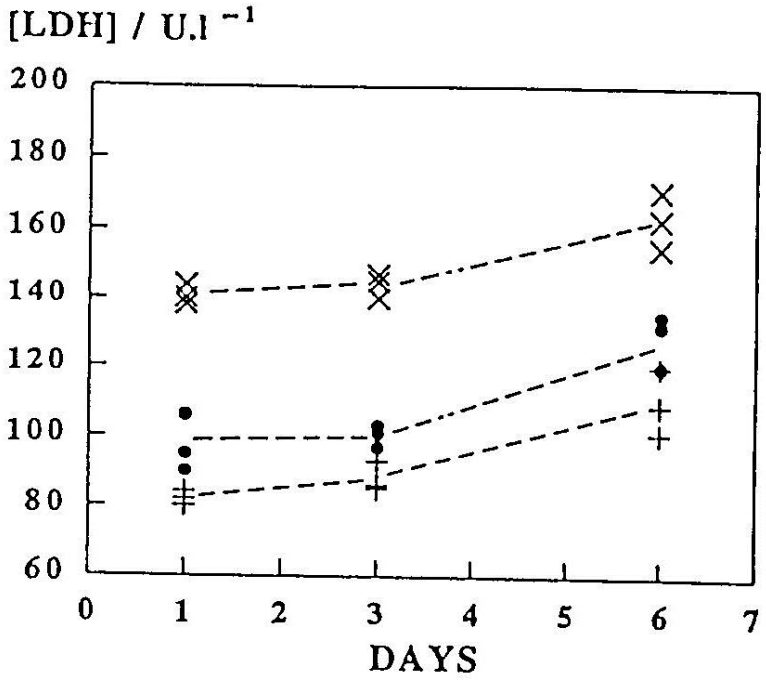

FIG 2 - LDH excretion (International Units per litre of supernatant). (Experimental conditions and symbols, as in figure 1 caption).

To visualize intra-individual response intensity variation, we performed the same experiment three times with the same donor. The results obtained were in agreement with those shown in Fig. 1.

To check inter-individual variations we repeated the same experiments with monocytes collected from 4 healthy donors. Results obtained at J = 1 are summarized in Table 1. Despite response intensity variation, [60] fullerene effects remain identical to those of Fig. 1: strong positive response with LPS, positive response

Table 1 - **Human monocytes IL-1β secretion (pg/ml) as per donor.** Monocytes cultured in supplemented RPMI alone (*) and with LPS (**) (3 mg/ml in supplemented RPMI), graphite (+) and [60] fullerene (++) (both 1 mg/ml in supplemented RPMI).

Donor n°	1 (woman)	2 (woman)	3 (man)	4 (man)	5 (man)
cells/ml	2.10^6	2.10^6	2.10^6	2.10^6	2.10^6
monocytes (%)	25	15	25	33	9
cytokin	IL-1β	IL-1β	IL-1β	IL-1β	IL-1β
RPMI *	68	33	62	140	<15
LPS **	>1000	425	>1000	>1000	275
graphite +	200	450	320	448	75
C_{60} ++	52	51	68	160	<15

with graphite, negative response with (60) fullerene (powdered or micronized[3]).

Although monocytes were activated by [60] fullerene particles, the increase of IL-1β secretion is not observed. Preliminary measurements show the same response as to what concerns interleukin-6.

These results could be connected with the obvious lack of inflammatory reaction observed in Swiss mice having received large amounts of micronized [60] fullerene[3].

The understanding of this phenomenon (indifference, specific or unspecific inhibition, ...) remains to be clarified.

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