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Candida albicans polysaccharide extract (MPPS) and PPD stimulate the production of interleukin-1 and lymphocyte proliferation

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SUMMARY

Peripheral blood mononuclear cells (PBMC) were stimulated *in vitro* either with *Candida albicans* polysaccharide extract (MPPS) or with PPD. Both MPPS and PPD driven lymphocyte proliferation was strictly dependent on the presence of macrophages. In fact purified T cells failed to proliferate unless adherent cells were added. The ability of monocytes to produce interleukin-1 (IL-1) was then investigated. Both MPPS and PPD caused the release IL-1 into the culture supernatant, as measured in a direct thymocyte proliferative assay. MPPS and PPD also stimulated the production of IL-1 by the mouse macrophage like line $P388D_1$. These data support the view that the antigen specific activation of human T cells by MPPS and PPD requires both antigen presentation and IL-1 production.

Keywords *Candida albicans* polysaccharide extract PPD monocytes interleukin-1

INTRODUCTION

The macrophage has two distinct functions in the antigen specific activation of T cells. The first function is the binding, processing and presentation of antigen in an immunogenic form to T cells (Farr, Kiely & Unanue, 1979). This ability is confined to those macrophages which carry class II antigen of the major histocompatibility complex (Schwartz, Yano & Paul, 1978). The second macrophage function is the production of a low molecular weight peptide termed interleukin-1 (IL-1) which is a maturation signal for T cells (reviewed by Mizel, 1982; Lachman, 1983).

Human peripheral blood mononuclear cells (PBMC) from healthy donors show increased DNA synthesis when stimulated *in vitro* for 5 days with antigenic preparations of *Candida albicans* (MPPS) (Piccolella, Lombardi & Morelli, 1980). This paper investigates MPPS and PPD driven T cell proliferation and evaluates the role of monocytes in antigen presentation and IL-1 production. The results show that monocytes are required for T cell proliferation and that MPPS and PPD stimulate the production of IL-1 by human mononuclear cells and from a mouse macrophage like cell line.

MATERIALS AND METHODS

Cell preparation. PBMC from healthy donors were isolated on Ficoll-Hypaque gradient. PBMC were separated into T and non-T cell subpopulations by E rosetting (Piccolella *et al.*, 1980). To

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obtain an enriched monocyte population, rosette negative cells (2×10^6) were plated on Petri dishes in 5 ml of RPMI 1640 medium with 10% heat-inactivated fetal calf serum, glutamine (2 mM) and gentamicin (40 μg/ml). Two cycles of 1 h of incubation at 37°C were performed. The non-adherent cells were removed and the adherent population was recovered. The adherent cells did not respond to T and B cell mitogens and are referred as the monocyte enriched population. This cell population (10^6/ml) was then pulsed for 1 h at 37°C with MPPS (100 μg) or PPD (50 μg), washed twice and used as antigen presenting cells in a specific proliferative assay.

Microbial antigens. Polysaccharide fraction (MPPS) obtained from *C. albicans* (Piccolella *et al.*, 1980) and purified protein derivative (PPD) from *Mycobacterium tuberculosis* (Statens Seruminstitut, Copenhagen, Denmark) were used.

Proliferative cell assay. Unfractionated PBMC or purified T lymphocytes were suspended in medium and cultured (10^5 cells/well) using microtitre flat bottom plates (Falcon, 3040) in a volume of 200 μl. Different concentrations of soluble MPPS and PPD or different number of MPPS and PPD pulsed macrophages were added. The cells were cultured in a CO_2 incubator and ^{3}H-thymidine (0·5 μCi, Amersham International) was added 12 h before harvesting. Radioactivity was expressed as counts per minutes (ct/min) of triplicate cultures.

Production of human and mouse IL-1. Unfractionated PBMC, and a mouse macrophage line $P388D_1$ (Mizel & Mizel, 1981) were cultured with soluble MPPS, PPD or lipopolysaccharide (LPS, 20 μg/ml, *E. coli* 127 B 8, DIFCO, Michigan, USA). The supernatants were collected at 24 h passed through a Millipore filter (0·45 μm) and stored at −20°C.

IL-1 assay. A direct thymocyte proliferative assay was used (Mizel, 1982). Thymus cells (10^7/ml) from C3H mice, 5–7 weeks of age were cultured for 24 h in microtitre plates in triplicate in presence of 0·1 ml of serial dilutions of the IL-1 containing supernatants. In some experiments a submitogenic dose of PHA (DIFCO P_1 1 μg/ml) was added and the cells harvested at 48 h. Six hours prior to harvesting, 0·5 μCi ^{3}H-thymidine was added. There was a dose-dependent response of thymocytes for stimulation by IL-1 containing supernatants, while MPPS and PPD alone failed to stimulate DNA synthesis. In the Results section, only the dilution of IL-1 supernatant giving maximal thymocyte stimulation is reported.

Interleukin-2 (IL-2) assay. IL-2 was assayed by adding 100 μl of CTL-L (2×10^4) to 100 μl of a 1/4 dilution of culture supernatant. ^{3}H-thymidine was added at 24 h and the cells harvested at 30 h. In the experiment in Fig. 4, IL-2 (10% final concentration) produced by stimulating normal PBMC with PHA for 48 h was added at the beginning of some of the cultures.

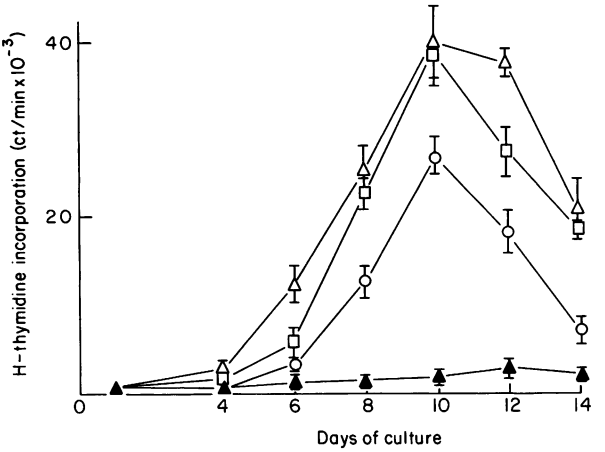

Fig. 1. Incorporation of ^{3}H-thymidine at different days of culture by normal PBMC (10^5/well) stimulated with various doses of candida polysaccharide (MPPS) Δ = 10 μg/ml; □ = 5 μg/ml; ○ = 1 μg/ml. Control (▲) indicates ^{3}H-thymidine incorporation of PBMC cultured alone. Data are expressed as counts per minute (ct/min) ± standard deviation (s.d.).

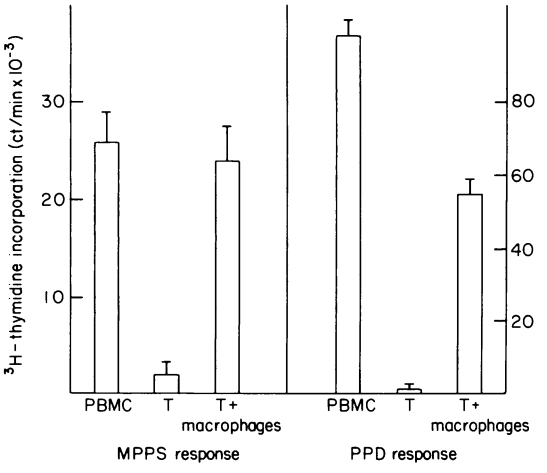

Fig. 2. Incorporation of ^{3}H-thymidine at day 7 by normal PBMC or T cells depleted of adherent cells, in presence of soluble MPPS (10 μg/ml) or PPD (20 μg/ml). T cells were also cultured for 7 days with MPPS or PPD pulsed macrophages (15×10^4/well).

RESULTS

Fig. 1 shows the ability of different concentration of polysaccharide extract from *C. albicans* (MPPS) to induce cell proliferation. The peak of thymidine incorporation occurred at day 10. This is an unusual finding as most microbial antigens such as PPD induce blast transformation in 5–6 days. For this reason MPPS and PPD were studied in parallel.

To investigate the role of antigen presenting cells in antigen specific T cell proliferation, adherent cells were pulsed *in vitro* either with MPPS or PPD and cultured for 5 days with purified T cells. Fig. 2 shows that unfractionated mononuclear cells are stimulated by soluble MPPS or PPD. In contrast, purified T cells which lack adherent cells fail to react to soluble MPPS and PPD. However,

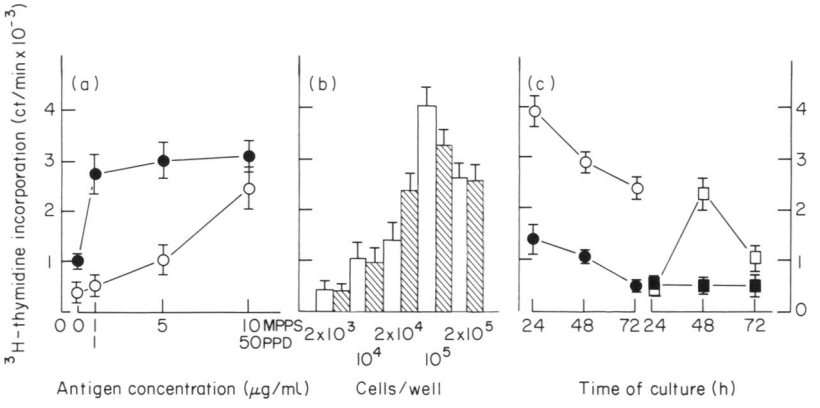

Fig. 3. Incorporation of ^{3}H-thymidine of mouse thymocytes (10^6/well) in presence of different IL-1 containing supernatants from human mononuclear cells. (a) Effect of supernatants obtained by culturing mononuclear cells with various doses of soluble MPPS or PPD. The culture supernatants were collected 48 h (MPPS) or 24 h (PPD) afterwards and tested in a direct thymocyte assay. See Materials and Methods. (b) The IL-1 production by various numbers of mononuclear cells stimulated with PPD (20 μg/ml, □ column) or by MPPS (10 μg/ml ■). (c) Time course of the production of IL-1 by 4×10^4 cells in the presence (○) or absence (●) of 20 μg/ml of PPD or by 2×10^4 cells in presence (□) or absence (■) of 10 μg/ml of MPPS.

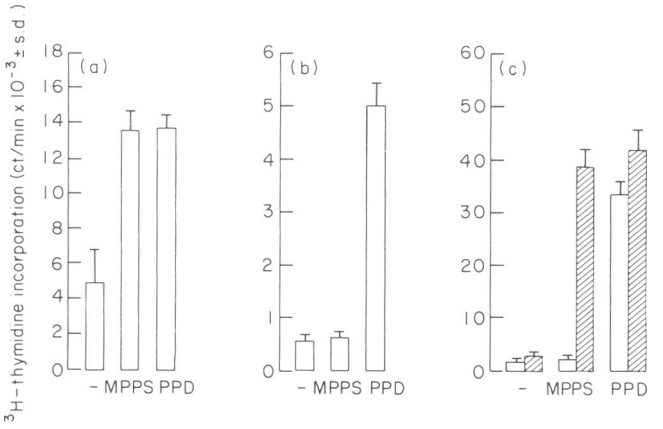

Fig. 4. (a) Thymocyte and (b) CTL-L (IL-2) assays on supernatants of PBMC stimulated with MPPS or PPD. The first two panels show the thymocyte and CTL-L (IL-2) assay on supernatants of PBMC taken 2 days after stimulation. The thymocyte assay was undertaken in the presence of suboptimal amount of PHA. The third panel (c) shows lymphocyte proliferation on day 6 in the absence (□) or presence (▨) of added exogenous IL-2.

MPPS or PPD pulsed monocytes cause strong T cell proliferation. Other experiments showed that unpulsed macrophages fail to cause proliferation (data not shown).

The previous experiment illustrated the role of adherent cells in antigen presentation to T cells. The following experiment investigated whether human mononuclear cells make IL-1 when stimulated by MPPS and PPD. Fig. 3a shows that both MPPS and PPD induce the production of IL-1 by mononuclear cells as measured in the thymocyte assay. The optimal cell concentration was 5×10^5 cells/ml both in the MPPS and PPD system (Fig. 3b). However, a striking difference in the time course of IL-1 production between the two microbial antigens was observed. Fig. 3c shows that

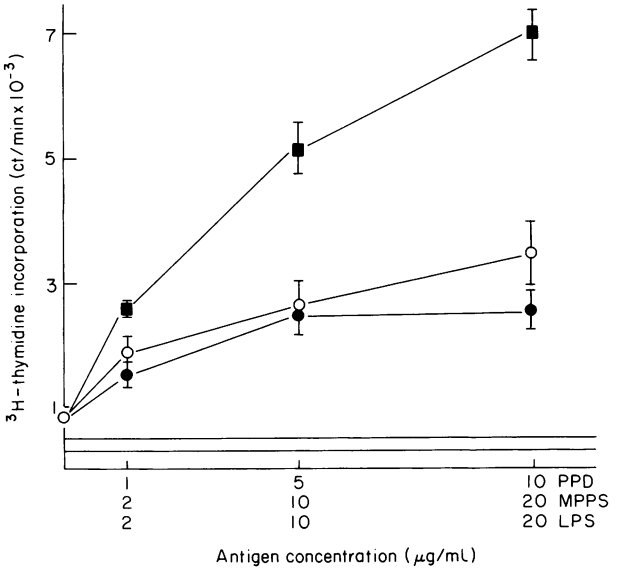

Fig. 5. Incorporation of ^{3}H-thymidine of murine thymocytes in the presence of IL-1 containing supernatants from the mouse macrophage-like cell line P388D_1. The line was cultured (10^6 cells/ml) in presence of various doses of MPPS (●) PPD (○) or LPS (■). The supernatants were collected at 24 h and tested for the presence of IL-1 in the thymocyte assay.

PPD induces the production of IL-1 within 24 h, while MPPS only induces appreciable amounts of IL-1 at 48 h.

A possible criticism of these results is that the thymocyte assay does not distinguish between IL-1 and IL-2. The following study of three normal subjects who fail to proliferate in response to MMPS but have normal responses to PPD indicates that IL-1 production can occur in the absence of IL-2 production. Fig. 4 shows a typical experiment. Supernatant taken 2 days after stimulation with MPPS is strongly positive in the thymocyte assay but negative in the IL-2 assay. In keeping with this no proliferation to MPPS was seen on day 6. This dissociation of the thymocyte and the IL-2 assays implies that at least in the case of MPPS the thymocyte assay was measuring IL-1. The failure of PBMC proliferation to MPPS may have been due to a failure of IL-2 production as good proliferation occurred when exogenous IL-2 was added. In contrast two days after stimulation with PPD both the thymocyte and the IL-2 assay were positive and in this situation it is not possible to be certain whether IL-1 or IL-2 is being measured by the thymocyte assay.

To confirm that both antigen were able to stimulate the production of IL-1, the effect of MPPS and PPD on a macrophage like cell line was studied. This line ($P388D_1$) produces high levels of IL-1 when stimulated with bacterial lipopolysaccharide (LPS) (Mizel, 1982). Fig. 5 shows that both MDPS and PPD induce the production of mouse IL-1 although to a lesser extent than LPS.

DISCUSSION

It is widely believed that monocytes have two functions in T cell proliferation. They bind and process the antigen and present it together with major histocompatibility complex products to T cells (Schwartz *et al.*, 1978; Farr *et al.*, 1979). They also produce a lymphocyte activating factor(s) called IL-1 (Mizel, 1982; Oppenheim & Gery, 1982; Lachman, 1983). It is likely that these two signals are required for the activation of T cells by MPPS and PPD. Although the requirement of two signals has been extensively studied in mitogen- and alloantigen-induced T cell activation, no clear data are available in antigen-induced human T cell proliferation. Purified polysaccharide extract from *C. albicans* (MPPS) and purified protein derivative from *M. tuberculosis* (PPD) are two 'recall' microbial antigens able to induce a secondary proliferative response of peripheral T lymphocytes from donors that have been previously exposed to Mycobacteria or Candida (Wirtz *et al.*, 1984). The kinetics of the proliferative response and the failure of cord blood lymphocytes to recognize PPD and MPPS (unpublished results) indicate that this response is antigen driven.

In the thymocyte proliferative assay for IL-1, the IL-1 induces IL-2 production which is regarded as the immediate cause of increased DNA synthesis. For this reason the assay does not distinguish between IL-1 and IL-2. There are two ways of overcoming this problem with the thymocyte assay. One approach is to use populations lacking T cells so that IL-2 cannot be produced. In the present experiments both MPPS and PPD released IL-1 from a macrophage like murine cell line. Only small quantities of IL-1 were released from adherent macrophages, perhaps because adherent macrophages are reported to be poor IL-1 producers (Treves *et al.*, 1983).

The alternative approach is to find individuals with a dissociation between IL-1 and IL-2 production. In fact three normal individuals out of about 20 tested failed to proliferate in response to MPPS and produced IL-1 but not IL-2. These individuals were not generally anergic as they gave normal responses to PPD. Hence it may be asserted that MPPS causes IL-1 production from both mouse macrophages and human monocytes, while PPD causes IL-1 production from mouse macrophages and presumably from human monocytes.

It has been recently shown that PPD fails to induce the proliferation of T cells depleted of macrophages unless IL-1 is provided (Treves *et al.*, 1983). Our present findings confirm the need for macrophages or related antigen presenting cells in the induction of antigen specific T cell proliferation. Moreover the correlation between the time course of IL-1 production and of cell proliferation in the PPD and Candida systems suggests that the kinetics of IL-1 production may determine the kinetics of cell proliferation. In fact, cultures stimulated with PPD show a peak of Il-1 production on day 1 and of proliferation on day 5. In contrast cultures stimulated with Candida show a peak of IL-1 production on day 2 and of proliferation on day 10. The kinetics of IL-1

production are in keeping with the findings of Mayernik, Haq & Rinehart (1983) that human monocytes lose the ability to produce IL-1 after 3 days in culture.

The present results add further weight to the view that T cells and their products may augment but are not always essential for the production of IL-1. On the one hand activated T cells greatly increase IL-1 production by human macrophages (Lachman, 1983) and the mouse cell line P338D_1 (Mizel, 1982). On the other hand the present results show that the mouse macrophage like cell line produces IL-1 when stimulated with PPD and Candida polysaccharide in the absence of T cell products. There are several possible ways in which the macrophage might recognize these antigens as stimuli for IL-1 production. The macrophage may have receptors for the polysaccharide or other part of the antigen. Alternatively, the antigen may activate complement components secreted by the macrophage and these activated components may be the immediate stimulus for IL-1 production. Finally these antigens may be interferon inducers and the interferon may in turn activate the macrophage. The finding that macrophages produce interferon and that interferon enhances the secretion of IL-1 is in keeping with this view (Arenzana-Seisdedos & Virelizier, 1983).

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REFERENCES

ARENZANA-SEISDEDOS, F. & VIRELIZIER, J.L. (1983) Interferon as macrophage-activating factors. II. Enhancement secretion of interleukin 1 by lipopolysaccharide stimulated human monocytes. *Eur. J. Immunol.* **13,** 437.

FARR, A.G., KIELY, J.M. & UNANUE, E.R. (1979) Macrophage–T cell interactions involving *Listeria monocytogenes*: the role of the H-2 gene complex. *J. Immunol.* **122,** 2395.

LACHMAN, L.B. (1983) Human interleukin 1: purification and properties. *Fed. Proc.* **42,** 2639.

MAYERNIK, D.G., HAW, A. & RINEHART, J.J. (1983) Evaluation of human monocytes–macrophage interleukin 1 secretion. *Fed. Proc.* **42,** 455.

MIZEL, S.B. (1982) Interleukin 1 and T cell activation. *Immunol. Rev.* **63,** 51.

MIZEL, S.B. & MIZEL, D. (1981) Purification to apparent homogeneity of murine interleukin 1. *J. Immunol.* **126,** 834.

OPPENHEIM, J.J. & GERY, J. (1982) Interleukin 1 is more than one interleukin. *Immunol. Tod.* **3,** 133.

PICCOLELLA, E., LOMBARDI, G. & MORELLI, R. (1980) Human lymphocyte-activating properties of a purified polysaccharide from *Candida albicans*: B and T cell cooperation in the mitogenic responses. *J. Immunol.* **125,** 2082.

SCHWARTZ, R.H., YANO, A. & PAUL, W.E. (1978) Interaction between antigen presenting cells and primed T lymphocytes: an assessment of Ir gene expression in the antigen-presenting cell. *Immunol. Rev.* **40,** 153.

TREVES, A.J., BARAK, V., TAL, T. & FUKS, Z. (1983) Constitutive secretion of interleukin 1 by human monocytes. *Eur. J. Immunol.* **13,** 647.

WIRTZ, P., LOMBARDI, G., PUGLIESE, R., MORELLI, M. & PICCOLELLA, E. (1984) Anti-Candida antibody production and specific blast transformation in patients with *Candida albicans* infection. *Clin. Immunol. Immunopath.* (In press.)