Clin. exp. Immunol. (1980) **41**, 309–316.

Activation of human monocytes for nitroblue tetrazolium reduction and the suppression of lymphocyte response to mitogens

M. ZEMBALA, E. M. LEMMEL* & W. URACZ *Division of Clinical Immunology, Institute of Paediatrics, Copernicus School of Medicine, Cracow, Poland, and*
* *Institute of Medical Microbiology, University Medical School of Mainz, Mainz, FRG*

(Accepted for publication 28 February 1980)

SUMMARY

Human peripheral blood mononuclear cells freed from polymorphs reduce nitroblue tetrazolium (NBT). This reduction is due to monocytes, i.e. adherent, phagocytic, esterase-positive cells with Fc receptors. Monocytes allowed to phagocytose zymosan show increased NBT reduction which under optimal conditions is $12{\cdot}2 \pm 2{\cdot}4 \times 10^{-9}$ $mol \cdot hr^{-1} \cdot 10^{-6}$ monocytes. Monocytes which have phagocytosed zymosan depress the mitogen response of human lymphocytes to PHA. This effect of 'activated' monocytes is due to a soluble inhibitory mediator which appears in the supernatant after culture for 24 hr. Its appearance requires protein synthesis. It is suggested that NBT reduction of peripheral blood mononuclear cells can be used as a test for the state of monocyte activation in disease. The possibility that activated monocytes may depress blast transformation *in vitro* in disease states is discussed.

INTRODUCTION

The role of macrophages in the regulation of different forms of cell-mediated immunity is well established (Allison, 1978; Unanue, 1978; Ptak, Zembala & Gershon, 1978). It is also known that the presence of monocytes is required for the response of human lymphocytes to mitogens and antigens *in vitro* (Bergholtz & Thorsby, 1977; Levis & Robbins, 1970; Schmidtke & Hatfield, 1976; de Vries *et al.*, 1979). The modulation of lymphocyte response to mitogen or specific antigens *in vitro* may be due to the release of various soluble mediators with both enhancing and inhibitory properties (Avala-Chaves *et al.*, 1978; Rinehart, Orser & Kaplan, 1979). Studies of monocyte function in cancer patients are of considerable interest. Monocyte defective chemotaxis, enhanced cytotoxicity against opsonized human red cells and the altered Fc receptor expression have been demonstrated (Hausman *et al.*, 1975; Rhodes, Bishop & Benfield, 1979; Uracz *et al.*, 1978). The role of monocytes in the suppression of lymphocyte responsiveness has also been shown (Berlinger, Lopez & Good, 1976; Quan & Burtin, 1978; Zembala *et al.*, 1977). These studies have implicated monocyte 'activation' (Rhodes *et al.*, 1979) but there is no formal proof for this, partially due to difficulties in determining the state of monocyte activity.

The purpose of the present investigation was to adapt the NBT reduction test for quantitative determinations of human monocyte activity. The effect of monocyte activation with a phagocytic stimulus on the NBT reduction and the response of autologous peripheral blood lymphocytes to mitogens was also studied.

Correspondence: Marek Zembala, Division of Clinical Immunology, Institute of Paediatrics, Wielicka 265, 30–663 Cracow, Poland.

0099-9104/80/0800-0309$02.00

The present paper shows that human monocytes stimulated with zymosan exhibit an increased NBT reduction. The intracellular reduction of NBT correlates with hexose–monophosphate shunt activity (Nathan, Karnovsky, & Davis, 1971) which is known to be increased in activated mononuclear phagocytes (Cohn, 1978). Thus the NBT test can be used as a measure of the state of monocyte activation. Monocytes activated with zymosan exert a suppressive effect on autologous lymphocyte response to mitogen, presumably via a soluble mediator(s).

MATERIAL AND METHODS

Isolation of mononuclear cells. Peripheral blood mononuclear cells (PBM) were isolated from heparinized peripheral blood of healthy donors on a standard Isopaque/Ficoll gradient following Böyum (1968). The cells were washed once with Eagle's minimum essential medium (MEM, Difco) without serum, and the cell concentration was adjusted to 1×10^7/ml. Cell suspensions containing > 1% granulocytes were excluded.

NBT test. An equal volume of 0·1% *p*-nitroblue tetrazolium (Roth, Karlsruhe) solution in 0·15 M NaCl was added to 0·2 ml of PBM cell suspension. The cells were incubated for different periods of time. Various concentrations of either boiled and washed zymosan (Sigma) resuspended in MEM, or lipopolysaccharide (LPS) from *E. coli* 055:B5 (Difco) were added to the test-tubes. In a standard test PBM cells were preincubated for 1 hr with 400 μg/ml of zymosan and then NBT was added for a further 1 hr. The reaction was stopped by adding an equal volume of 0·1 N HCl. The cells were washed, centrifuged at 800 ***g***, and the cell pellet was allowed to dry at 37°C in the dark. To extract the formazan, 1 ml of dioxane (Roth, Karlsruhe) was added to each tube and the tubes were transferred to a water bath at 85°C for 20 min. Cell debris and zymosan particles were removed by centrifugation and the optical density (OD), of the supernatant was read at 580 nm in a Zeiss spectrophotometer. Dioxane was used as a blank. All tests were run in triplicate.

Adherent cells. In some experiments adherent cells (AC) were used for the NBT test. Thermanox 13-mm round plastic coverslips (Lux Scientific Corp.) were covered with 0·1 ml of PBM cell suspension. The cells were incubated for 30 min, non-adherent cells removed and the coverslips were washed three times. Zymosan and NBT were then added as specified above. At the end of incubation, the coverslips were washed in saline, dried, placed in tubes and extracted with dioxane. The total cell numbers and the percentage of non-specific esterase-positive cells were estimated on other slides run in parallel.

Monocyte Fc receptor determination. The AC monolayers on coverslips, obtained as above, were treated with zymosan (400 μg/ml) for 1 hr, washed three times and covered with 1% human O, Rh^+ erythrocytes coated with hyperimmune IgG anti-D serum (see Uracz *et al.*, 1978). After incubation for 30 min at 37°C in a CO_2 incubator, the coverslips were washed and the percentage of cells forming rosettes (EA-RFC) was determined. Non-specific esterase staining was performed on separate slides run in parallel.

Staining for non-specific esterase. Non-specific esterase staining was performed according to Yam, Li & Crosby (1971).

Lymphocyte cultures. Isolated PBM cells were cultured in flat-bottomed microplates (Greiner) in 0·2 ml of Eagle's MEM supplemented with 5% human AB serum containing 2×10^5 cells per well. For stimulation 5 μg/ml of phytohaemagglutinin (PHA-P, Difco) was used. The cells were cultured for 3 days at 37°C in a CO_2 incubator with a 4-hr terminal pulse of 1 μCi/ml of ^{3}H-thymidine (Radiochemical Centre, Amersham; specific activity 5,000 mCi/mmol). The cells were harvested with an automatic Scatron cell harvester. The radioactivity was determined using Rotiszint 11 (Roth, Karlsruhe) in a Tricarb scintillation counter (Packard).

Preparation of PBM cell subpopulations. Adherent cells (AC) were obtained by the incubation of 2×10^5 PBM cells per microplate well for 45 min. Non-adherent cells (NAC) were removed and adherent cells were washed three times with Eagle's MEM. This yielded $0{\cdot}5–1{\cdot}5 \times 10^4$ AC cells per well. The adherent fraction contained 65–95% of esterase-positive, 70–95% phagocytic and 85–98% NBT reducing cells. Lymphocyte contamination was 2–17%, usually less than 5% (cf. Rinehart *et*

al., 1979). These cells did not respond to stimulation with PHA and their spontaneous ^{3}H-thymidine incorporation was less than 250 c.p.m.

In some experiments AC were pretreated with different concentrations of zymosan for 45 min, then rinsed three times. Autologous NAC or unseparated PBM cells (2×10^5 per well) and PHA were then added to wells containing untreated or zymosan-treated AC. For blocking protein synthesis 1 μmol of emetine (Sigma) was added to untreated or zymosan-treated AC cells for 1 hr. In a separate experiment it was shown that this concentration of emetine blocked protein synthesis almost completely (*ca* 95%) as judged from incorporation of ^{3}H-leucine, in unstimulated or PHA-stimulated lymphocytes. After incubation with emetine, AC were washed three times before addition of the lymphocytes.

NAC removed from the wells after 45 min of incubation were further purified by additional adherence for 45 min to small plastic petri dishes (internal diameter 4·6 cm; Sterilin). NAC were removed, washed, resuspended in culture medium, adjusted to a concentration of 1×10^6/ml and kept on ice until use. These cells, when cultured alone, responded poorly to PHA and their contamination with esterase-positive cells was usually less than 1%.

Monocyte supernatants. AC obtained from 3×10^6/ml PBM cells were cultured for 24 hr in small petri dishes in Eagle's MEM corresponding to half the volume used for cell seeding. This gave approximately 4×10^5 monocytes/ml. Supernatants were obtained from the following cultures: (a) zymosan-stimulated monocytes; (b) zymosan-stimulated monocytes treated with emetine; (c) untreated monocytes; (d) untreated monocytes exposed to emetine. Supernatants were centrifuged at 800 ***g*** and kept frozen at −30°C till use. They were added to PBM cell cultures at a final concentration of 20%.

Analysis of results. Statistical analysis was done using Student's *t*-test.

RESULTS

NBT reduction by unstimulated and zymosan-stimulated MN cells

Isolated PBM cells were preincubated either with zymosan (400 μg/ml) or left untreated for different periods of time. NBT was then added to all tubes and the cells incubated for 1 hr before measuring the reduction of NBT. The washed and dried cell pellet was extracted with dioxane.

Fig. 1 shows that zymosan-stimulated NBT reduction by PBM cells reached a peak at 1 hr and then decreased steadily. The spontaneous reduction changed little with time. In subsequent experiments a 1-hr preincubation with zymosan was used for stimulation.

In the next experiment various concentrations of zymosan and LPS were used for stimulation. Fig. 2 shows that the optimal concentration of zymosan was around 400 μg/ml, whereas higher concentrations caused less NBT reduction. LPS caused no stimulation.

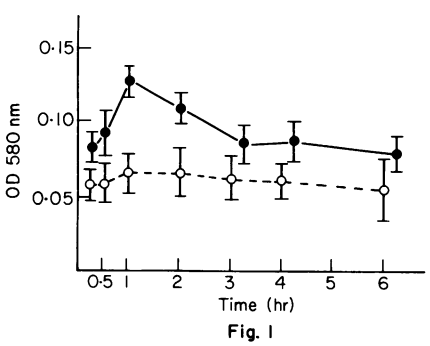

Fig. 1

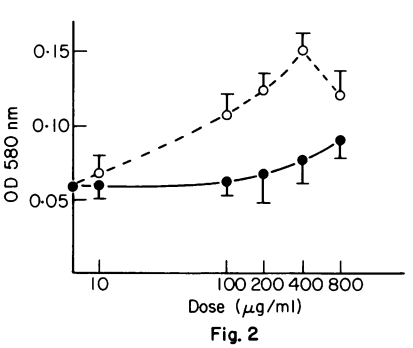

Fig. 2

Fig. 1. The NBT reduction by unstimulated PBM cells (o – – – o) and cells stimulated with 400 μg/ml zymosan (●——●) for different periods of time. The mean ± s.d. of the optical density (OD) in five different experiments is shown.

Fig. 2. NBT reduction by PBM cells stimulated with different doses of zymosan (o – – – o) and LPS (●——●). The mean ± s.d. of three experiments is shown.

It was concluded that PBM cells were able to reduce NBT. The cell responsible for the reduction was probably a phagocytic cell as zymosan led to the increased reduction. The next section shows that the monocyte was responsible.

Characterization of the NBT-reducing cell in PBM suspensions

The number of non-specific esterase-positive cells in each PBM suspension tested for NBT reduction was determined. Fig. 3 shows that there was a relationship between the number of esterase-positive cells and the spontaneous and zymosan-activated NBT-reduction capacity of PBM cells.

In the next experiments the NBT reduction by non-adherent cells was compared. Fig. 4 demonstrates that even in small numbers AC were able to reduce NBT while there was virtually no activity in the NAC fraction. It was concluded that the NBT-reducing cell in the PBM population was a monocyte because the correlative evidence indicated that it was esterase-positive and adherent to plastic.

Since there was a correlation between the number of esterase-positive cells and the extent of NBT reduction by different PBM populations used at a constant cell concentration, it was possible to express the results of NBT reduction per unit number of esterase-positive cells. The NBT-reduction activity of unstimulated and zymosan-stimulated PBM cell suspensions obtained from twelve normal donors was determined (cultures with $> 1\%$ granulocytes were excluded). The amount of reduced NBT was calculated from a standardization curve obtained after the reduction of NBT with ascorbic acid (Alföldi & Lemmel, 1979). It was found that spontaneous mean reduction

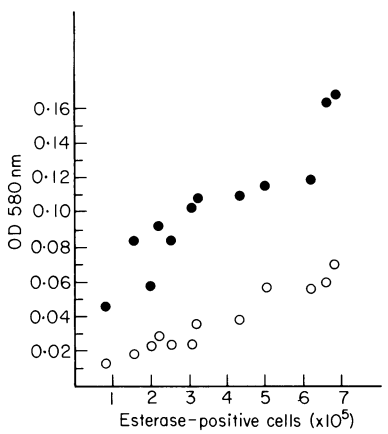

Fig. 3. Correlation between the number of esterase-positive cells and NBT reduction by unstimulated (○) and zymosan-stimulated (●) PBM cells from twelve normal persons.

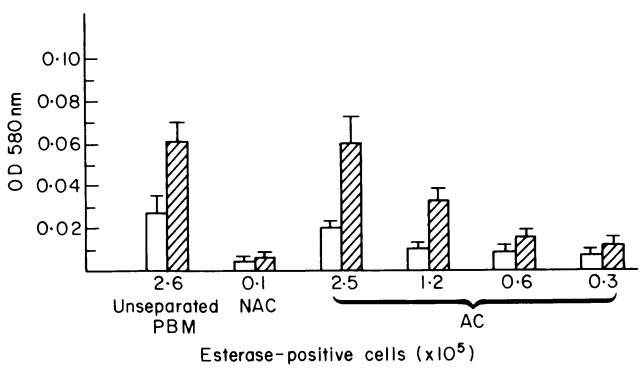

Fig. 4. NBT reduction by unseparated PBM cells, NAC and AC fractions unstimulated (□) or stimulated with zymosan (▨). The number of esterase-positive cells is shown below the columns.

($\pm$s.d.) was $4{\cdot}5 \pm 1{\cdot}1 \times 10^{-9}$ mol/10^6 esterase-positive cells and $12{\cdot}2 \pm 2{\cdot}4 \times 10^{-9}$ mol/10^6 cells stimulated with zymosan.

Effect of monocyte activation with zymosan on Fc receptor expression

The relative number of EA rosette-forming cells was determined in AC populations preincubated with zymosan or left untreated. The mean value for untreated cells from eight normal donors was 18% (range 1–39%) and 48% (range 30–67%) for cells pretreated with zymosan. Differences were observed between donors in the detection of EA rosette-forming cells. However, the cells obtained from one donor always showed a consistent increase after zymosan pretreatment (Fig. 5).

It was concluded that activation of monocytes with zymosan led to increased Fc receptor expression.

Effect of zymosan-stimulated adherent cells on the response of lymphocytes to mitogens

The fact that zymosan-activated monocytes showed increased NBT reduction suggested that they might be functionally altered. The influence of these monocytes on the lymphocyte mitogenic response was checked in the following experiment. AC were incubated with various concentrations of zymosan for 1 hr. The cells were washed three times; autologous NAC and PHA were then added. Fig. 6 shows that the response of autologous lymphocytes added to AC preincubated with zymosan was decreased. The extent of inhibition was dependent on the amount of zymosan used for AC stimulation.

To exclude the possibility that zymosan was toxic for monocytes or blocked their accessory effect, either unseparated PBM or NAC were added to AC pretreated with zymosan. As shown in Fig. 7 zymosan-pretreated AC caused the inhibition of the mitogenic response of both unseparated PBM and NAC. Untreated AC were not inhibitory.

It was concluded that the activation of monocytes was due to active suppression and not to the inhibition of their normal accessory function, since unseparated PBM cells possessing functionally competent monocytes were also inhibited.

In a separate experiment it was demonstrated that the effect of zymosan was not observed when monocytes were subsequently treated with emetine. This suggested that the inhibition required active protein synthesis in monocytes. To check whether zymosan-activated monocytes acted via

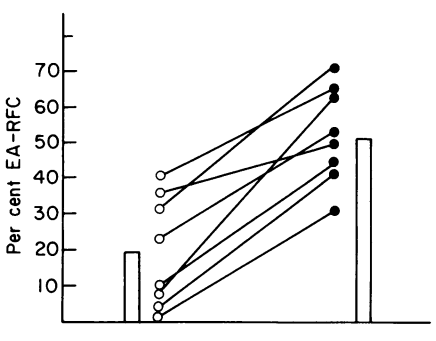

Fig. 5

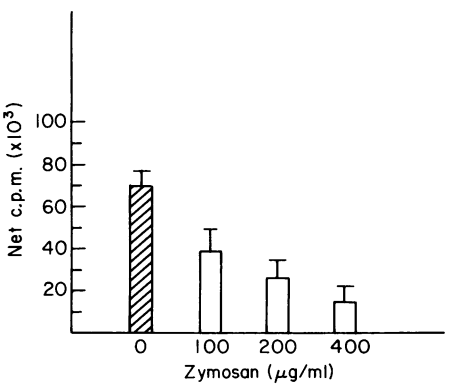

Fig. 6

Fig. 5. The effect of stimulation of AC with zymosan on Fc receptor expression. The AC monolayers were treated with 400 μg/ml zymosan for 1 hr and washed or left unstimulated. The percentage of AC forming EA rosettes was determined after 30 min of incubation with human O, Rh^+ (D) erythrocytes coated with IgG anti-D serum. (o) Untreated AC, (•) zymosan-treated AC, $0{\cdot}02 < P < 0{\cdot}01$.

Fig. 6. The effect of stimulation of AC with zymosan on the response of autologous lymphocytes to PHA. AC separated from PBM suspension were incubated for 1 hr with different concentrations of zymosan and washed. Autologous NAC (2×10^5 per well) and PHA were then added. Lymphocyte stimulation was assessed after 3 days of culture by incorporation of ^{3}H-thymidine.

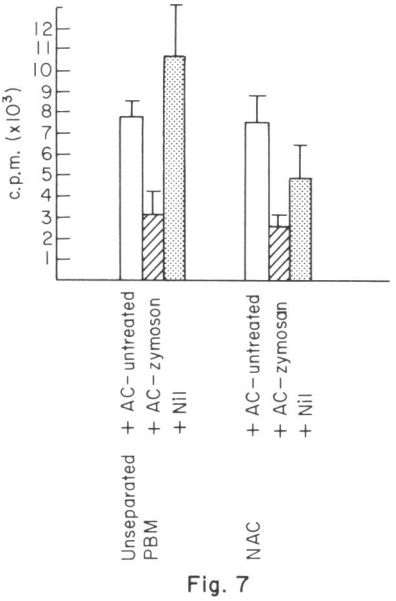

Fig. 7

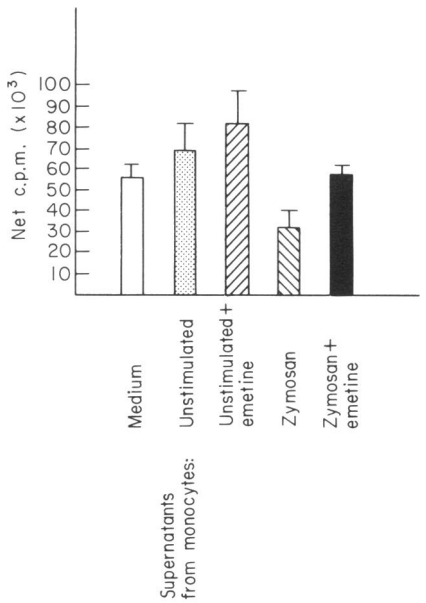

Fig. 8

Fig. 7. The effect of adherent cells pretreated with zymosan on the PHA response of unseparated PBM cells or NAC fraction. Unseparated PBM or NAC cells (2×10^5 per well) were added to monolayers of monocytes treated with zymosan (400 μg/ml) for 1 hr and washed. NAC or whole PBM cells cultured without addition of monocytes were used as control. The lymphocyte response to PHA was assessed in a 3-day culture. The columns show the mean $\pm$ s.d. of two experiments. The response of PBM or NAC cultured with zymosan-pretreated AC is significantly different from the response of the same cells cultured with untreated AC ($0{\cdot}01 < P < 0{\cdot}001$).

Fig. 8. Effects of monocyte supernatants on the response of lymphocytes to PHA. Supernatants were prepared from either unstimulated or zymosan-stimulated monocytes and emetine was used to block protein synthesis. These supernatants were added to normal PBM cells and the response to PHA assessed 3 days later. The mean $\pm$ s.d. of four experiments is shown. The response of cells cultured in the presence of supernatant from zymosan-stimulated monocytes is significantly different from the response of cells cultured in MEM or in the supernatant from zymosan-stimulated, emetine-treated monocytes ($0{\cdot}01 < P < 0{\cdot}001$).

soluble factors, supernatants from monocyte cultures exposed to zymosan were prepared. Control supernatants were obtained from non-stimulated monocytes and monocytes treated with zymosan and then subsequently with emetine. Fig. 8 demonstrates that supernatants from monocytes preincubated with zymosan and emetine did not cause the suppression. The supernatants from unstimulated monocytes were also not inhibitory.

It was concluded that the inhibitory effect of zymosan-stimulated monocytes on the lymphocyte response to PHA was due to the release of a soluble inhibitory factor(s) into the medium.

DISCUSSION

This paper confirms and extends the observations of Weston, Dustin & Hecht (1975) and Hedley & Currie (1978) that human peripheral blood PBM cells are able to reduce NBT. The present observations show that the stimulation with zymosan leads to an increased NBT reduction. The NBT-reducing cell in the PBM population is an adherent, phagocytic and esterase-positive cell—presumably a monocyte. Since there was a linear relationship between the number of esterase-positive cells and the amount of reduced NBT, the NBT-reducing activity can be expressed per unit number of monocytes. This makes it possible to compare the NBT-reducing capacity of monocytes from different individuals. The amount of NBT reduced by unstimulated monocytes was found to

be lower than that observed by Hedley & Currie (1978), while the values for stimulated cells have been similar, although a different stimulus was used in the present studies. The reasons for these discrepancies are not clear but may be related to different techniques employed. In any case, the NBT-reducing activity of normal monocytes is a consistent finding.

It is of interest that the spontaneous NBT reduction is significantly increased in some patients with carcinoma (unpublished observations). Recently, Hedley & Currie (1978) have found that latex-stimulated monocyte NBT reduction activity was increased in micrometastatic but not in disseminated melanoma.

In the present studies the activation of monocytes with zymosan was used to investigate the role of altered monocyte function in the response of lymphocytes to PHA. Zymosan-stimulated monocytes suppressed the lymphocyte response to mitogen. The demonstration that the supernatants of zymosan-pretreated monocytes were inhibitory for lymphocytes suggested that the suppressive effect of 'activated' monocytes was due to a soluble mediator(s). It is likely that the factor is produced during culture as emetine, an irreversible inhibitor of protein synthesis, prevented its appearance in the supernatant. It is known that normal monocytes produce both inhibitory and enhancing factors affecting lymphocytes (Avala-Chaves *et al.*, 1978). In the present system zymosan provided a non-specific signal which caused monocytes to liberate an inhibitory factor(s). There are other situations in which macrophages liberate non-specific factors. In particular macrophages 'armed' with specific T cell-suppressor factor (which blocks the contact sensitivity reaction) liberate a non-specific factor when exposed to antigen (Zembala *et al.*, 1978). It would be interesting to characterize this inhibitory monokine as activated macrophages inhibit lymphocyte response by several different factors (Allison, 1978). It is unlikely that the inhibitory factor is thymidine since the supernatants from 'activated' monocytes also cause inhibition of lymphocyte response to PHA as assessed by ^{3}H-leucine incorporation. Prostaglandins are also a possibility (Passwell, Dayer & Meler, 1978) and further studies are in progress.

Activation of monocytes by zymosan leads to increased NBT reduction, increased expression of Fc receptor and the ability to depress the lymphocyte response to mitogen. Similar changes occur in some patients with carcinoma. We have confirmed the observations of Rhodes *et al.* (1979) that monocyte Fc receptor expression is increased and have found that NBT reduction by the monocytes of patients is also raised (unpublished observations). Moreover the poor response of patients' lymphocytes to mitogens is due at least in part to the inhibitory activity of their monocytes (Zembala *et al.*, 1977). These considerations suggest that investigation of the activation of monocytes by zymosan may throw light on the altered state of monocytes in patients with carcinoma.

This investigation received financial support from the Polish Cancer programme (grant no. PR-6), the Polish Academy of Sciences (grant no. 10.5) and the Deutsche Forschungsgemeinschaft (SFB 107). M.Z. wishes to thank Alexander von Humboldt-Stiftung for a fellowship. We wish to acknowledge with thanks the excellent technical assistance of Ms Irena Ruggiero and Susanne Wegener.

REFERENCES

ALFÖLDI, O. & LEMMEL, E.M. (1979) Reduction of nitroblue tetrazolium for functional evaluation of activated macrophages in the cell-mediated immune reactions. *Clin. Immunol. Immunopathol.* **12,** 263.

ALLISON, A.C. (1978) Mechanism by which activated macrophages inhibit lymphocyte response. *Immunol. Rev.* **40,** 1.

AVALA-CHAVES, M.P., KEY, M., FETT, J.W., PORTO, M.T. & FUDENBERG, H.H. (1978) The complex role of target cells in the effects of soluble substances released by monocyte–macrophage cell populations. *Scand. J. Immunol.* **8,** 81.

BERGHOLTZ, B.O. & THORSBY, E. (1977) Macrophage-dependent response of human T lymphocytes to PPD *in vitro*. Influence of HLA-D histocompatibility. *Scand. J. Immunol.* **6,** 779.

BERLINGER, N.T., LOPEZ, C. & GOOD, R.A. (1976) Facilitation or attenuation of mixed leucocyte culture responsiveness by adherent cells. *Nature,* **260,** 145.

BÖYUM, A. (1968) Isolation of mononuclear cells and granulocytes from human blood. *Scand. J. clin. Lab. Invest.* **21,** 77.

COHN, Z.A. (1978) The activation of mononuclear phagocytes: fact, fancy and future. *J. Immunol.* **121,** 813.

DE VRIES, J.E., CAVILES, A.P., BONT, W.S. & MENDEL-

SOHN, J. (1979) The role of monocytes in human lymphocyte activation by mitogens. *J. Immunol.* **122,** 1099.

HAUSMAN, M.S., BROSMAN, S., SNYDERMAN, R., MICKEY, M.R. & FAHEY, J. (1975) Defective monocyte function in patients with genitourinary carcinoma. *J. Natl. Cancer Inst.* **55,** 1047.

HEDLEY, D.W. & CURRIE, G.A. (1978) Monocytes and macrophages in malignant melanoma. III. Reduction of nitroblue tetrazolium by peripheral blood monocytes. *Br. J. Cancer*, **37,** 747.

LEVIS, W.R. & ROBBINS, J.H. (1970) The function of glass-adherent cells in the human mixed lymphocyte culture. *Transplantation*, **9,** 515.

NATHAN, C.F., KARNOVSKY, ML. & DAVIS, J.R. (1971) Alterations of macrophage function by mediators from lymphocytes. *J. exp. Med.* **133,** 1356.

PASSWELL, J.H., DYER, J.M. & MERLER, E. (1979) Increased prostaglandin production by human monocytes after membrane receptor activation. *J. Immunol.* **123,** 115.

PTAK, W., ZEMBALA, M. & GERSHON, R.K. (1978) Intermediary role of macrophages in the passage of suppressor signals between T cell subsets. *J. exp. Med.* **148,** 424.

QUAN, P.C. & BURTIN, P. (1978) Demonstration of non-specific suppressor cells in peripheral; blood lymphocytes of cancer patients. *Cancer Res.* **28,** 288.

RINEHART, J.J., ORSER, M. & KAPLAN, M.E. (1979) Human monocyte and macrophage modulation of lymphocyte proliferation. *Cell. Immunol.* **44,** 131.

RHODES, J., BISHOP, M. & BENFIELD, J. (1979) Tumor surveillance: how tumors may resist macrophage-mediated host defence. *Science*, **1,** 179.

SCHMIDTKE, J.R. & HATFIELD, S. (1976) Activation of purified human thymus-derived (T) cells by mitogens. II. Monocyte-macrophage potentiation of mitogen-induced DNA synthesis. *J. Immunol.* **116,** 357.

UNANUE, E.R. (1978) Macrophage functions. (Introduction.) *Fed. Proc.* **37,** 77.

URACZ, W., PITUCH, A., MYTAR, B., NOWOROLSKI, J. & ZEMBALA, M. (1978) Monocyte changes in cancer patients and their role in mitogen induced lymphocyte responses. *Arch. Immunol. Ther. exp.* **26,** 429.

WESTON, W.L., DUSTIN, R.D. & HECHT, S.K. (1975) Quantitative assays of human monocyte–macrophage function. *J. Immunol. Methods*, **8,** 213.

YAM, L.T., LI, C.Y. & CROSBY, W.H. (1971) Cytochemical identification of monocytes and granulocytes. *Am. J. clin. Pathol.* **55,** 283.

ZEMBALA, M., MYTAR, B., POPIELA, T. & ASHERSON, G.L. (1977) Depressed *in vitro* peripheral blood lymphocyte response to mitogens in cancer patients: the role of suppressor cells. *Int. J. Cancer*, **19,** 605.

ZEMBALA, M., ASHERSON, G.L., PTAK, W. & KOWALCZYK, D. (1978) Suppressor cell control of contact sensitivity. In *The Pharmacology of Immunoregulation* (ed. by G. H. Werner and F. Floch), p. 113. Academic Press, New York and London.