*Clin. exp. Immunol.* (1980) **41**, 326–335.

# Effect of autologous and homologous serum and circulating immune complexes on monocyte functions of patients with solid tumours

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*(Accepted for publication 20 February 1980)*

## SUMMARY

Some functions of monocytes (phagocytosis, bactericidal capacity, handling of endocytosed $^{51}$Cr-SRBC and chemotaxis) were studied in fifty patients with solid tumours and in fifty controls. In the presence of autologous serum, the catabolism of endocytosed $^{51}$Cr-SRBC and the phagocytic capacity were similar in tumour and control monocytes, while the bactericidal capacity of tumour monocytes was increased. In the presence of pooled AB sera the catabolism and the bactericidal capacity were decreased in tumour monocytes as compared with autologous serum. Tumour sera did not enhance the functions of normal monocytes. Inactivation of pooled tumour or AB sera resulted in a decrease of bactericidal capacity in tumour and control monocytes. Using the C1q-binding test, we detected circulating immune complexes in 36% of sera. The presence or absence and the quantity of such complexes did not correlate with the different functions studied in either tumour or normal monocytes. Finally, the chemotactic activity of monocytes was studied using the migration under agarose technique. No difference was found between tumour and control monocytes. On the other hand, the presence of a chemotactic inhibitor was not revealed in tumour sera. These observations suggest that monocytes from tumour patients require factor(s) present in autologous serum as well as autologous cellular component(s) to achieve normal functions.

## INTRODUCTION

It is well recognized that tumoral processes can develop as a consequence of a deficiency in the immune system. In this system, macrophages play a key role, not only in the induction of immune response, but also in the effects of the immune response on the tumoral cell growth. Tumours that produce a strong lymphoreticular reaction are associated with a more favourable clinical prognosis than those which are not accompanied by such reactions (Ioachim, Dorsett & Paluck, 1976).

In some tumoral diseases that directly involve the reticuloendothelial system, i.e. myelo-monocytic leukaemia, lymphomas, etc., a deficiency in the macrophage functions has been described (Cline, 1973). However, relatively little is known about the functional capacity of macrophages in patients with solid tumours.

A methodology has been developed recently (Roth, Celada & Cruchaud, 1979) in order to allow the *in vitro* study of the functional activity of monocytes, these being the circulating precursors of

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0099-9104/80/0800-0326$02.00 

tissue macrophages. In the present work, we have compared some functions of monocytes obtained from patients suffering from solid tumours with those of normal individuals. These functions were studied in various situations combining populations of monocytes with different sera. We also analysed the effect of the presence of circulating immune complexes on the functional capacity of these cells.

## MATERIALS AND METHODS

*Patient population.* This consisted of fifty patients, thirty-two males and eighteen females (age range 45 to 87 years; mean $67{\cdot}6 \pm 11{\cdot}3$ years) with solid tumours such as carcinomas of the respiratory tract (seventeen cases), as well as genito-urinary (sixteen cases), gastrointestinal (eight cases) and breast carcinomas (seven cases) and melanoma (two cases). The diagnosis was based in all cases on histological examination. All patients had an active disease and did not receive any treatment for their tumoral disorder at the time the study was undertaken. Patients also received no drugs such as prednisone, anti-inflammatory or antibiotics which could alter monocyte functions (Rinehart *et al.*, 1975; Norris, Weston & Sams, 1977). None of the patients had active infection at the time of investigation. In every case, the presence of bone, hepatic, cutaneous or other metastasis was documented by bone and/or hepatic scanning, biopsy or autopsy.

Fifty healthy subjects of either sex (age range 24 to 65 years; mean $43{\cdot}2 \pm 15{\cdot}6$ years) consisting of laboratory technicians, hospital workers and blood donors were used as controls. Informed consent was obtained from all participants.

*Separation of monocytes.* Several 25-ml samples of heparinized blood were layered on 12·5 ml sterile Ficoll–Hypaque (Pharmacia Fine Chemicals, Uppsala, Sweden), according to Böyum's technique (Böyum, 1968). Total and differential leucocyte counts were performed on Giemsa and peroxidase-stained (Kaplow, 1965), cytocentrifuged smears (Cytospin, Shandon Elliot Scientific Company, London). After three washes, cells were resuspended in Eagle's minimum essential medium (MEM) (Flow Laboratories, Irvine, Scotland) supplemented with 10% inactivated foetal calf serum (FCS). This will hereafter be referred to as 'medium'. Cell suspensions consisted essentially of mixtures of monocytes and lymphocytes. Peroxidase-positive cells which do not belong to the mononuclear phagocyte population (i.e. promyelocytes, myelocytes, metamyelocytes and segmented neutrophils) were excluded on Giemsa-stained smears. Using this classification, the number of monocytes in normal subjects and patients was similar to that obtained with the non-specific esterase stain. Patients with more than 5% contamination by granulocytes or their precursors were excluded from the study. Using this method we could not find a sizeable contamination by granulocyte precursors as described by Currie *et al.* (1978). The concentration of monocytes was adjusted to $10^7$/ml; viability as assessed by the trypan blue dye exclusion test was always more than 95%.

*Bacteria. S. aureus* Wood 46 was used in all experiments because the lack of protein A in the cell wall allows rapid phagocytosis (Peterson *et al.*, 1976). It was maintained by incubation in glucosated broth and used after overnight growth on blood agar at 37°C.

*Bactericidal capacity.* This was determined as described in detail elsewhere (Roth *et al.*, 1979). In brief, we prepared in each case a *test* tube containing monocytes, bacteria (ten bacteria/monocyte) and serum, and a *control* tube containing only bacteria and serum. From each tube a sample was obtained at time 0 and after incubation at 37°C for 1 hr. Cells were lysed in distilled water and bacteria were enumerated by pour-plate counting of colonies. Relative bactericidal activity of monocytes was calculated, taking into account the growth of bacteria during the 60-min incubation and using the formula: $R = A/E \times 100$, where A is the absolute bactericidal activity and E the theoretical number of bacteria expected at time 60 min in the test-tube. All tests were done in duplicate.

*Phagocytic activity.* This was expressed as the percentage of monocytes having phagocytized one or more bacteria, by counting 500 cells on the cytocentrifuged peroxidase- and Giemsa-stained smears of the final suspension of the bactericidal assay (after 60 min of incubation).

*Catabolism of endocytosed sheep erythrocytes.* This was measured according to the method

previously described (Roth *et al.*, 1979). Briefly, sheep red blood cells (SRBC) that had been labelled with $^{51}Cr$ and opsonized were incubated with a suspension of monocytes for 1 hr. Non-phagocytized SRBC were lysed by hypotonic shock. Monocytes were then incubated for 18 hr at 37°C. The radioactivity associated with cells or present in culture medium was measured. The extracellular radioactivity was considered as *excreted* material and the part of it that was not precipitated by trichloroacetic acid was designated *degraded* material (free or non-protein-bound material). All tests were done in duplicate.

*Chemotaxis.* This assay was performed under agarose according to a procedure developed by Nelson, Quie & Simmons (1975), with slight modifications. In each plate of agarose, four series of three wells 3 mm in diameter and spaced 3 mm were cut. Monocytes were adjusted to a concentration of $25 \times 10^6$/ml in medium. The centre well of each three-well series received 10 $\mu$l of chemotactic factor (zymosan-activated serum). The inner well received a 10-$\mu$l volume of non-chemotactic control medium. In some experiments, the monocyte suspension or the chemotactic factor was supplemented with 10% fresh or inactivated either autologous or homologous serum, pooled AB sera or serum from one patient with solid tumour. The dishes were incubated for 18 hr at 37°C. The cells were then fixed in methanol and buffered formalin and stained with Giemsa. Quantitation of migration was obtained by measuring (in millimetres) the linear distance the cells had moved from the margin of the well towards the chemotactic factor and the linear distance the cells had moved from the margin of the well towards the control medium. Chemotactic differential and chemotactic index are the difference and the coefficient between these two values respectively.

*Serological studies.* The serum of each individual included in the study was divided into several aliquots and stored at −70°C. For every patient and control studied, analyses for the presence of circulating immune complexes in serum were made using the C1q-binding test (Zubler *et al.*, 1976); the total haemolytic complement was determined with a continuous-flow system (Nydegger *et al.*, 1972) and the C3 level was assayed by radial immunodiffusion.

*Statistical analysis.* For all calculations non-parametric tests were used: the Wilcoxon for paired differences, Mann–Whitney ranking for unpaired values and the Spearman rank correlation (Snedecor & Cochran, 1967).

## RESULTS

*Yield of monocytes from peripheral blood*

After Ficoll–Hypaque centrifugation, the number of mononuclear cells positive for peroxidase was $50{\cdot}68 \pm 11{\cdot}98\%$ (mean ± 1 s.d.) after excluding the granulocytes and their precursors in the fifty cancer patients and $38{\cdot}69 \pm 7{\cdot}83\%$ in the fifty controls; the difference between these two groups is significant ($P < 0{\cdot}0001$).

*Circulating immune complexes and complement levels*

The mean $^{125}I$-C1q-binding activity in serum was $5{\cdot}32 \pm 5{\cdot}66\%$ in the cancer patients and $2{\cdot}55 \pm 1{\cdot}46\%$ in the controls ($P < 0{\cdot}0001$). In eighteen of fifty cancer sera the value of C1q-binding activity was above the 2 standard deviation limit of the control group. The CH50 in the group of cancer patients was $137{\cdot}10 \pm 45{\cdot}52\%$ and in the controls $102{\cdot}42 \pm 16{\cdot}09\%$ ($P < 0{\cdot}0001$). The level of C3 in the cancer group was $110{\cdot}50 \pm 23{\cdot}45\%$ and in the controls $98{\cdot}10 \pm 16{\cdot}58\%$ ($P < 0{\cdot}01$). The values of CH50 and C3 correlated slightly ($r = 0{\cdot}36$; $P < 0{\cdot}05$). Nevertheless the values of the C1q-binding test did not correlate with CH50 or C3 levels.

*Catabolism of endocytosed $^{51}Cr$-SRBC*

Figs 1 and 2 show the results of the handling of endocytosed SRBC by the monocytes from forty patients with solid tumours (which will be referred to as 'tumour monocytes') and from forty normal subjects (normal monocytes), both in the presence of autologous and pooled AB sera. The amount of material released by monocytes of cancer patients in the presence of pooled sera was lower than that observed with normal monocytes ($P < 0{\cdot}01$). In the presence of autologous serum, monocytes from cancer patients and normal subjects released a similar amount of catabolized

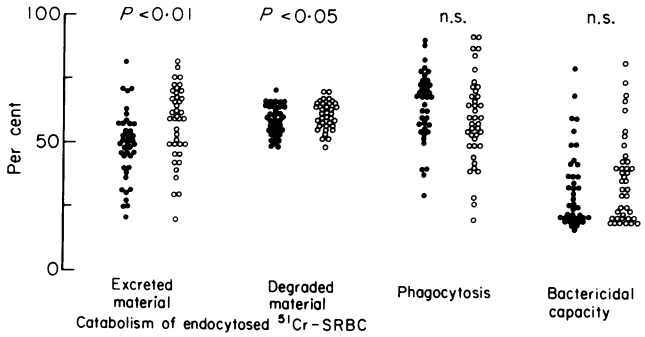


**Fig. 1.** Functions of monocytes from forty patients with solid tumours and from forty controls in the presence of pooled AB sera. (•) Individual values of tumour-bearing patients and (o) individual values of normal subjects. Excreted material is the percentage of radiactive material from the endocytosed $^{51}$Cr-SRBC that was released by monocytes into the medium after 18 hr of incubation. Degraded material is the percentage of excreted material that is not precipitable by TCA. Phagocytosis is expressed as the percentage of monocytes containing one or more bacterias in their cytoplasm after 1 hr of incubation. The bactericidal capacity represents the percentage of bacterias killed after 1 hr of incubation with monocytes. For statistical calculations the Mann–Whitney ranking for unpaired values was used.

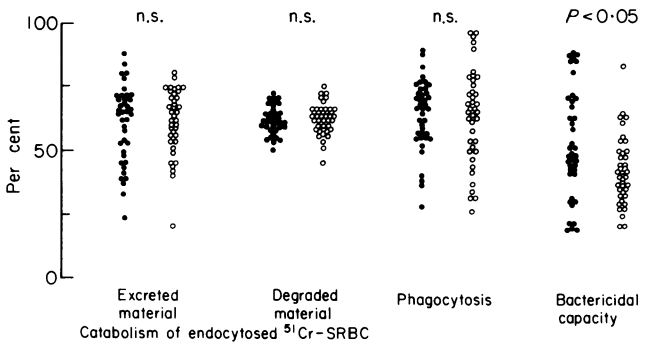


**Fig. 2.** Functions of monocytes from forty patients with solid tumours and from forty controls in the presence of autologous serum. For details see Fig. 1.

material ($P>0{\cdot}05$). The material excreted by tumour monocytes in the presence of autologous serum was significantly increased ($P<0{\cdot}001$), as compared with the pooled AB sera (Fig. 3). These values are correlated ($r=0{\cdot}34$; $P<0{\cdot}05$). The normal monocytes did not show any difference in the presence of either autologous or pooled sera.

In order to investigate the effect of cancer serum on the functions of normal monocytes, we compared the monocytes from forty normal individuals in the presence either of autologous serum or of one individual tumour serum. There was no difference between the release of catabolized material by these normal monocytes in the presence of autologous ($61{\cdot}99 \pm 12{\cdot}42\%$) or tumoral serum ($63{\cdot}72 \pm 12{\cdot}32\%$; $P>0{\cdot}05$). A good correlation was found between these two values ($r=0{\cdot}50$; $P<0{\cdot}01$).

The quantity of material degraded by tumour monocytes in the presence of pooled sera was slightly decreased as compared with normal monocytes ($P<0{\cdot}05$) (Fig. 2). In the presence of autologous serum the proportion of material degraded by tumour and normal monocytes was not significantly different ($P>0{\cdot}05$) (Fig. 1). The material degraded by tumour monocytes was greater when incubated in autologous serum than in pooled sera ($P<0{\cdot}02$) (Fig. 3). These values are well correlated ($r=0{\cdot}75$; $P<0{\cdot}001$). For normal monocytes, there was no difference in the amount of degraded material whether they were in autologous or in pooled sera ($P>0{\cdot}05$), and there was a

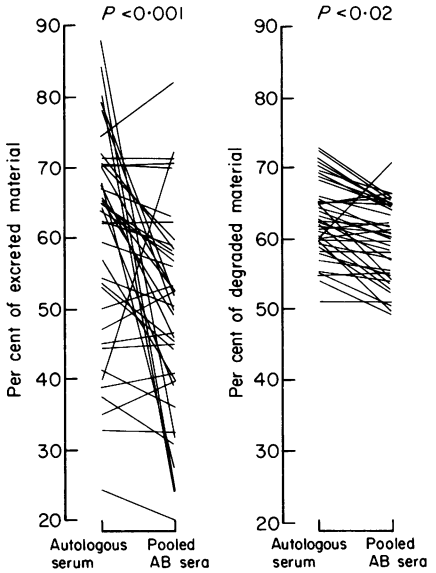


Fig. 3

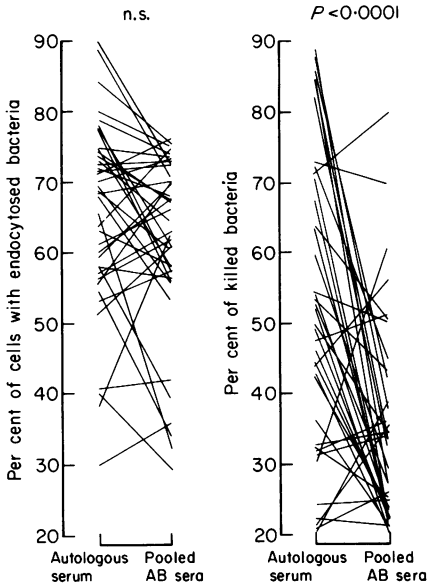


Fig. 4

**Fig. 3** Catabolism (left, excreted material; right, degraded material) of endocytosed $^{51}$Cr-SRBC by the monocytes of forty patients with solid tumours in the presence of autologous or pooled AB sera. For statistical calculations the Wilcoxon test for paired differences was used.

**Fig. 4.** Phagocytic (left) and bactericidal (right) capacity of monocytes from forty patients with solid tumours in the presence of autologous or pooled AB sera. For statistical calculations the Wilcoxon test for paired differences was used.

good correlation ($r=0{\cdot}40$; $P<0{\cdot}01$). In the presence of autologous serum, the material degraded by normal monocytes ($63{\cdot}58\pm5{\cdot}99\%$) was not significantly different ($P>0{\cdot}05$) from that degraded in the presence of one individual tumour serum ($62{\cdot}51\pm5{\cdot}03\%$). These values are correlated ($r=0{\cdot}59$; $P<0{\cdot}001$).

*Phagocytic activity*

The phagocytic capacity of monocytes from cancer patients and that of normal monocytes was similar in the presence of either autologous or pooled sera (Figs 1 and 2). The phagocytic capacity of tumour monocytes was not different in the presence of autologous or pooled sera ($P>0{\cdot}05$); the two values are correlated ($r=0{\cdot}51$; $P<0{\cdot}01$) (Fig. 4). The normal monocytes did not show different phagocytic activity in the presence of autologous or pooled sera; these values are correlated ($r=0{\cdot}64$; $P<0{\cdot}01$). The phagocytic activity of normal monocytes in the presence of autologous serum was $65{\cdot}71\pm19{\cdot}26\%$; in the presence of individual tumour serum it was $63{\cdot}77\pm14{\cdot}70\%$; these values are not different ($P>0{\cdot}05$) and they are correlated ($r=0{\cdot}68$; $P<0{\cdot}001$).

*Bactericidal capacity*

The bactericidal capacity was similar when tumour and normal monocytes were in the presence of pooled sera (Fig. 2). However, in the presence of autologous serum the bactericidal capacity of tumour monocytes was significantly increased as compared with normal monocytes ($P<0{\cdot}05$) (Fig. 1). The bactericidal activity of tumour monocytes was also more important in the presence of autologous serum than with pooled sera ($P<0{\cdot}0001$) (Fig. 4): there is no correlation between these two values ($r=0{\cdot}18$; $P>0{\cdot}05$). The bactericidal capacity of normal monocytes in the presence of autologous serum was increased as compared to pooled sera ($P<0{\cdot}02$); there is no correlation between these two values ($r=0{\cdot}03$; $P>0{\cdot}05$). There was no difference between the bactericidal capacity of normal monocytes in the presence of autologous serum ($44{\cdot}51\pm14{\cdot}90\%$) or in the

**Table 1.** Effect of fresh or inactivated pooled sera from normal donors or tumour patients on monocyte functions of normal donors or patients with solid tumours

| Serum | Normal monocytes ($n=10$) | Tumour monocytes ($n=10$) |
|---|---|---|
| Catabolism: excreted material (% of endocytosed material) | | |
| Fresh pooled AB sera | 58·16 ± 9·86* | 60·63 ± 9·64 |
| Inactivated pooled AB sera | 57·26 ± 10·36 | 58·71 ± 9·19 |
| Fresh pooled tumour sera | 59·00 ± 10·89 | 62·30 ± 9·71 |
| Inactivated pooled tumour sera | 59·08 ± 10·39 | 59·33 ± 9·59 |
| Catabolism: degraded material (% of excreted material) | | |
| Fresh pooled AB sera | 67·15 ± 4·76 | 65·66 ± 5·28 |
| Inactivated pooled AB sera | 65·81 ± 4·13 | 64·26 ± 5·09 |
| Fresh pooled tumour sera | 68·54 ± 3·99 | 64·79 ± 3·14 |
| Inactivated pooled tumour sera | 67·13 ± 4·66 | 64·05 ± 4·09 |
| Phagocytic capacity (% of phagocytozing cells) | | |
| Fresh pooled AB sera | 56·70 ± 16·77 | 63·45 ± 17·88 |
| Inactivated pooled AB sera | 46·05 ± 16·79 | 56·55 ± 18·95 |
| Fresh pooled tumour sera | 54·40 ± 17·90 | 64·20 ± 19·41 |
| Inactivated pooled tumour sera | 41·65 ± 12·06 | 58·50 ± 15·30 |
| Bactericidal capacity (% of killed bacteria) | | |
| Fresh pooled AB sera | 41·72 ± 17·67† | 55·21 ± 19·39† |
| Inactivated pooled AB sera | 28·06 ± 13·98 | 37·07 ± 22·82 |
| Fresh pooled tumour sera | 48·17 ± 17·38† | 51·93 ± 24·17† |
| Inactivated pooled tumour sera | 28·59 ± 17·34 | 38·24 ± 18·62 |

* Mean ± 1 s.d.

† $P<0·05$, difference between fresh and inactivated sera.

presence of one of the forty tumour sera (46·45 ± 16·07%). A significant correlation was found between these two values ($r=0·32$; $P<0·05$).

Finally, no correlation was found between the different functions (catabolism, phagocytosis and bactericidal capacity) of normal or tumour monocytes and the serum levels of total complement, C3, C1q-binding capacity and the percentage of monocytes after Ficoll–Hypaque separation.

*Effect of different pools of sera and complement on monocyte functions*

In Table 1 are summarized the effects of different pools of sera, either fresh or inactivated, on monocyte functions. In the presence of pooled sera from ten patients with solid tumours or from ten normal donors, the catabolism and the phagocytic activity of both populations were similar. A decrease in bactericidal capacity of normal or tumour monocytes was observed only when the normal or tumour pooled sera were inactivated.

*Studies on chemotaxis*

In Table 2 the results of chemotaxis are shown. No difference was found between normal and tumour monocytes for the differential or index chemotaxis. In some experiments, the serum from one donor (the same as that of the cells used in this particular experiment), pooled sera or serum from one cancer patient (the same as that of the monocytes employed for chemotaxis experiments), all fresh or inactivated, was added in the well containing the cells or in the well containing the chemoattractant (C5a). No differences were shown between these various experiments or between normal or tumour monocytes in each experiment.

**Table 2.** Chemotaxis of monocytes

| Serum added | Differential chemotaxis (mm) | | Chemotaxis index (%) | |
|---|---|---|---|---|
| | Tumour monocytes ($n=10$) | Normal monocytes ($n=10$) | Tumour monocytes ($n=10$) | Normal monocytes ($n=10$) |
| None | 7·56 ± 2·83 | 7·98 ± 2·16 | 2·37 ± 0·63 | 2·59 ± 0·78 |
| Serum added to the cell well | | | | |
| Fresh normal serum* | 6·05 ± 3·98 | 7·31 ± 3·53 | 2·21 ± 1·25 | 2·22 ± 0·70 |
| Inactivated normal serum* | 7·30 ± 3·78 | 6·57 ± 3·21 | 2·17 ± 0·58 | 2·13 ± 0·49 |
| Fresh pooled AB sera | 7·33 ± 4·94 | 6·61 ± 3·16 | 2·27 ± 1·00 | 2·13 ± 0·43 |
| Inactivated pooled AB sera | 7·08 ± 5·29 | 7·38 ± 3·38 | 2·16 ± 0·50 | 2·34 ± 0·73 |
| Fresh tumour serum† | 8·21 ± 5·89 | 7·51 ± 4·06 | 2·42 ± 1·27 | 2·32 ± 0·98 |
| Inactivated tumour serum† | 7·41 ± 5·64 | 7·46 ± 3·35 | 2·09 ± 0·50 | 2·32 ± 0·51 |
| Serum added to the chemoattractant well | | | | |
| Fresh normal serum* | 7·21 ± 3·51 | 6·31 ± 3·43 | 2·28 ± 0·76 | 2·10 ± 0·68 |
| Inactivated normal serum* | 8·08 ± 4·44 | 7·01 ± 1·87 | 2·38 ± 0·82 | 2·22 ± 0·89 |
| Fresh pooled AB sera | 6·73 ± 3·13 | 7·22 ± 2·69 | 2·19 ± 0·50 | 2·26 ± 0·78 |
| Inactivated pooled AB sera | 7·37 ± 4·38 | 6·77 ± 2·53 | 2·28 ± 0·63 | 2·23 ± 0·63 |
| Fresh tumour serum† | 8·43 ± 3·65 | 6·49 ± 3·07 | 2·19 ± 0·50 | 1·89 ± 0·52 |
| Inactivated tumour serum† | 8·54 ± 4·83 | 7·86 ± 3·61 | 2·29 ± 0·81 | 2·33 ± 0·90 |

* In each experiment the serum was obtained from the donor of normal monocytes.
† In each experiment the serum was obtained from the donor of tumour monocytes.

## DISCUSSION

Using the Ficoll–Hypaque technique for isolating mononuclear cells, we found that the number of peroxidase-positive cells was increased in patients with solid tumours as compared with controls. It is unlikely that the increase of mononuclear phagocytes in the cancer patient group is related to the different mean ages between this and the control group (Munan & Kelly, 1979). An increased number of macrophages has been described in the tumour (Carr, 1977) or the blood (Barrett, 1970) of patients suffering from cancer. It must be stressed that in our investigations the number of monocytes was, in each case, adjusted to the same concentration.

From published data it appears that the functions of monocytes obtained from patients with solid tumours are mostly normal or even enhanced. For instance, monocytes from tumour patients were reported to show normal staphylocidal (King, Bain & Lo Buglio, 1975), phagocytic (Kuntz, Kuntz & Albert, 1978) and metabolic activity (Kitahara, Eyre & Hill, 1979), as well as increased lytic activity of antibody-coated erythrocytes (Nyholm & Currie, 1978), enhanced reduction of nitroblue tetrazolium (Hedley & Currie, 1978) and augmented density of membrane Fc receptors (Rhodes, 1977). Chemotactic activity was the only function that was found regularly decreased and the presence of a chemotactic inhibitor in the serum of these patients has been reported (Boetcher & Leonard, 1974; Hausman *et al.*, 1975; Hausman & Brosman, 1976; Rubin, Cosimi & Goetzl, 1976; Kay & McVie, 1977; Snyderman, Seigler & Meadows, 1977; Kjeldsberg & Pay, 1978; Snyderman *et al.*, 1978).

Our experiments were designed to analyse the functions of normal and tumour monocytes in the presence of autologous and pooled AB sera. When populations of monocytes from tumour-bearing patients were compared with normal monocytes, it appeared that, in the presence of autologous serum, tumour monocytes had greater bactericidal activity than normal monocytes, whereas in the presence of AB serum they catabolized endocytosed erythrocytes less efficiently than normal cells. On average, these differences were modest and phagocytosis was in no case modified. When monocyte functions were analysed within the same population exposed to either autologous or AB serum, functions of normal cells were similar with both types of sera, while tumour monocytes had better catabolytic and bactericidal activity in the presence of autologous serum. This suggests the presence of an enhancing factor in the serum of tumour patients. However, functions of normal monocytes were not modified by tumour sera; in addition, incubation of normal and tumour monocytes with pooled AB or tumour sera, either fresh or inactivated, did not result in significant changes in phagocytosis and catabolism in either population. Only bactericidal capacity was decreased in both normal and tumour monocytes when either tumour or AB sera were inactivated.

These observations suggest that monocytes from tumour patients require factor(s) present in autologous serum as well as autologous cellular component(s) to achieve normal functions. The decrease in bactericidal activity noticed when sera were inactivated is in keeping with the observations of Leijh *et al.* (1979) who found that extracellular complement was required for intracellular killing of micro-organisms by human monocytes.

Circulating tumour antigen–antibody complexes may play a role in modulating cell-mediated immune responses against autologous tumours through the blockade of lymphocyte transformation and the generation of tumour-specific cytotoxic lymphocytes (Hellström *et al.*, 1977). The presence of circulating immune complexes has been reported in patients with different types of solid tumours in the same proportion as in our study (Heier *et al.*, 1977; Rossen *et al.*, 1977; Shepherd, 1979). Finally, it is known that immune complexes may alter monocyte functions (Rabinovitch, Manejias & Nussenzweig, 1975). In our work no correlation could be established between the different parameters studied and the presence or the amount of circulating immune complexes. This suggests that these complexes have little influence if any on the monocyte functions that were analysed. If a factor present in the serum of cancer patients stimulates functions of autologous tumour monocytes but not of homologous or normal monocytes, this factor does not appear to be related to circulating immune complexes or to complement levels.

In chemotaxis studies no difference could be demonstrated between normal and tumour monocytes whether they were incubated in medium with 10% autologous serum, pooled AB sera or homologous serum. These results show that in our experimental conditions chemotactic properties

of tumour monocytes are not altered. In addition, a chemotactic inhibitor was not demonstrable in the serum of our patients. The difference with previously reported results may be related to the different methodology employed for chemotaxis assay. In other reports, the method used was the Boyden chamber, or a modification of this technique, using a micropore filter with a diameter from 5 to 8 $\mu$m. As the monocyte population is increased in tumour patients, it is possible that this population is composed of young and old cells that are more heterogeneous than normal monocytes and may therefore have a different migration pattern through the micropore filter. For these reasons we chose the method of chemotaxis under agarose, in which the monocytes can be identified after migration (Nelson *et al.*, 1975) and in which the difference of cell maturity should not play an important role.

In conclusion, we showed that in patients with solid tumours, phagocytosis, catabolism of endocytosed SRBC and chemotaxis of monocytes in the presence of autologous serum are not different from the controls while bactericidal capacity is enhanced. Tumour serum increases the catabolism and the bactericidal capacity of autologous monocytes alone as compared with homologous serum. This helper effect does not appear to be related to the presence of circulating immune complexes or to complement levels.

We wish to thank Mrs Tecla Dudez and Carolyn Zavadil for valuable technical assistance, and Mrs Marie-Claire Seydoux for secretarial help.

This work was supported by Grant 3.470.1.75 from the Swiss National Science Foundation.

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