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# DISTINCT TUMOR CELL MEMBRANE CONSTITUENTS ACTIVATE HUMAN MONOCYTES FOR TUMOR NECROSIS FACTOR SYNTHESIS

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**Several lines of evidence point to an activation mechanism of monocytes/macrophages by tumor cells. In this study we present data for distinct surface structures on K562 and Jurkat cells to directly induce TNF-mRNA expression and TNF production by human peripheral blood monocytes. Northern analysis showed that incubation of monocytes with either K562 or Jurkat cells led to a significant increase in TNF-mRNA expression. In addition, enhanced TNF production was detected in supernatants of monocyte cultures activated by Jurkat cells. Not only viable tumor cells but also metabolically inactivated tumor cells, cytoblasts, and membrane preparations from Jurkat and K562 cells induced TNF-mRNA expression. We identified two different membrane protein fractions with relative molecular mass of 32 to 38 kDa for Jurkat cells and 46 to 54 kDa for K562 cells that were responsible for monocyte activation.**

Considerable evidence suggests that monocytes and macrophages play an important role in host defense against tumor growth (1–3). Monocytes and macrophages release inflammatory mediators like TNF when activated by bacterial, parasitic, or viral agents. Production and release of TNF is one of the cytolytic effector mechanisms by which the activated monocytes and macrophages destroy certain neoplastic cells in vitro (4, 5). However, it is unclear whether monocytes directly respond to tumor cells and if so how they distinguish between normal and neoplastic cells. Recent studies demonstrated elevated TNF levels in serum of cancer patients (6, 7). After resection of the tumors the TNF levels returned to normal (7). In addition, tumor cytostatic activity of monocytes derived from cancer patients was enhanced compared to healthy controls (8, 9). Since some tumor cell lines were found to produce TNF (10) these reports could not distinguish whether enhanced TNF serum levels were due to TNF released by activated monocytes/macrophages or by tumor cells. In this study we present data that distinct surface structures of tumor cells can directly activate human monocytes for TNF production. We used two human tumor cell lines K562, a proerythromyeloid cell line and Jurkat, a T cell line to show increased TNF-mRNA and TNF protein production in human monocytes after exposure to these cells. Two different membrane protein fractions were responsible for induction of TNF production by human peripheral blood monocytes.

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## MATERIALS AND METHODS

*Culture methods.* All cell lines were cultured in RPMI 1640 (GIBCO, Glasgow, Scotland) supplemented with 10% heat-inactivated FCS (GIBCO) and 50 μg/ml gentamycin (GIBCO), under 5% $CO_2$ in air at 37°C. K562, a proerythromyeloide cell line and Jurkat, a T cell line were regularly tested for *Mycoplasma* contamination and also the absence of viral structures was established (11).

*Mycoplasma test. Mycoplasma* growth in culture medium of tumor cells was tested by spreading it on PPLO[1] agar (Difco, Detroit, MI) supplemented with 20% horse serum and 1% yeast autolysate for 10 days at 37°C and 5% $CO_2$. Alternatively, the culture medium was first incubated in PPLO broth for 7 to 10 days and then spreaded on PPLO agar. *Mycoplasma* growth in these cultures was examined microscopically.

In parallel, cells were spun down in a cytocentrifuge (Shandon, England) at 600 rpm for 5 min on glass slides, fixed for 15 min in methanol/acetic acid 3:1 and stained for 30 min with the fluorescent dye (Hoechst 33258, Riedel de Haen, Hannover, FRG) at a concentration of 0.05 to 0.5 μg/ml. The preparations were inspected by fluorescence microscopy.

*Isolation of monocytes.* Human peripheral blood monocytes were isolated from blood of healthy donors by density gradient centrifugation (12). The interphase was washed twice in RPMI 1640 and the mononuclear cells were allowed to adhere for 1 h either in 24-well Costar plates ($4 \times 10^6$/well) for dot blot analyses or in 100-$mm^2$ petri dishes (6 to $8 \times 10^7$/dish) when Northern blotting analyses were performed. Contaminating lymphocytes were washed off and the remaining cells (>95% monocytes as determined by phagocytosis and differential staining) were cultured in RPMI 1640/10% FCS/gentamycin for the indicated times in the absence or presence of various stimuli.

*L929-Bioassay and TNF-ELISA.* The supernatants from activated monocyte cultures were collected at the indicated times and stored at −20°C until they were assayed for TNF activity in the L929 bioassay (13) (in the presence of actinomycin D) and in the TNF-specific ELISA system (14). In both assay systems the absorption curves obtained with the test samples were compared with a standard curve obtained with human rTNF (Knoll/BASF AG, Ludwigshafen, FRG) and the TNF content expressed as nanograms per milliliter.

*Preparation of cytoblasts.* Enucleated cells (cytoblasts) were prepared as previously described (15). Briefly, cells ($2 \times 10^7$/ml) were suspended in 12.5% Ficoll 400 (Pharmacia, Freiburg, FRG) in gradient medium consisting of PBS, 10 μg/ml cytochalasin B (Sigma, München, FRG), 0.5% DMSO, and 10 mM HEPES (pH 7.2). Aliquots of 3 ml of the cell suspension were layered onto a discontinuous Ficoll gradient, centrifuged at 25,000 rpm for 60 min at 31°C, and the cytoblasts were collected from the interphase and washed twice with RPMI 1640 containing 10% FCS.

*Membrane preparation and electroelution.* For membrane preparations (16), cells (2 to $4 \times 10^8$) were homogenized with a Polytron homogenizer in 10 mM sodium phosphate buffer (pH 7.4) containing 1 mM MgCl, 30 mM NaCl, 1 mM dithiothreitol, 0.005 mM PMSF, and 0.1 μg/ml DNase (without $NaN_3$). The homogenate was layered onto a 41% solution of sucrose (in PBS) and centrifuged at 95,000 × *g*. After 1 h the white interfacial band of membranes was collected and washed twice with homogenization buffer.

These membrane preparations were either used directly for activating monocytes or analyzed in a 12.5% SDS-PAGE. After electrophoresis the gel was cut in 13 to 15 small slices, transferred into

[1] Abbreviations used in this paper: PPLO, pleuro pneumonia-like organisms; RT, room temperature.

dialysis tubes containing 1 ml elution buffer (50 mM Tris, 0.38 M glycine, and 0.1% deoxycholate), and the proteins were electroeluted for 1 h at 100 V. The samples were dialyzed against PBS and the appropriate molecular weight for each fraction was determined by 12.5% SDS-PAGE.

*RNA-Dot blot analysis.* RNA-dot blots were performed by the guanidine-HCl method (17). The RNA was blotted on nylon filters (Genofit, Heidelberg, FRG) and hybridized as described (18).

*Northern blot analysis.* Cytoplasmic RNA was prepared (19), electrophoresed on 1% agarose/formaldehyde gels, transferred to nylon filters and hybridized as previously described (18). A TNF cDNA probe (425-bp *Pst*I-fragment of the nontranslated 3′-region of human TNF) was labeled by the random primer method (20). After exposure to x-ray film at −70°C, the filters were stripped and hybridized for control with a human β-actin cDNA probe (560-bp *Sal*I-*Eco*RI cDNA fragment) (21).

*In situ hybridization.* In situ hybridization was based on the method of Haase et al. (22). Aliquots of 100 μl of a cell suspension ($5 \times 10^5$/ml) were deposited on precoated glass slides by spinning in a cytocentrifuge (Shandon, England) at 600 rpm for 5 min. After air drying, the preparations were fixed for 5 min with 4% paraformaldehyde in PBS and stored at −20°C in 70% ethanol. For prehybridization the preparations were rehydrated at RT in PBS for 20 min, incubated in PBS/5 mM $MgCl_2$ (10 min), 0.2 M Tris/0.1 M glycine (10 min), and at 42°C in 2 × SSC/50% formamide (10 min). The cell preparations were hybridized with ~1 to 3 ng of probe ($1 \times 10^6$ dpm) labeled by the random primer method with 50 μCi of α-$^{35}$S-labeled dATP and 50 μCi of α-$^{35}$S-labeled dCTP (NEN, Dreieich, FRG) in 10 μl of a solution consisting of deionized 50% formamide/2 × SSC/10% dexran sulfate/1% RNase free BSA (Böhringer, Mannheim, FRG)/10 mM dithiothreitol/sonicated salmon sperm DNA (250 μg/ml) and *Escherichia coli* t-RNA (2 mg/ml). The solution was placed on the cells and covered with a siliconized glass coverslip, the edges of which were sealed with rubber cement. Hybridization was carried out at 42°C for 16 to 24 h. Coverslips were removed, the slides were rinsed in 2 × SSC, and transferred for 20 min to 1 × SSC/50% formamide at RT. Subsequent washings were carried out at 42°C in 1 × SSC/50% formamide for 2 h (change buffer after each 30 min) and 5 to 10 min at RT in 1 × SSC. The preparations were dehydrated through 70% and 95% ethanol containing 0.3 M ammonium acetate. For autoradiography, slides were dipped into Kodak NTB-2 nuclear track emulsion, dried in an upright position, and stored in a lightproof box at 4°C for 10 to 15 days. The slides were developed in Kodak D-19 developer at 15°C for 3 min and stained with the Diff-quick solutions (Merz and Dade, Basel, Switzerland).

## RESULTS

To test whether TNF is involved in immune surveillance, we investigated whether tumor cells could directly induce TNF production by human monocytes. With both TNF detection systems, the bioassay for TNF cytotoxic activity, as well as the TNF-specific ELISA, significant TNF activity and protein was detected in culture supernatants of monocytes stimulated with Jurkat cells (Table I). Optimal TNF release in monocyte/Jurkat cell cocultures was obtained at a producer:activator ratio of 5:1. Significant TNF levels were detected as early as 2 h after stimulation and reached a maximum after 8 to 12 h (data not shown). Surprisingly, in supernatants of monocyte cultures stimulated with K562 cells, no TNF was measured (Table I). An explanation for this seeming lack of TNF secretion could be that TNF receptors present on K562 cells (23) in a relatively high number (2 to $3 \times 10^3$/cell) might absorb TNF from the supernatant. In agreement with this hypothesis it was found that increasing numbers of K562 cells progressively reduced LPS-induced TNF levels (Table I). Reduced TNF levels were not observed when glutaraldehyde-fixed K562 cells saturated with TNF were cultured with LPS-activated monocytes (data not shown). Furthermore, K562 cells also absorb rTNF from culture dishes (data not shown). On the other hand, when Jurkat cells which express few TNF receptors (2 to $3 \times 10^2$/cell) (24) were added to LPS-activated monocytes even an enhanced TNF production was detected (Table I). These data support the assumption that released TNF in monocyte/K562 cultures was absorbed by TNF receptors on K562 cells.

TABLE I

*TNF levels in human monocyte cultures stimulated by tumor cells with or without LPS*[a]

| Stimulus | Cell No. | Activity (ng/ml TNF) | | | |
|---|---|---|---|---|---|
| | | No LPS | | With LPS (10 μg/ml) | |
| | | Bioassay[b] | ELISA[c] | Bioassay[b] | ELISA[c] |
| Control | | <1 | <1 | 10 | 10 |
| K562 | $1 \times 10^4$ | <1 | <1 | 10 | 10 |
| K562 | $3 \times 10^4$ | <1 | <1 | 5 | 10 |
| K562 | $1 \times 10^5$ | <1 | <1 | 2.5 | 5 |
| K562 | $3 \times 10^5$ | <1 | <1 | 1.25 | 2.5 |
| Jurkat | $1 \times 10^4$ | <1 | 1 | 10 | 10 |
| Jurkat | $3 \times 10^4$ | 10 | 2.5 | 10 | 10 |
| Jurkat | $1 \times 10^5$ | 20 | 5 | 10 | 10 |
| Jurkat | $3 \times 10^5$ | 5 | 20 | 20 | 10 |
| PBL | $1 \times 10^5$ | <1 | <1 | 10 | 10 |

[a] Monocytes (4 to $8 \times 10^5$/culture) were cultured in the absence or presence of LPS (*Salmonella minnesota*) together with tumor cells. The supernatants were collected after 16 h and assayed for TNF activity and TNF protein as described in *Materials and Methods*.
[b] Experiment 1.
[c] Experiment 2.

TNF protein detected in culture supernatants of human monocytes activated with Jurkat cells was produced by the monocytes and not by contaminating T cells or NK cells as shown in Table II. The indicated cell fractions were either stimulated with 50 μg/ml *Staphylococcus aureus* or not. A population enriched for monocytes (>95%) and also the unfractionated peripheral blood lymphocytes (PBL including monocytes) activated with *S. aureus* showed equally high TNF titers (40 to 80 ng/ml) in the culture supernatants. No TNF was detected when the cells were not activated by *S. aureus*. In contrast, even after stimulation with *S. aureus* the nonadherent PBL fraction (depleted of monocytes) was not able to produce TNF protein. Under those experimental conditions only monocytes and not T cells or NK cells were the source of TNF.

To investigate the findings of tumor cell-induced TNF on TNF-mRNA level, human monocytes were cultivated either with K562 or Jurkat cells for 2 h. Subsequent Northern blotting analyses revealed that cells of both tested tumor cell lines provided activation signals for human monocytes. Significant increase of TNF-mRNA expression was observed when monocytes were cultivated with Jurkat cells (Fig. 1*B*, *lane 2*; see also Fig. 2, *lane 2*) as well as with K562 cells (Fig. 1*A*, *lane 3*). Both tumor cell lines alone failed to express TNF-mRNA (Fig. 1*A*, *lanes 4* and *5* for K562 cells; Fig. 1*B*, *lane 5* for Jurkat cells). Treatment with either PMA or TNF of the tumor cells did not lead to TNF-mRNA expression (data not shown). Taken together, these data indicated that not only Jurkat cells but also K562 cells were able to induce TNF synthesis by human monocytes. Furthermore, Raji cells, another lymphoid cell line and melanoma cells were found to induce TNF expression in monocytes (data not shown). Therefore, it seems likely that tumor cell mediated monocyte activation is not restricted to only a few tumor cell lines.

To investigate whether viable cells are required for monocyte activation, tumor cells were metabolically inactivated by UV-irradiation and tested for their ability to induce TNF-mRNA in monocytes. Figure 1*B*, *lane 3*,

TABLE II

*Monocytes as the source of TNF*[a]

| Cells | TNF (ng/ml) | | | |
|---|---|---|---|---|
| | ELISA | | Bioassay | |
| | No stimulus | *S. aureus*, 50 μg/ml | No stimulus | *S. aureus*, 50 μg/ml |
| Monocytes | <1 | 40 | <1 | 80 |
| PBL (unfractionated) | 1.25 | 40 | <1 | 80 |
| PBL (depleted of monocytes) | <1 | 5 | <1 | <1 |

[a] The $8 \times 10^5$ monocytes (adherent cells), $4 \times 10^6$ unfractionated PBL (containing approximately $8 \times 10^5$ monocytes), and the nonadherent fraction of $4 \times 10^6$ PBL (monocyte depleted) were cultured in the absence or presence of 50 μg/ml fixed *S. aureus*. The culture supernatants were collected after 16 h and assayed for TNF activity and TNF protein as indicated.

shows that even UV-irradiated Jurkat cells were able to induce TNF-mRNA in the monocytes. Cocultivation of viable Jurkat cells with UV-irradiated monocytes in contrast did not lead to TNF-mRNA expression (Fig. 1*B*, *lane 4*).

To determine on a single cell level TNF-mRNA expression in human monocytes after activation with tumor cells, in situ hybridizations were performed. Tumor cells were cultivated with unfractionated PBL under nonadherent conditions. These cell mixtures were used for in situ hybridizations. Figure 2, *A* and *B* (PBL/K562 cell cocultures) show that cells in these cultures expressed significant TNF-mRNA levels. Similar results were obtained when PBL/Jurkat cocultures were used for in situ hybridizations (data not shown). Both types of tumor cells did not show any positive hybridization signal which confirms the results of Figure 1. Furthermore, Figure 2*B* shows that only a small percentage of the cells which by morphology looked like monocytes produced the TNF-mRNA. These findings are in agreement with our results obtained with the PMA-activated promyeloid tumor cell line HL60 where only 10 to 20% of the cells showed positive signals for TNF-mRNA expression under in situ hybridization conditions (data not shown).

The specificity of tumor cell mediated monocyte activation was tested by a coculture of monocytes with allogeneic PBL. As Figure 3, *lane 3*, shows these PBL only led to a slight increase in TNF-mRNA expression in this experiment, which could be due to contaminating monocytes in the PBL preparation. In five other independent experiments, no enhancement of TNF-mRNA was seen with allogeneic PBL. Moreover, no TNF protein was detected in supernatants of monocytes cultured in the presence of PBL (Table I). When various Ag-specific T cell clones were investigated for monocyte stimulating capacity, it was found that two of three clones led to a slight increase (1.5-fold) in TNF-mRNA expression. Therefore, it seems possible that the ability to stimulate monocytes for TNF expression might not only be restricted to tumor cells but may be a quality of proliferating cells. Furthermore, monocytes were cultured in cell free tumor cell supernatant to test whether soluble products from the tumor cells caused the observed monocyte activation. Neither enhanced TNF-mRNA levels nor soluble TNF protein was detected in such cultures (data not shown). Taken together, these results demonstrate that the observed TNF-mRNA was expressed in monocytes and not in tumor cells cocultured with monocytes and further suggests that surface structures on the outer membrane of the tumor cells might provide activation signals for human monocytes.

To further support the hypothesis of tumor cell surface structures which account for monocyte activation, enucleated tumor cells and tumor cell membrane preparations were prepared. These cytoblasts represent spheres similar to the original cells but without nuclei. They are enclosed by the intact cell membrane outside facing out. Figure 3, *lanes 4* and *5*, show that these tumor cytoblasts induced enhanced TNF-mRNA production compared with untreated monocytes (Fig. 3, *lane 1*). Furthermore, monocytes also respond to membrane preparations from tumor cells with TNF-mRNA expression (Fig. 3, *lane 6*), indicating the possibility that isolated surface structures might cause this effect.

To characterize these structures Jurkat and allogeneic PBL cell membrane preparations were size fractionated on PAGE. Human monocytes were treated with the eluted protein fractions. TNF-mRNA expression was determined by RNA preparations of the stimulated cells and subsequent RNA-dot blot analyses (Fig. 4*a*). Stimulation of TNF release was analyzed by assaying the supernatants for TNF protein (Fig. 4*b*). As shown in Figure 4*a*, monocytes cultured with protein fractions of relative molecular mass 32 to 38 kDa from Jurkat cells expressed enhanced TNF-mRNA levels. Control protein fractions from PBL had no effect. In addition, the supernatants of the corresponding cultures obtained significant TNF protein (Fig. 4*b*). Besides the 32- to 38-kDa protein fractions from Jurkat cells a 77-kDa protein fraction also induced TNF production (Fig. 4*b*) in human monocytes. TNF protein induction after stimulation with the 77-kDa protein fraction was reproducible, whereas TNF-mRNA induction with the 77-kDa fraction could be observed only in some experiments.

Comparable results were obtained when the same experiment was performed with K562 cell membrane fractions. However, the 46- to 54-kDa protein fractions were identified as monocyte-activating preparations. TNF protein and TNF-mRNA were induced by the same fractions (Fig. 4, *a* and *b*). Protease treatment of these fractions abrogated monocyte activation capacity, indicating the protein nature of these tumor cell-derived membrane structures (data not shown). The observation that the surface structures from K562 and Jurkat cells which are responsible for monocyte activation differ in their molecular mass might be due to tumor heterogeneity.

## DISCUSSION

Although several reports demonstrated elevated TNF levels in serum of cancer patients (6, 7) no proof could be obtained that monocytes/macrophages respond directly to tumor cells with TNF production. Besides monocytes/macrophages also T and NK cells (24, 25) and a variety of tumor cells (10) are able to produce TNF. Therefore,

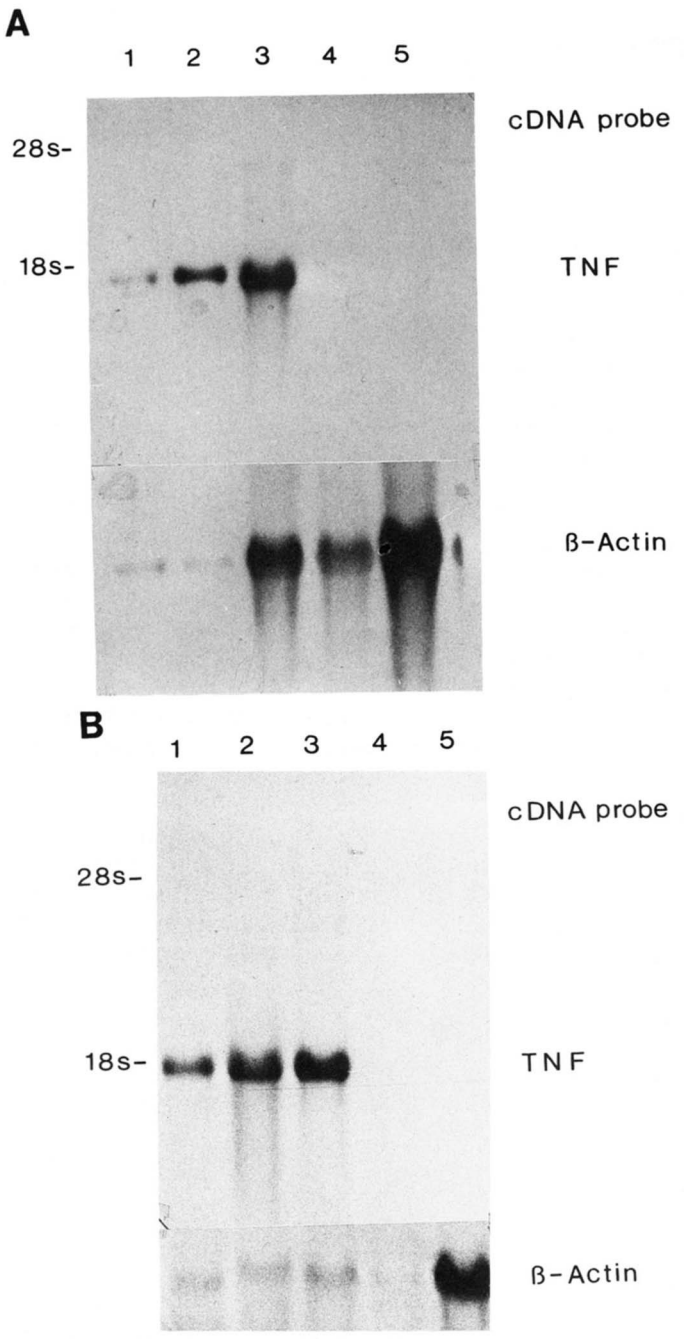


*Figure 1.* Northern blot analysis of human monocytes treated for 2 h. *a*, With culture medium alone (*lane 1*); 10 μg/ml fixed *S. aureus* (Pansorbin, Calbiochem) as a positive control for TNF-mRNA expression (*lane 2*); $5\times10^6$ K562 cells (*lane 3*). *Lanes 4* and *5* represent mRNA from $3\times10^6$ and $1\times10^7$ K562 cells, respectively (without monocytes). *b*, With culture medium alone (*lane 1*); $5\times10^6$ Jurkat cells (*lane 2*); $5\times10^6$ UV-irradiated Jurkat cells (*lane 3*). For control, monocytes were UV-irradiated and treated for 2 h with $5\times10^6$ viable Jurkat cells (*lane 4*). Tumor cells were washed off before mRNA extraction (*lanes 2* to *4*). *Lane 5* represents mRNA from $5\times10^6$ Jurkat cells (without monocytes).

these studies could not identify the cellular origin of the observed TNF activity. The results presented here demonstrate that both tested tumor cell lines K562 and Jurkat provide direct activation signals for human monocytes on the surface of their outer membranes in vitro.

In addition to the two tumor cell lines used for the experiments described above we found two additional tumor lines, Raji, another lymphoid cell line, and melanoma cells with the capacity to induce TNF-mRNA expression in human monocytes. Recently, TNF-mRNA

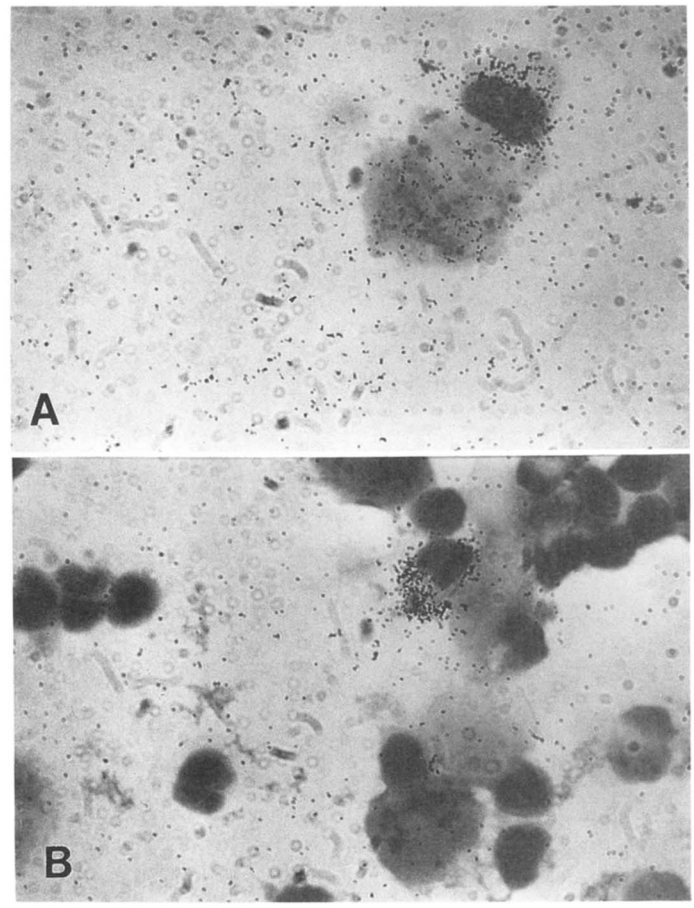


*Figure 2.* In situ hybridization of K562-activated human PBL *lymphocytes* (with the TNF-cDNA probe), 2 h after onset of induction (*A*) × 1000 and (*B*) × 400.

has been demonstrated in situ by Beissert et al. (26) in tumor infiltrating macrophages in coloncarcinoma biopsies.

When human monocytes were cultured together with either K562 or Jurkat cells for 2 h a significant increase of TNF-mRNA was observed (Fig. 1). The TNF-mRNA was exclusively produced by the monocytes and not by the tumor cells as shown in control experiments. Firstly, both tumor cell lines alone did not express the TNF-mRNA (Fig. 1). Also neither PMA nor TNF activation lead to any TNF-mRNA signal in these tumor cells as determined by dot blot analyses (data not shown). Secondly, not only viable tumor cells but also UV-irradiated tumor cells (Fig. 1), cytoblasts and even membrane preparations (Figs. 3 and 4) from both tumor cell lines were able to induce TNF-mRNA expression in human monocytes. And finally, in situ hybridizations showed that only monocytes and not the tumor cells expressed the TNF-mRNA (Fig. 2). The fact that only a small proportion of cells showed positive hybridization signals could be explained by our own unpublished data. Even in the cloned myeloid cell line HL60 only 10 to 20% of the cells expressed the TNF-mRNA after activation by 10 ng/ml PMA as detected by in situ hybridizations.

Also the possibility that contaminating lymphocytes (T cells and NK cells) rather than monocytes might be the source of tumor cell-stimulated TNF could be excluded. Only the adherent monocyte population and unfractionated PBL (including monocytes) were able to produce TNF protein after activation by *S. aureus* (Table II). No TNF

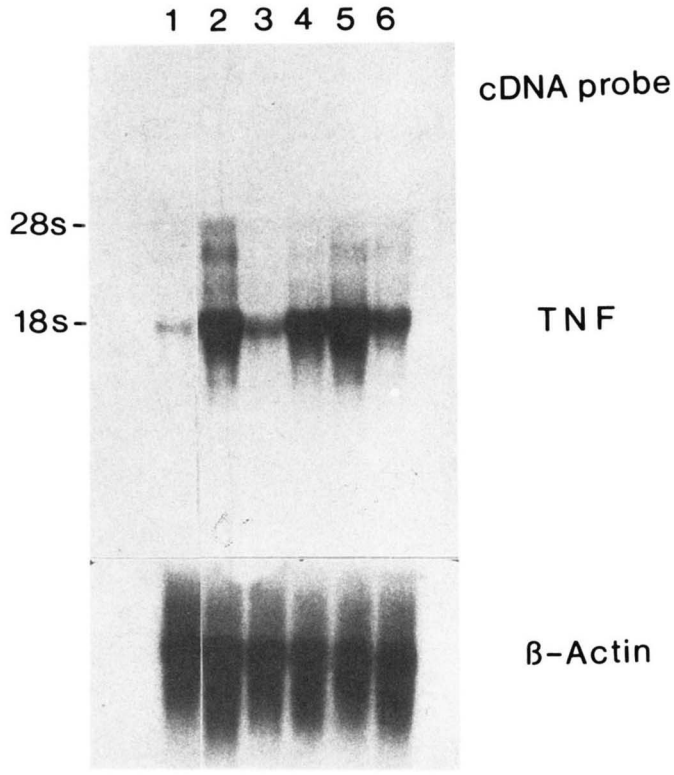


*Figure 3.* Northern blot analysis of human monocytes treated for 2 h with culture medium alone (*lane 1*); $5\times10^6$ Jurkat cells (*lane 2*); $5\times10^6$ allogeneic peripheral blood lymphocytes (*lane 3*); $3\times10^6$ K562-cytoblasts (*lane 4*); $9\times10^6$ K562-cytoblasts (*lane 5*) and with a K562-membrane preparation (*lane 6*).

could be detected in culture supernatants of monocyte-depleted nonadherent PBL even after the activation by *S. aureus* (Table II). In addition, NK-sensitive targets such as K562 cells were not killed by stimulated cultures that produced TNF (data not shown).

When the supernatants of tumor cell-activated monocytes were harvested and assayed for TNF activity in the L929 bioassay and in the TNF specific ELISA system, only the activation with Jurkat cells led to significant TNF release by the monocytes. No soluble TNF activity could be observed when K562 cells were used (Table I). Earlier experiments by Uchida et al. (27) have shown that K562 cells induced a monocyte derived cytotoxic factor only when the tumor cells were cultured together with the monocytes on autologous serum-precoated plastic dishes. When FCS-precoated plastic dishes were used, no monocyte cytotoxic factor was observed. However, in our hands under identical experimental conditions with autologous serum-coated dishes no TNF release could be measured (data not shown). The hypothesis that TNF receptors present in a relatively high number on K562 cells (2 to $3\times10^3$/cell) but not on Jurkat cells (2 to $3\times10^2$) (10) might rapidly absorb released TNF from the supernatant was consistent with our findings that increasing numbers of K562 cells progressively reduced LPS-induced TNF levels (Table I). This was not observed when Jurkat cells were cultured together with LPS-activated monocytes. This hypothesis was also supported by the findings that TNF-saturated glutaraldehyde-fixed K562 cells did not lead to reduced TNF levels (data not shown). Immunoprecipitations revealed that K562 cells induced similar levels of the 26-kDa membrane-bound TNF in the monocytes as it was observed with the Jurkat cells (data not shown). Since also the level of recombinant TNF added to K562 cultures was reduced within 16 h (data not shown) it seems conceivable that TNF receptors on K562 cells were responsible for the lack of soluble TNF in the supernatant of human monocytes activated with K562 cells.

Some viruses, such as Sendai virus (28) of influenza virus (29) have also been reported to induce TNF production in human monocytes. Both tumor cell lines (K562 and Jurkat) have been tested regularly for the absence of viral structures and mycoplasma contamination. Therefore, neither mycoplasma components nor viral proteins seemed to be responsible for the observed monocyte activation. In addition, allogeneic structures as activation signals were excluded. Allogeneic PBL from different donors (with different HLA-types) were tested for their ability to induce TNF synthesis in human monocytes in six independent experiments. Neither TNF production (Table I) nor TNF-mRNA expression (Figs. 3 and 4) was observed after coculture of monocytes with allogeneic PBL in any experiment. However, two of three Ag-specific T cell clones led to a slight increase of TNF-mRNA when cultured together with monocytes. Even though contamination was not excluded rigorously in these cells, this could indicate that not only tumor cells but proliferating cells could have the capacity to induce monocyte activation.

The finding of TNF-mRNA expression in human monocytes induced by metabolically inactivated tumor cells but not by tumor cell supernatants indicated that the monocyte activating structures were exposed on the surface of the tumor cells. This hypothesis was confirmed by the induction of TNF-mRNA and protein expression by tumor cytoblasts and even membrane preparations from tumor cells (Fig. 3). Two distinct membrane fractions from the tumor cell lines were identified which accounted for the observed monocyte activation (Fig. 4).

LPS contamination of only certain membrane fractions could be excluded because these experiments were repeated several times with the same molecular weight fractions being positive in each experiment. Therefore, it is very unlikely that only the 32- to 38-kDa membrane fractions from Jurkat cells and the 46- to 54-kDa fractions from K562 cells and no fraction from the PBL preparation was contaminated by LPS. Furthermore, protease treatment of the monocyte activating membrane fractions abrogated the activity, indicating the protein nature of these structures. If LPS was the responsible agent also the protease-treated fractions should be able to induce TNF-mRNA expression.

The fact that these proteins differed in their molecular mass (32- to 38-kDa for Jurkat cells and 46- to 54-kDa for K562 cells) cannot be explained at the moment and might be due to tumor heterogeneity. The observation that besides the 32- to 38-kDa protein fraction from Jurkat cells also a 77-kDa protein induced TNF production in human monocytes could be due to a dimeric structure. Further molecular characterization studies of these activation structures might help to answer the question of whether this represents the mechanism by which tumor cells are recognized by monocytes/macrophages and thus distinguished from normal cells in vivo.

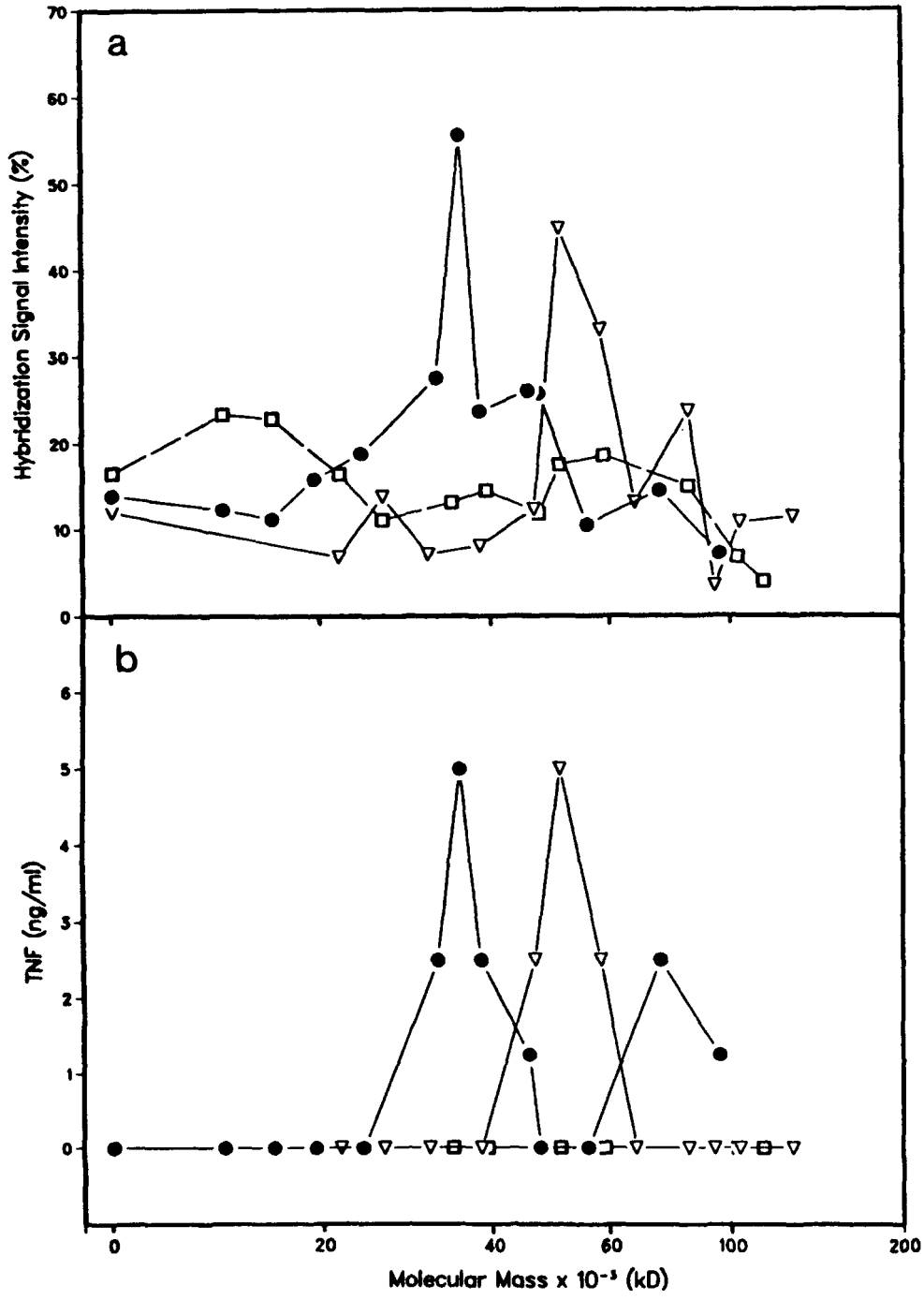


*Figure 4.* Isolated protein fractions from tumor cell membranes induced TNF production in human monocytes. (*a*) TNF-mRNA expression in human monocytes and (*b*) TNF protein release from human monocytes after activation for 2 h with Jurkat cell (*closed circles*), K562 cell (*triangles*), and allogeneic PBL (*squares*) membrane protein fractions. In (a) RNA-dot blots were made, hybridized with the TNF cDNA probe and for control also with the β-actin cDNA probe. The autoradiographs were measured densitometrically with an Elscript 400 film scanner and each signal was expressed as hybridization signal intensity in percent compared to a positive control in which the monocytes were treated with 50 μg/ml *S. aureus* for 2 h. In (*b*) TNF protein was determined by the TNF-specific ELISA.

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