Characterization of Monocyte-Activating Tumour Cell Membrane Structures

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Tumour cells are known to activate monocytes/macrophages and it has been shown that this stimulation was conferred by tumour-cell membranes. In order to analyse the relevant structures for tumour cell-specific TNF-induction monocytes from healthy donors were cultured in the presence of plasma membrane preparations from Jurkat or K562 cells. Both tumour cell lines revealed a monocyte-stimulating plasma membrane component of about 45 kDa. The TNF-inducing factor exhibited characteristics of a glycoprotein with the carbohydrate moiety as the structure responsible for stimulation. CD2, a glycosylated T-cell specific membrane component, was identified as being involved in monocyte activation in the case of the Jurkat cells whereas the identity of the activating structure on K562 cells is still unknown. From the data presented here indicating the importance of carbohydrate structures for monocyte activation we conclude that altered glycosylation of cell surface molecules of tumour cells might be responsible for tumour cell-induced monocyte stimulation.

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INTRODUCTION

Monocytes and macrophages can be activated [1, 2] by a variety of substances like bacterial and [3–5] viral [6] agents or tumour cells [7, 8]. They are able to discriminate between normal and neoplastic cells and selectively destroy the neoplastic cells [9–12]. However, the mechanism of this selectivity is not clear. Earlier work demonstrated that plasma membrane constituents might contribute to the difference between normal and tumour cells [9], but so far it has not been possible to attribute specific recognition of tumour cells by monocytes/macrophages and the subsequent activation of the mononuclear phagocytes to a tumour-specific antigen. Activation of monocytes is accompanied by production and secretion of cytokines, like tumour necrosis factor (TNF). Thus, the tumour cell-induced activated state of monocytes can be monitored by their secretion of TNF into the culture supernatant [8, 13]. In this study we present data indicating that glycosylated tumour-cell membrane structures are responsible for tumour cell-specific monocyte stimulation and that in the case of Jurkat cells this carbohydrate structure is associated with the CD2 molecule.

MATERIALS AND METHODS

Cell cultures. Culture methods of tumour cells and the isolation procedure for human peripheral blood monocytes from buffy coats of healthy donors by density gradient centrifugation on Ficoll-Paque (Pharmacia, Freiburg, Germany) have been described recently [9]. The cell lines used for this study were Jurkat, a lymphoid CD4+-cell line [14], and K562, an erythroleukaemia cell line. The cells were cultured in RPMI 1640 (Gibco, Glasgow, UK) supplemented with 10% heat-inactivated fetal calf serum (FCS) (Gibco) and 50 μg/ml Gentamycin (Gibco).

UV-irradiation. Cells were irradiated in tissue culture plates, with 500 mJ using a Stratalinker UV1800 (Stratagene, La Jolla, CA, USA).

T-cell clones. The human T-cell clones used, C3F8 (CD4+) and 3025 (CD8+, CD2+, CD3+, TCRαβ, LDCC) [15], were derived from U. Möbius (DKFZ, Heidelberg, Germany). The T-cells (3×10^6) were restimulated every 10–14 days with irradiated (2000 rad) human peripheral blood leukocytes (PBL) (6×10^6), irradiated (6000 rad) Laz-509 cells (3.5×10^6) [16] in 20 ml RPMI/FCS supplemented with IL-2 (10 U/ml).

Glutaraldehyde treatment. Cells were washed in phosphate buffered saline (PBS) and incubated with PBS containing 1% glutaraldehyde for 30 min on ice. Subsequently cells were washed with PBS at least 6 times.

Periodate treatment. Cells or plasma membranes blotted on nitrocellulose membranes (NC) (Schleicher und Schüll, Dassel, Germany) were incubated with sodium periodate (10 mM) in a sodium acetate buffer (50 mM, pH 4.5) for 60 min at room temperature (RT) in the dark. Subsequently the same volume of PBS with 1% glycine was added and the incubation was carried on for an additional 30 min. At the end of the treatment cells were washed with PBS at least twice.

Mycoplasma test. Presence of mycoplasma in culture medium was tested with a mycoplasma-DNA-hybridization kit according to the manufacturer's instructions (Gen Probe, San Diego, CA, USA).

TNF assay. Monocytes (purified by plastic adherence from 5×10^6 PBL) were cultured in 0.5 ml RPMI 1640 supplemented with 5% FCS for 18–20 h in a 24-well tissue culture plate (Costar, Cambridge, MA, USA). Cell-free supernatants were harvested and the TNF concentrations were measured immediately.

TNF concentrations were determined by an enzyme-linked immunosorbent assay (ELISA). Unless noted otherwise, all reactions were carried out at RT. Wells (96-well flat-bottom, Titertek Immuno Assay-Plate, Flow Laboratories, Meckenheim, Germany) were coated with affinity-purified (Protein A-Diasorb, Diagen, Düsseldorf, Germany) polyclonal rabbit anti-rhTNF antibodies (Ab) at 5 µg/ml in NaHCO₃ buffer (50 mM, pH 9) overnight at 4°C. Serial dilutions of the supernatants in PBS/0.1% bovine serum albumin (BSA) were applied to the wells (0.1 ml/well) for 2 h after blocking the wells with PBS/1% BSA for 2 h. Wells were washed with PBS/0.05% Tween20. Subsequently mouse anti-rhTNF MoAb 14B3 were added at 1 µg/ml in PBS/0.1% BSA to the wells and incubated for 2 h. Wells were washed and rabbit anti-mouse-IgG peroxidase conjugate (Sigma, St. Louis MO, USA) at a 1:2000 dilution in PBS was applied for 2 h. Wells were washed intensively and the substrate solution (3,3′,5,5′-Tetramethylbenzidine (10 mg) in 100 ml sodium acetate/citric acid buffer (100 mM, pH 4,9) and 15 µl 30% H₂O₂) was added (50 µl/well). The reaction was stopped with 2 M H₂SO₄ (50 µl/well) and absorption was measured at 450 nm in an EASY-Reader (SLT-Instruments), compared to a standard curve obtained with rhTNF. The MoAb 14B3 are specific for rhTNF (no cross reaction with h-Lymphotoxin).

Membrane preparation. Cells (10⁹) were incubated with 10 ml lysis buffer (Tris/HCL 25 mM, pH 7.4; NaCl 140 mM; PSMF 1 mM; Tween40 2.5%) for 1 h at 4°C. Subsequently, the cells were homogenized with a Potter apparatus and the suspension was centrifuged for 10 min at $1500 \times g$ to remove the nuclei. The supernatant was centrifuged again for 1 h at $100,000 \times g$. This supernatant was discarded and the pellet was resuspended with 1 ml Tris/HCl (10 mM, pH 8) containing 2% sodium deoxycholate (DOC). This suspension was centrifuged for 1 h at $100,000 \times g$. This time the pellet was discarded and the supernatant was either used immediately or frozen at $-70°C$.

Gel-filtration. HPLC gel permeation chromatography was performed using Beckman System Gold equipment with a Beckman TSK 3000SW column. Tris/HCl buffer (150 mM, pH 8) at a flow rate of 0.5 ml/min was used for elution. Collected fractions were dialysed in RPMI 1640 (100-fold excess). The proteins used for calibration were BSA (67 kDa), ovalbumin (43 kDa), and α-chymotrypsinogen (25 kDa).

Glycosylation inhibitors. Jurkat cells (2×10^8) were cultured in RPMI/FCS culture medium supplemented with swainsonine (10 µM), castanospermine (530 µM), 1-deoxymannojirimycin (DMM) (150 µM) or tunicamycin (0.5 µg/ml) for 20 h to inhibit N-glycosylation. All inhibitors were purchased from Boehringer Mannheim GmbH,
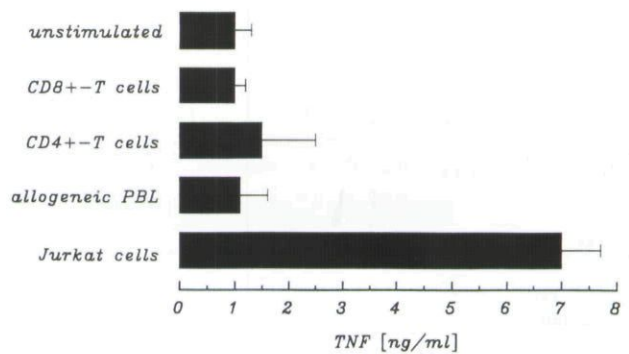

Fig. 1. TNF release of monocytes stimulated with normal and neoplastic cells. Monocytes (10^6/culture) were cultured with viable Jurkat cells, CD8⁺-T-cells (3025), CD4⁺-T-cells (C3F8) and PBL from a different donor (2×10^5 each/culture) for 18 h at 37°C. TNF concentrations in the supernatants were measured by ELISA. The data were obtained from quadruplicate cultures and are expressed as mean ± SD.

Mannheim, Germany. After the culture period cell viability was greater than 95% as tested by trypan blue exclusion.

SDS-PAGE and Western blot. Plasma membrane preparations of Jurkat cells (2×10^8/lane) were electrophoresed on a 12% polyacrylamide gel (PAGE) and transferred to NC by electroblotting (2 h, 150 mA) [17]. The NC-sheet was cut into appropriate pieces according to the molecular weight markers, placed in wells of a 24-well culture plate (Costar) and the pieces were dissolved with 0.5 ml methanol. The methanol was evaporated overnight and PBL (5×10^6/well) were seeded into the wells. After 18 h of incubation TNF in the supernatant was measured by ELISA.

FACS analysis. Cells (10^6/analysis) were incubated with FITC-coupled anti-CD2 Ab (Clon IOT-11, Dianova GmbH, Hamburg, Germany) for 30 min on ice, washed and analysed with a FACScan (Becton-Dickinson, San Jose, CA, USA).

CD2-Depletion. The samples from the HPLC gel permeation run were incubated with anti-CD2 MoAb (1 µg/ml; Clon 8E5, Dr. B. Schraven, DKFZ, Heidelberg, Germany) for 30 min. Subsequently Protein A conjugated to Sepharose (10 mg/ml, Pharmacia) was added and after 30 min the sepharose beads were removed by centrifugation.

RESULTS

Stimulation of human monocytes for TNF production by neoplastic but not by normal cells

It has been shown by several groups that tumour cells can stimulate monocytes to secrete TNF into the culture supernatant, whereas normal cells cannot. In our experimental system we cultured human peripheral blood monocytes from healthy donors with viable Jurkat cells. Cells from a CD8⁺-T-cell clone (3025) and from a CD4⁺-T-cell clone (C3F8), as well as PBL derived from a different donor were used as control. Only the Jurkat cells induced TNF-secretion from monocytes, while the other cells did not induce TNF levels greater than background (Fig. 1).
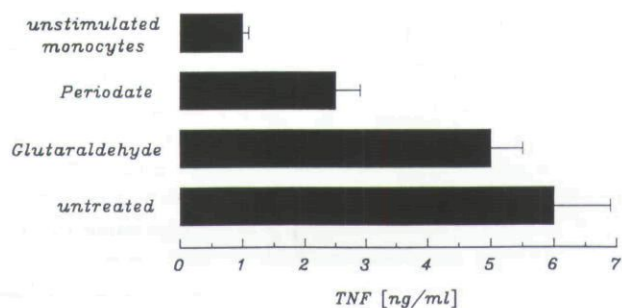
Fig. 2. TNF release of monocytes stimulated with treated Jurkat cells. Monocytes (10^6/culture) were incubated with UV-irradiated Jurkat cells (2×10^5/culture) treated with sodium periodate or glutaraldehyde. After 20 h the TNF concentrations in the supernatants were measured by ELISA. The experiment was performed as triplicate cultures and the data are expressed as mean $\pm$ SD.
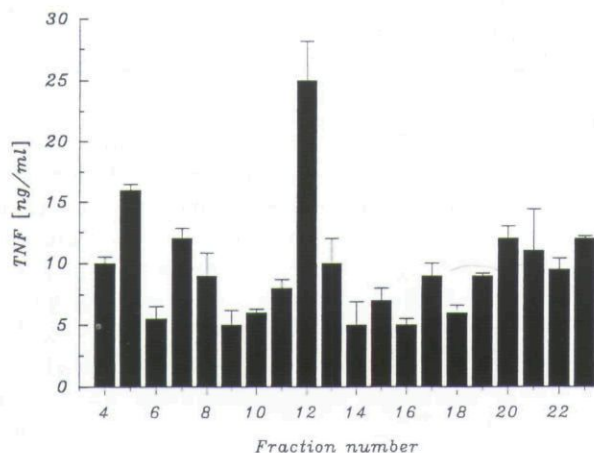

Fig. 4. TNF release of monocytes stimulated with membrane fractions from Jurkat cells cultured with glycosylation inhibitors. Jurkat cells (3×10^8 each) were cultured with (■) swainsonine, (●) DMM, (▼) tunicamycin, (▲) castanospermine or (○) without any inhibitor. Plasma membranes were prepared, HPLC-sized and incubated with monocytes (10^6/culture). After 18 h the TNF concentrations in the supernatants were measured by ELISA. The experiment was performed three times and the data shown are from one representative experiment. The dashed line represents samples of a control run with ovalbumin (43 kDa) eluting in fraction no. 12.

Fig. 3. TNF release of monocytes stimulated with size-fractionated Jurkat cell membrane preparations. Plasma membranes from Jurkat cells (5×10^8) were prepared and subjected to HPLC gel-filtration. The samples were dialysed and incubated with monocytes (10^6/culture). After 20 h the TNF concentrations in the supernatants were measured by ELISA. The experiment was performed in triplicate cultures and the data are expressed as mean $\pm$ SD.

Fig. 5. TNF release of monocytes stimulated with α-CD2 MoAb treated Jurkat cells. Monocytes (10^6/culture) were incubated with Jurkat cells (solid bars) or K562 cells (hatched bars) (2×10^5 each/culture). Tumour cells were treated with mouse α-hCD2 MoAb (8E5; IgG2a) and monocytes were treated with mouse α-hLFA-3 MoAb (TS2/9; IgG1) for 30 min before stimulation. Each MoAb was used at 10 μg/ml. After 20 h the TNF concentrations in the supernatants were measured by ELISA. The experiment was performed as quadruplicate cultures and the data are expressed as mean $\pm$ SD. The dashed line represents TNF levels in isotype control experiments.

Previous experiments [9] showed that Jurkat cells themselves neither express TNF mRNA nor TNF protein and, therefore, cannot account for TNF production. Thus the question arose of whether Jurkat cells release a TNF inducing factor (TIF) or whether they express TIF on their plasma membranes. In order to destroy the metabolic activity of the tumour cells, Jurkat cells were killed by UV-irradiation. Again, only tumour cells but not normal cells induced TNF secretion (data not shown). This supported the idea that monocytes recognize tumour cells by a constituent exposed on the plasma membrane.
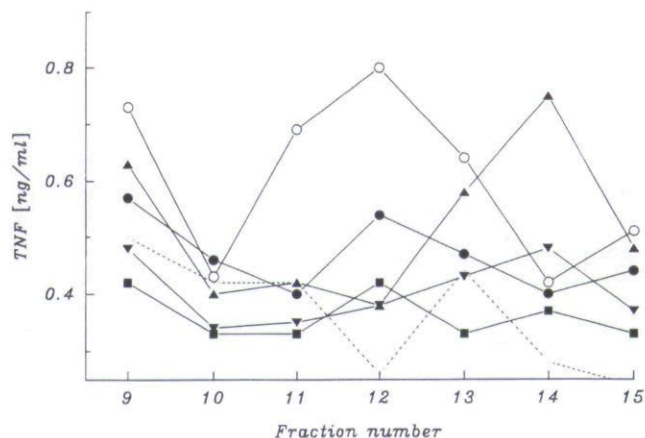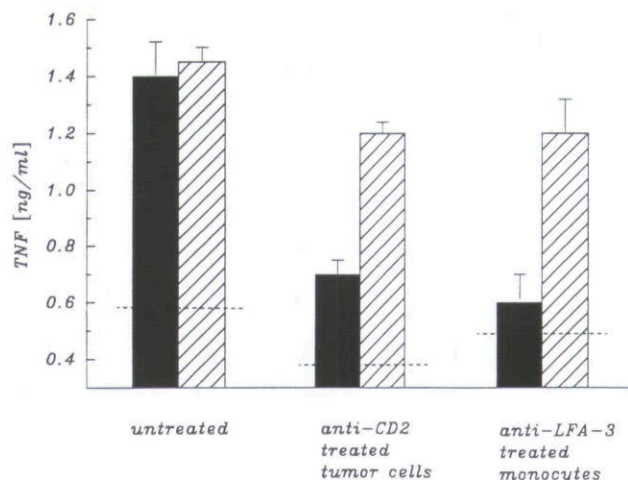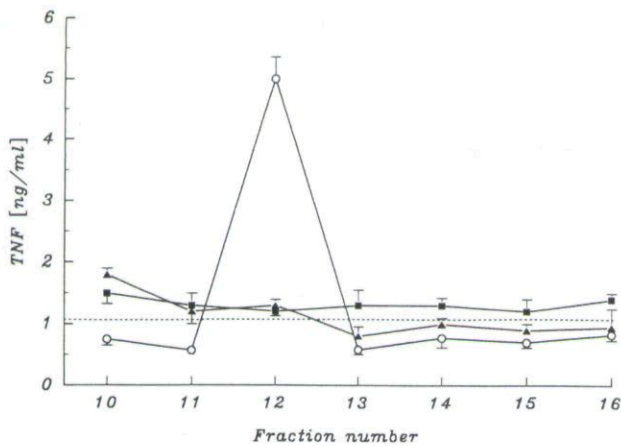
Fig. 6. TNF release of monocytes treated with CD2-depleted Jurkat cell membrane fractions. Plasma membranes from Jurkat cells (10^9) were prepared and size fractionated using HPLC. 1/3 of the samples were (■) CD2-depleted (α-CD2 MoAb concentration 5 $\mu g/ml$). Monocytes (10^6/culture) were either pretreated with (▲) α-LFA-3 MoAb (5 $\mu g/ml$) or (○) no Ab. After 20 h the TNF concentrations in the supernatants were measured by ELISA. The experiment was performed twice. Results from duplicate cultures are expressed as mean + SD. The dashed line represents the TNF background released by unstimulated monocytes. Ovalbumin (43 kDa) eluted in fraction no. 12.

Table 1. PH-stability of TIF. Plasma membranes from Jurkat cells (2×10^9) were prepared, sized and the fractions were tested for activity. The active fraction was aliquoted, dialysed for 12 h with isotonic buffers of different pH and redialysed for 4 h with RPMI 1640. The treated aliquots were then incubated with monocytes (10^6/culture). After 18 h the TNF concentrations in the supernatants were measured by ELISA. The experiment was performed as triplicate cultures and the data are expressed as mean $\pm$ SD.

pH	2	3	4	5	6	7	8	9	10	11	12
TNF [ng/ml]	2.3	2.1	2.0	2.7	9.2	8.4	9.0	8.4	9.4	7.8	6.6
SD	0.2	0.1	0.2	0.2	0.5	0.2	0.4	0.1	0.3	0.4	0.1

Biochemical modification of the tumour cell surface

Experiments with protein or carbohydrate-modifying chemicals were performed to answer the question of whether a protein or a carbohydrate structure was responsible for monocyte activation. For this reason, UV-irradiated Jurkat cells were treated with either glutaraldehyde- or sodium periodate solution. The results revealed that the monocyte-stimulating properties were destroyed after periodate treatment (40% of the untreated control), while the glutaraldehyde treatment left the stimulating capacity of the tumour cells

Table 2. TNF release kinetics from monocytes. Plasma membranes from Jurkat cells (10^9) were prepared, sized, and the fractions were tested for activity. The active fraction was aliquoted and incubated with monocytes (10^6/monocytes). In parallel monocytes were incubated with LPS from S. minnesota at 5 $\mu g/ml$. After 1, 2, 3, 4, 6, 9, 19, 24 h supernatants were collected and frozen at $-20°C$. The TNF concentrations in the supernatants were measured by ELISA. The experiment was performed as triplicate cultures and the data are expressed as mean $\pm$ SD.

| Incubation period [h] | TNF [ng/ml] | | |
	Membrane preparation	LPS	Unstimulated monocytes
1	0.9 ± 0.1	0.9 ± 0.2	0.6 ± 0.1
2	0.9 ± 0.2	0.9 ± 0.2	0.3 ± 0.1
3	0.9 ± 0.1	2.2 ± 0.2	0.3 ± 0.1
4	0.9 ± 0.2	0.9 ± 0.3	0.4 ± 0.1
6	0.9 ± 0.3	7.0 ± 0.5	0.9 ± 0.1
9	2.7 ± 0.4	14.5 ± 0.9	1.9 ± 0.1
19	7.3 ± 0.3	15.8 ± 1.0	2.6 ± 0.2
24	7.5 ± 1.0	15.0 ± 0.9	2.2 ± 0.2

nearly unaffected (77% of the untreated control; Fig. 2). Figure 2 shows data of a representative experiment. The remaining stimulatory activity of tumour cells in repeated experiments varied from 10% to 40% after periodate treatment and from 77% to 90% after glutaraldehyde treatment. In experiments where Jurkat cell membrane constituents had been separated by PAGE the active fraction recovered from the gel lost its monocyte-activating capacity completely after periodate treatment (data not shown). This was the first indication of carbohydrates playing a role in tumour-specific monocyte stimulation. Similar results were obtained at the mRNA level. After glutaraldehyde treatment Jurkat cells as well as K562 cells were capable of inducing TNF mRNA production whereas periodate treatment of the tumour cells destroyed this activity (data not shown).

Separation of tumour cell membranes by HPLC gel-filtration

In prelimininary experiments Jurkat cell membranes were separated by SDS-PAGE, blotted onto Nitrocellulose, and thereafter applied to monocyte cultures by dissolving the nitrocellulose pieces in methanol. It could be demonstrated that membrane constituents of the molecular size in the range of 37–47 kDa stimulated monocytes for TNF production and release. To confirm this and in order to gather further information on the biochemical and physical structure of TIF Jurkat cell membranes were size-chromatographed by HPLC gel-filtration to obtain TIF in larger quantities. The fractions were tested for their monocyte-stimulating capability after elution from the sizing column and dialysis. Fraction number 12 (corresponding to the apparent molecular weight of 43

kDa) activated monocytes for TNF secretion (Fig. 3). The fractions that contained TIF were used for pH-stability tests and TNF release kinetics. Plasma membranes from K562 cells were equally sized-separated on HPLC and tested for monocyte stimulation. The membranes prepared from K562 cells also displayed TIF at a molecular size of about 45 kDa (data not shown).

pH-stability and TNF release kinetics of TIF

Sized plasma membrane fractions from Jurkat cells were tested for TNF-inducing capacity and subsequently used for TNF release kinetics or for testing the pH-stability. To check the resistance of TIF to pH-changes the TIF bearing fraction was dialysed against buffers covering pH-values from 2 to 12 for 12 h and redialysed into RPMI 1640 for 8 h. Table 1 shows TNF secretion by human monocytes after incubation with such treated fractions. TIF was stable in a range from pH 6 to 10. Incubation of monocytes with TIF for various periods of time showed that TIF induced TNF levels greater than background after 9 h of incubation (LPS, in comparison, induced TNF secretion after 6 h of incubation) and reached a plateau after 19 h (LPS 9 h) (Table 2).

Effect of glycosylation inhibitors

Jurkat cells were cultured in the presence of glycosylation inhibitors to investigate the role of carbohydrates in the tumour-specific stimulation process. Swainsonine, 1-deoxymannojirimycin, castanospermine or tunicamycin were used. These substances block the addition and trimming of sugar structures at different stages during the N-glycosylation process of mammalian cells. After a 20 h culture period the Jurkat cells were harvested, plasma membranes were prepared, sized on HPLC, and incubated with monocytes for 20 h. The TNF concentrations in the supernatants are shown in Fig. 4. Plasma membranes derived from Jurkat cells cultured with non-supplemented medium stimulated monocytes for TNF release. In contrast, none of the 43 kDa fractions of the membrane preparations with a glycosylation pattern absent, incomplete or changed due to the inhibitors were able to induce TNF secretion.

For control purposes the presence of typical membrane structures on the cell surface was tested in parallel. Flow cytometrical analysis revealed that Jurkat cells were positive for the CD2 molecule whether or not they had been cultured with a glycosylation inhibitor. This indicated the correct transport of glycosylated membrane molecules to the cell surface even when glycosylation was disturbed.

Effects of anti-CD2 (α-CD2 MoAb) and anti-LFA-3 antibodies (α-LFA-3 MoAb) on the stimulating potency of Jurkat cells

Deduced from the results shown above and in view of recently published data [18] the role of CD2 in the monocyte activation process was investigated. For this reason UV-irradiated Jurkat cells were incubated with α-CD2 MoAb and then incubated with monocytes. In addition, the ligand of CD2, the lymphocyte function-associated antigen-3 (LFA-3) on the surface of monocytes was blocked with α-LFA-3 MoAb. TNF secreted by the monocytes was measured after a 20 h culture period. UV-irradiated K562 cells were used as CD2-negative control. As can be seen from Fig. 5, both MoAbs blocked the stimulation of monocytes. The capacity of the K562 cells for TNF induction was not affected by the presence of the MoAb. This led to the conclusion that CD2 was involved in the stimulation of monocytes by Jurkat cells. It also demonstrated that monocyte stimulation by K562 cells was different from the CD2-LFA-3 mechanism. Similar results were obtained when plasma membrane preparations were used instead of complete Jurkat cells (Fig. 6). Size-fractionated membranes from Jurkat cells were CD2-depleted by immunoprecipitation and used for stimulation. Also, non CD2-depleted active Jurkat cell membrane fractions were used to stimulate monocytes bearing MoAb-blocked LFA-3. Again, untreated membrane preparations of about 43 kDa stimulated monocytes to secrete TNF. However, CD2-depletion of membrane or anti-LFA-3 treatment of the monocytes abolished TNF release.

DISCUSSION

Despite the fact that specific recognition of tumour cells by monocytes has been described in many systems, it is still unclear how monocytes/macrophages distinguish between normal and neoplastic cells. Results from Fidler [10], Shimizu [11], Hasday [12], Jänicke & Männel [9], and Chong *et al.* [19] showed that monocytes specifically bind and destroy tumour cells of syn-, allo-, and xenogeneic origin. We support this observation in our system demonstrating that only tumour cells, not allogeneic PBL or non-neoplastic T-cell lines, caused TNF secretion by monocytes. Jänicke & Männel [9] and Chong *et al.* [19] also showed that the activating principle was located on the tumour cell surface. To date little is known about the molecular mechanism of action and the biochemical nature of this TNF-inducing factor. Chong *et al.* found that the TNF-inducing capacity of tumour cells was lost after periodate treatment. Fidler and coworkers discussed an altered lipid-composition on tumour cells as cause for the selective recognition process [20]. This hypothesis is not supported by the data of Hasday *et al.* who recovered TNF-inducing membrane molecules from murine tumours in the aqueous phase after methanol:water:chloroform extraction [12]. The sensitivity to periodate as well as the solubility in water (HPLC runs were performed without detergents) was also shown in our experiments. The importance of carbohydrate structures for the activation process was stressed by the use of glycosylation inhibitors. The glycosylation inhibitors used in our experiments affect the glycosylation of proteins at different stages: Tunicamycin inhibits N-glycosylation completely by inhibiting the enzyme glycosyltransferase.

Castanospermin inhibits glucosidase I resulting in modified complex or hybrid carbohydrate structures. DMM is a mannose analogon leading to high-mannose structures. Swainsonine inhibits the building of complex structures by trimming the glycoproteins but does not affect the development of high-mannose or hybrid structures. After treatment with each of these glycosylation inhibitors all TIF activity had disappeared in the molecular weight range of 43 kDa. The reason for the appearance of a TNF-inducing fraction in the molecular weight range of 35 kDa (Fig. 4, fraction No. 14) after castanospermin incubation of the Jurkat cells is unclear but could be due to synthesis of biologically active partial structures of TIF. However, the appearance of this peak of activity was not reproducible in repeated experiments. The presence of CD2 was measured by immunofluorescence to rule out the possibility that the stimulation of the monocytes was lost after inhibition of glycosylation because the non-glycosylated proteins might not be transported to and expressed on the cell surface. The CD2 molecule was still detectable at comparable intensities on more than 94% of the cells whether cultured in the presence or absence of inhibitors.

Furthermore, Jurkat cells were cultured without FCS to minimize the possibility that serum components were responsible for the TNF release. The plasma membrane preparations from such cells activated monocytes as well as preparations from Jurkat cells cultured with FCS (data not shown). This indicated that TIF is an intrinsic factor of the tumour cells. Contaminating bacterial endotoxin, mycoplasma or viral components could not account for the observed monocyte activation for several reasons: Both tumour cell lines (Jurkat and K562) were tested regularly for the absence of viral structures and mycoplasma. Endotoxin contamination was excluded since control cells were cultured and subjected to identical fractionating procedures as the tumour cells and did not activate the monocytes. Furthermore, the difference in kinetics for TIF-induced versus LPS-induced TNF release also indicated different signalling pathways for the two stimuli.

Recently Webb *et al.* described TNF stimulating capability of CD2 isolated from Jurkat cells [18] and magrophage-colony-stimulating factor production subsequent to LFA-3 triggering [21]. However, the question remains whether Jurkat cell-CD2 is identical with T-cell CD2 or whether it is glycosylated in a different way. Reinherz *et al.* demonstrated the importance of glycosylation of the CD2 molecule for the functional three-dimensional structure of the protein part [22]. Also, the quantity of CD2 expressed by some tumour cells could differ from the quantity on normal T-cells and the stimulating phenomenon could be a matter of CD2-concentrations on the tumour cell surface seen by the monocytes. Investigations to answer these questions are in progress.

It is of interest that the CD2-negative K562 tumour cells also exposed carbohydrate structures on the membrane that activated monocytes. This structure was also inactivated by periodate treatment but not affected by glutaraldehyde (data

not shown). Therefore, a carbohydrate conformation common to tumour cells might be responsible for activation of mononuclear phagocytes rather than a protein tumour marker. Quantitative isolation, purification and comparative biochemical analysis of the monocyte-activating carbohydrate structures from different tumour cells will be required to solve this problem.

In summary, the characteristics of the monocyte activating structures determined in our system are: (1) they are plasma membrane constituents and exposed to the outside; (2) in the case of K562 and Jurkat cells the molecular weight is about 45,000 Da; (3) the biologically active part of these structures resides most likely in carbohydrate moieties; and (4) in the case of Jurkat cells the activating structure seems to be part of the CD2-molecule.

The possibility of finding common features of the monocyte-activating principle from different tumour cells and pinpointing those to molecularly defined structures is intriguing and worthy of further investigation with the aim of gaining a better understanding of tumour cell-monocyte communication with possible consequences for future diagnostic and therapeutic purposes.

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