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Induction of Monokine Production by Tumor Cells

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ABSTRACT

Cells of the proerythromyeloid cell line K562 and of the T cell line Jurkat were able to induce an increase in TNF-, IL1α-, and IL1β-mRNA expression in human peripheral blood derived monocytes *in vitro*. The activating principle of these tumor cells was associated with fractions of membrane preparations of distinct molecular mass, 32-38 kD for Jurkat cells and 46-54 kD for K562 cells, respectively. At least part of the activating constituent seemed to be protein in nature. Isolated membrane preparations of both cell types induced production and secretion of TNF. Stimulation of monocytes with the viable tumor cells led to TNF release only when Jurkat cells were used. Viable K562 cells induced enhanced TNFmRNA expression but seemed to absorb soluble TNF from the supernatant.

INTRODUCTION

It is a generally observed phenomenon that monocytes/macrophages are able to distinguish between neoplastic cells and normal cells *in vitro*. The macrophages/monocytes destroy tumor cells selectively as a result of activation. One means of tumor cell killing by activated macrophages is the production and utilization of tumor necrosis factor (TNF) as a cytotoxic effector molecule to which some tumor cells in contrast to normal cells are exquisitely sensitive. The question arose whether such a phenomenon takes also place *in vivo* and whether the tumor cells can directly activate macrophages/monocytes for monokine production. Several reports have documented an enhanced capability of TNF production by peripheral blood mononuclear cells of cancer patients (1), enhanced TNF levels in serum or plasma of cancer patients (2,3), and enhanced cytostatic activity of monocytes from cancer patients (4,5). We investigated in an *in vitro* system whether tumor cells from two cell lines, K562 and Jurkat, were capable of directly activating human peripheral blood monocytes for TNF-, interleukin 1 (IL1) α-, and IL1β-mRNA expression, and TNF release.

MATERIALS AND METHODS

Tissue Culture Conditions: All cell lines and the monocytes were cultured in RPMI

1640 (GIBCO), supplemented with 10% heat inactivated FCS (GIBCO) and 50μg/ml gentamycin (GIBCO). K562, and Jurkat were regularly tested for mycoplasma contamination and also for the absence of viral structures. Human peripheral blood monocytes were isolated from blood of healthy donors by Ficoll-Paque (Pharmacia) density gradient centrifugation and the peripheral blood mononuclear leucocytes (PBL) were washed twice with RPMI 1640. Monocytes were isolated by adherence on plastic for 1 h. The remaining cells were >95% monocytes as determined by differential staining.

Reagents: Fixed *Staphylococcus aureus (Staph. aureus)* cells (Pansorbin, Calbiochem) 10μg/ml or *S. minnesota* lipopolysaccharide (LPS) (Difco Laboratories) 10μg/ml was used for monocyte stimulation.

TNF Assay: Supernatants of the adherent cell fraction of $4x10^6$ mononuclear leucocytes per ml were tested in the TNF specific ELISA system as described recently (6).

Cytoblast Preparation, Membrane Preparation and Electroelution from SDS-PAGE: Enucleated cells (cytoblasts) were prepared as described (7). Tumor cell membranes were prepared by homogenisation of the cells in 10mM sodium phosphate buffer pH 7.4, containing 1mM MgCl, 30mM NaCl, 1mM DTT, 0.005mM PMSF, and 0.1μg/ml DNase, and subsequent centrifugation through a 41% sucrose gradient. The membrane preparations were separated on a 12.5% SDSPAGE, and portions of the gels corresponding to different size fractions were electroeluted in 50mM Tris, 0.38M glycine and 0.1% deoxycholate for 1 h at 100 Volt.

mRNA Detection: RNA extraction for dot blots was performed by isolating the RNA by the guanidine-HCl method (8) and blotting the RNA onto nylon filters (GENOFIT, Heidelberg, F.R.G.). For Northern analysis the RNA was prepared using the guanidine-thiocyanate method (9). After separation on 1% agarose-formaldehyd gels the RNA was transferred to nylon filters and hybridized as described (10). For hybridization the following probes were used: 425 bp PstI fragment of the nontranslated 3'region of human TNFcDNA (BASF, Ludwigshafen, F.R.G.), 460 bp human IL1α EcoRI-BamH1 cDNA fragment (p3-IL1α) of the coding region, 530 bp human IL1β BamH1-Nde1 cDNA fragment (p11-IL1β) of the coding region (both by U. Gubler, Hoffmann LaRoche, Nutley, New Jersey), 560 bp human β-actin Sall-Eco R1 fragment of the cDNA as described (11). The probes were labeled by the random primer method as described recently (12).

RESULTS AND DISCUSSION

Monocytes isolated from blood of healthy donors express baseline levels of TNFmRNA and IL1mRNA when cultured for 2 hours after isolation. The mRNA expression was markedly enhanced when the monocytes were cocultured with either K562 or Jurkat cells. Coculture with nonadherent cells from a different blood donor was unable to activate the monocytes exlcuding allogeneic determinants as a general stimulus (Fig.1). Cytoblasts of K562 cells or purified membrane preparations of both tumor cell lines were equally able to induce increased TNF-, IL1α- and IL1β-mRNA expression.

Dot blot analysis for gene activation by different size fractions of the tumor cell membrane preparations revealed activating constituents with relative molecular mass of 32-38 kD for Jurkat cells and 46-54 kD for K562 cells (Fig.2). Equivalent fractions of membrane preparations from allogeneic nonadherent peripheral blood leucocytes did not induce TNF-, IL1α- or IL1β-mRNA expression. The activating activity in tumor cell membrane fractions was destroyed when the fractions were treated with proteases (data not shown), indicating a protein structure responsible for activity.