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ARTICLES

Nonlymphoid Cultured Cells Possess a System Controlling Cellular Compatibility

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Abstract We show that various nonlymphoid cultured cells can activate the production of cytotoxic factors in response to direct contact with cells of a different kind. Accumulation of cytotoxic factors in the medium was detected 1 h after contact of K562 and L929 cells or after contact of L929 cells with purified membranes of K562 cells. TNF-α or immunologically related proteins, or both, but not Fas-ligand or lymphotoxin, were also accumulated in membranes of K562 and L929 cells shortly after these cells had been allowed to contact each other. The cytotoxic factors expressed by nonlymphoid cells trigger apoptosis of target cells. These observations strongly suggest that nonlymphoid cells possess molecular mechanisms controlling cellular compatibility. J. Cell. Biochem. 78:186–196, 2000.

Key words: tumor necrosis factor; cell co-cultivation; membrane-bound cytotoxic factors; apoptosis

Different types of lymphoid cells produce cytotoxic factors in response to contact with target cells. Some of these factors are secreted, and others are accumulated in cellular membranes [Arai et al., 1990; Herberman et al., 1986; Trapani, 1998; Wright and Bonavida, 1981; Young, 1989]. The best characterized cytotoxic factors expressed by monocytes, as well as by a variety of T-lymphocyte cell lines are, perhaps, the tumor necrosis factor (TNF-α) and the lymphotoxin (TNF-β) [Beutler and Cerami, 1989; Korner and Sedgwick, 1996; Paul and Ruddle, 1988; Ruddle, 1992; Vassalli, 1992]. Production of these and some other related cytotoxic factors by normal T lymphocytes and cultured lymphoid cells can be stimulated by interleukins, phorbol esters, and contact with target cells [Chong et al., 1989; Dett et al., 1991; Steffen et al., 1988; Sung et al., 1988)]. The factors produced by T and B lymphocytes are cytotoxic toward a variety of cells. In some cases, they trigger target cells to undergo apoptosis [Duke et al., 1986; Golstein et al., 1991; Laster et al., 1988; Vassalli, 1992]; in other cases, necrosis of target cells is observed [Laster et al., 1988; Sashchenko et al., 1993; Zychlinsky et al., 1991]. The ability to produce and release cytotoxic factors is not specific to lymphoid cells. It has been reported that TNF-α and lymphotoxin can be produced by different cultured tumor cells [Kronke et al., 1988; Spriggs et al., 1988], and some tumor cells express a membrane-bound cytotoxic protein, Fas-ligand, which permits them to counterattack and kill lymphoid cells [Gratas et al., 1998; Griffith et al., 1995; Rabinovich et al., 1998; Strand et al., 1996].

The ability to activate expression of cytotoxic factors in response to contact with target cells was assumed to be a characteristic feature of lymphoid cells. Our recent observations [Gnuchev et al., 1997] appear to question the generality of the above statement, as we demonstrated that nonlymphoid cells can also be induced to produce cytotoxic factors, including TNF-α, under conditions of co-cultivation with cells of a different type. This observation was made in experiments on co-cultivation of mouse fibroblasts (L-929 cells) with human erythroleukemia cells (K-562); under these conditions, the cells of both lines started to

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produce and to release several cytotoxic factors into the medium. In the present investigation, we have obtained further evidence that various nonlymphoid cells can express cytotoxic factors in response to direct physical contact with other types of cells. Furthermore, under these conditions, both soluble and membrane-bound cytotoxic proteins were expressed by nonlymphoid cells. Most if not all of these membrane-bound cytotoxic proteins were found to be immunologically related to TNF-α.

MATERIALS AND METHODS

Cell Cultures

All cell cultures, with the exception of HD3 (see below), were grown in Dulbecco's modified Eagle's medium (DMEM; Sigma Chemical Co.) supplemented with 10% fetal calf serum (FCS). Chicken erythroblastoid HD3 cells (clone A6 of line LSCC) [Beug et al., 1982] were grown in DMEM supplemented with 8% FCS and 2% chicken serum.

Co-cultivation

L929 cells, MCF-7 cells, NB41A3 cells, MG22A cells were grown in 6-well plates (Costar). As soon as the monolayer covered 50% of the surface, the medium was replaced by fresh serum-free medium containing K562 or HD3 cells grown in suspension at a concentration of 10^6 cells per ml. After cultivation for different time intervals, the medium was gently removed. The percentage of dead cells in the monolayer and in the medium was determined by counting under a light microscope after staining with Trypan blue [Sashchenko et al., 1988]. Cytotoxicity (P%) was calculated as described [Gnuchev et al., 1997].

Analysis of Cytotoxicity of Cell Membranes and of Proteins Released Into the Medium

Co-cultivation of L-929 cells and K-562 cells was carried out for different time intervals as described above. After co-cultivation for the desired time, the medium and the cells were collected. The medium was filtered through a of 0.2-μm filter (Sartorius).

To assay the medium for the presence of cytotoxic factors, 200-μl aliquots were added to target cells (L-929) growing in 96-well plates at a concentration of 4×10^4 cells per well after removal of growth medium. In control experiments 200 μl of fresh serum-free DMEM were added to the target cells. The cells were then cultured for 24 h and the percentage of dead cells determined.

To prepare cell membranes, cells collected after co-cultivation were disrupted in a Dounce homogenizer in hypotonic buffer. The cellular membranes were then purified by differential centrifugation, as described by Liu et al. [1989]. The cytotoxic activity of membranes was assayed in essentially the same way as that of medium. The concentration of protein was determined in each sample of membranes. Aliquots containing 10 μg of membrane protein were added to 4×10^4 target cells (L929 or K562) growing in 96-well plates and the percentage of dead cells was determined after cultivation for 24 h.

Immunological Experiments

Monoclonal antibodies against human TNF-α and human lymphotoxin (TNF-β) were purchased from Boehringer-Mannheim and polyclonal rabbit antibodies against rat Fas-ligand (cross-reactive with mouse and human Fas-ligand) from Santa Cruz Biotechnology. To suppress the cytotoxic activity of the corresponding antigens, the antibodies were added to samples of cell membranes at a dilution of 1:500. Immunoprecipitation was carried out according to a standard protocol [Firestone and Winguth, 1990]. The immunoprecipitated proteins were collected on protein A-Sepharose beads, washed, and eluted with sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer.

SDS-PAGE and Determination of Apparent Molecular Weights of Cytotoxic Factors

Cell membranes and conditioned medium were precipitated with acetone and proteins were separated by SDS-PAGE [Laemmli, 1970]. After electrophoresis, the proteins were transferred to polyvinylidene difluoride (PVDF) membranes (Millipore, Bedford, MA) as described by Towbin et al. [1979]. The membranes were cut into 2–3-mm-wide pieces corresponding to different molecular masses, and proteins were eluted by 16-h incubation in a solution containing 0.05 M ammonium bicarbonate, 50% acetonitrile, and bovine serum albumin (BSA) (0.5 mg/ml), as described by Chertov et al. [1992]. The eluted proteins were concentrated by lyophilization, dissolved in RPMI-1640 medium, and assayed

for cytotoxic activity as described above. For visualization of ^{14}C-labeled proteins, the gels were dried under vacuum and exposed with Kodak film for 3 weeks.

DNA Fragmentation Assay

Cells were lysed in a buffer containing 10 mM Tris-HCl (pH 8.0), 50 mM EDTA, 0.4% Triton X-100 and after 15-min incubation at 4°C the fast-sedimenting material was removed (5,000*g*, 15 min). The DNA remaining in the supernatant was precipitated with isopropanol and, after standard purification, analyzed by electrophoresis in a 1.5% agarose gel.

Microscopic Analysis

The cells were either grown on microscopic slides (monolayer cultures) or centrifuged onto slides (suspension cultures) using a cytospin centrifuge. The samples were fixed with 1% paraformaldehyde in phosphtae-bufferd saline (PBS) for 20 min at 37°C. This was followed by three washes with PBS and permeabilization with 0.2% Triton X100 in PBS for 10 min at room temperature. The cell nuclei were stained with DAPI (4′6-Diamine-2′-phenilidone dihydrochloride; Roche Molecular Biochemicals) at a concentration of 0.1 μg/ml in PBS for 30 min and embedded in fluorescence mounting medium (DAKO). The cells were examined using a Leitz Diaplan fluorescence microscope.

RESULTS

Nonlymphoid Cells Possess the Capability to Express Cytotoxic Factors Upon Co-Cultivation With Cells of a Different Type

We found previously that human erythroid cells (line K562) and mouse fibroblasts (line L929) start expression of cytotoxic factors upon co-cultivation in the same culture flask [Gnuchev et al., 1997]. To find out whether other nonlymphoid cells possess this ability, experiments on co-cultivation of the following cell lines were carried out: HD3 (chicken erythroblasts transformed by AEV virus) [Beug et al., 1982] and NB41A3 (transformed mouse neuroblasts) [Marchisio et al., 1978]; HD3 and MG22A (mouse hepatoma cell line) [Zakharian et al., 1985]; and HD3 and MCF-7 (transformed human epithelial cells) [Soule et al., 1973]. Aliquots of the medium were taken 24, 48, and 72 h after the beginning of co-cultivation and assayed for the presence of factors cytotoxic to either of the participating cell lines.

The data presented in Figure 1 demonstrate that cytotoxic factors were released into the medium upon co-cultivation of all the pairs of cell lines tested. With one exception (MCF-7 cells co-cultivated with HD3 cells), both participating cell lines were affected by the cytotoxic factors. Hence, the capability to produce and release cytotoxic factors under conditions of co-cultivation of cells of different kinds is not a unique property of K562 and L929 cells. In all cases, the cytotoxic effect of the medium was significantly suppressed (to 50% or more) by antibodies against TNF-α (not shown). In no case were the cytotoxic factors released in the medium upon separate cultivation in the absence of serum for up to 72 h of any of the cell lines tested in co-cultivation experiments (not shown).

Expression of Soluble Cytotoxic Factors by Nonlymphoid Cells Is Triggered by Direct Physical Contact With Cells of a Different Type

Expression of cytotoxic factors upon co-cultivation of various cell lines could be triggered either by direct contact or through an exchange of soluble factors. In order to distinguish between these possibilities, we cultivated K562 and L929 cells in two sections of a plastic flask separated by a membrane with 0.4-μm pores, i.e., when the direct cellular contacts were excluded, but the exchange of soluble factors was not restricted. As shown in Figure 2, no cytotoxic effect was observed in these conditions. In other experiments, cultivation of L929 cells in medium collected after cultivation of K562 cells, or vice-versa, did not trigger release of cytotoxic factors by either of the cell types (not shown).

Kinetics of Release of Soluble Cytotoxic Factors Upon Co-Cultivation of K562 and L929 Cells and Upon Treatment of L929 Cells With Isolated Membranes of K562 Cells

Although co-cultivation of K562 and L929 cells showed a considerable increase in the number of dead cells 24 h after the beginning of co-cultivation [Gnuchev et al., 1997], it was unclear how long it took for cells to start the production and release of cytotoxic factors in response to contact with cells of another kind. In order to clarify this question, we assayed the cytotoxicity of the medium collected at differ-

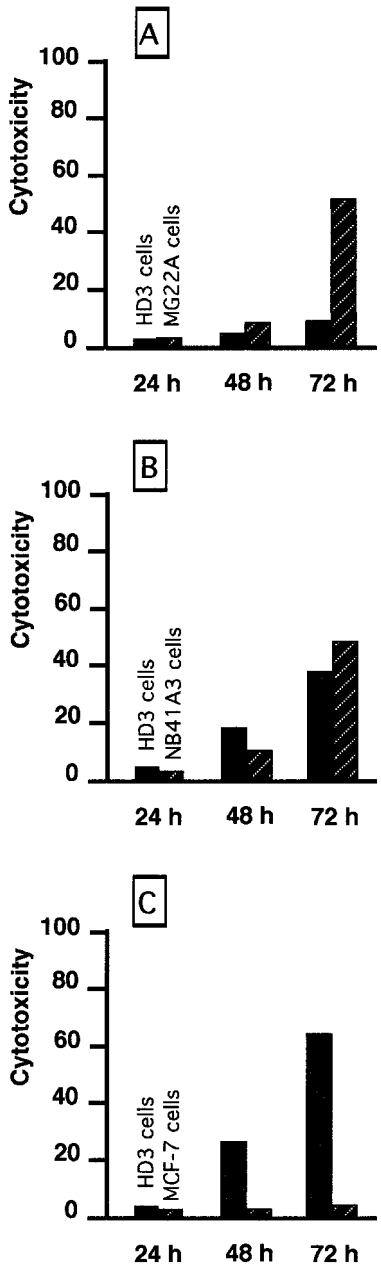

Fig. 1. Cytotoxicity of the medium collected after 24, 48, or 72 h of co-cultivation of several cell lines. **A:** HD3 and MG22A. **B:** HD3 and NB41A3. **C:** HD3 and MCF-7. The two different columns at each time point show cytotoxicity versus each the two cell lines tested in the co-cultivation experiment. The cytoxicity shown is a percentage of dead cells in an experiment with subtraction of the percentage of dead cells in the corresponding control experiment (incubation in a fresh serum-free DMEM). The percentage of dead cells in control experiments did not exceed 5% after 72-h incubation and was significantly lower after 24 or 48 h of incubation.

ent time intervals after the beginning of co-cultivation of K562 and L929 cells. The results of this experiment are shown in Table I. It is evident that cells begin to release cytotoxic factors a short time (1 h) after contact with cells of a different type. However, the level of cytotoxic activity in the medium gradually increased with time, reaching a maximum after 18 h. Similar kinetics of the release of cytotoxic factors into the medium were observed when L929 cells were allowed to contact purified membranes of K562 cells (1 mg of membrane protein per 4×10^5 cells; see Table I). Hence, L929 cells possess receptors capable of recognizing signals exposed on membranes of K562 cells.

Direct Contact of K562 and L929 Cells Triggers Expression by Both Cell Lines of a Variety of Membrane-Bound Cytotoxic Factors

Natural killer (NK) and other lymphoid cells express both soluble and membrane-bound cytotoxic factors [Herberman et al., 1986; Trapani, 1998]. In order to find out if nonlymphoid cells possess the same property, we assayed cytotoxic activity in the membranes of K562 and L929 cells isolated after co-cultivation for different time intervals. No cytotoxic activity was found in membranes of control cells (data not shown). However, upon co-cultivation both K562 and L929 cells expressed membrane-bound cytotoxic factors (Fig. 3). The cytotoxic activity of membranes of either K562 or L929 cells was already quite prominent after 3 h of co-cultivation and increased further with time. In some experiments, the cytotoxic activity of cell membranes of both K562 and L929 cells increased gradually for at least 48 h (Fig. 3), but in others it increased sharply for the first 4 h and then began to decrease (Fig. 3). Interestingly, this decrease of cytotoxic activity of cell membranes correlated with an increase of cytotoxic activity of the medium (not shown). Hence, it is likely that under certain conditions the membrane-bound cytotoxic factors may be released into the medium, possibly as a result of partial proteolysis.

In order to identify the cytotoxic factors accumulating in membranes of K562 and L929 cells under conditions of co-cultivation, we examined whether the cytotoxic activity of their membranes can be suppressed by antibodies against known cytotoxic factors. As shown in Figure 4, the cytotoxic activity of membranes of

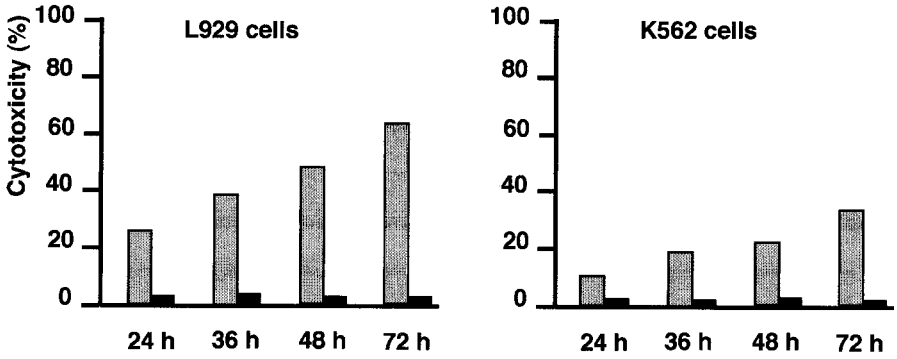

Fig. 2. Percentage of dead cells observed after co-cultivation of K562 and L929 cells together (gray columns) or in two sections of a plastic flask separated by a membrane with 0.4-μm pores (black columns).

TABLE I. Accumulation of Cytotoxic Activity in the Medium upon Joint Cultivation of L929 and K562 Cells for Different Time Intervals and upon Incubation for the Same Time Intervals of L929 Cells With Purified Membranes of K562 Cells*

Source of conditional medium	Target cells used to assay cytotoxicity	Time interval (after beginning of the experiment) before collection of the conditional medium			
		1 h	3 h	6 h	18 h
Experiment on joint cultivation of L929 and K562 cells	L929	14 ± 3%	16 ± 3%	24 ± 2%	36 ± 4%
Experiment on cultivation of L929 cells in the presence of membranes of K562 cells	L929	11 ± 2%	14 ± 4%	24 ± 4%	40 ± 5%

*In both experiments, aliquots of culture medium were collected at different time intervals after the beginning of the experiment. To estimate the cytotoxicity of medium in aliquots collected, the aliquots were added to fresh target cells; the percentage of dead cells was calculated after 24 h of cultivation in the absence of serum (see under Materials and Methods for details). The figures represent an average of three independent experiments.

both K562 and L929 cells was significantly suppressed by antibodies against TNF-α. Antibodies against lymphotoxin (TNF-β) and Fas-ligand showed little if any effect.

In order to characterize further the cytotoxic factors present in membranes of K562 and L929 cells, the size distribution of these factors was studied. The membrane proteins were separated by SDS-PAGE and transferred to a PVDF membrane. The latter was cut into a number of pieces containing proteins of different sizes. The proteins were eluted from each piece and assayed for cytotoxicity toward either L-929 cells (membrane proteins from K562 cells) or toward K562 cells (membrane proteins from L929 cells). The size distribution of membrane-bound cytotoxic factors appeared to be different in L929 and K562 cells (Fig. 5). Membranes of K562 cells contained cytotoxic factors with apparent molecular masses of 17–18, 26, 30, 40, and 53–54 kDa, while the cytotoxic factors present in membranes of L929 cells had apparent molecular masses of 26, 40, 53, and 70–90 kDa. The observed wide size distribution of membrane-bound cytotoxic factors was rather surprising, as the cytotoxic activity of both membrane samples was almost completely suppressed by TNF-α-specific antibodies (see above), and the molecular mass of the classical membrane form of TNF-α is 26 kDa [Kriegler et al., 1988; Perez et al., 1990]. Hence, in an alternative approach we isolated cytotoxic membranes from L929 and K562 cells prelabeled with ^{14}C amino acids. After solubilization of membrane proteins by a nonionic detergent (Triton X100), TNF-α and immunologically related proteins were immunoprecipitated using TNF-α-specific monoclonal antibodies. The size distribution of the precipitated proteins was then analyzed by SDS-PAGE followed by fluorographic visualization (Fig. 6). This experiment demonstrated that proteins with molecular masses of 40, 53–55, and 70-90 kDa present in membranes of L929 cells and proteins with molecular masses of 17, 40, and 53–55 kDa present in membranes of

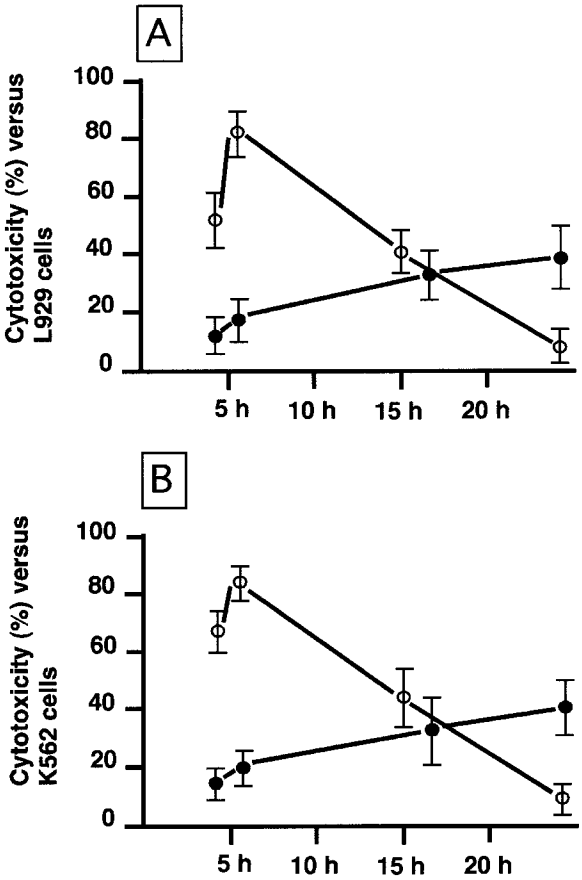

Fig. 3. Kinetics of accumulation of cytotoxic factors in membranes of K562 (**A**) and L929 (**B**) cells during the course of co-cultivation. Two typical kinetic curves obtained in different experiments (see the text) are shown by open and closed circles. Bars show maximal deviations from the average results (circles) observed in five independent experiments.

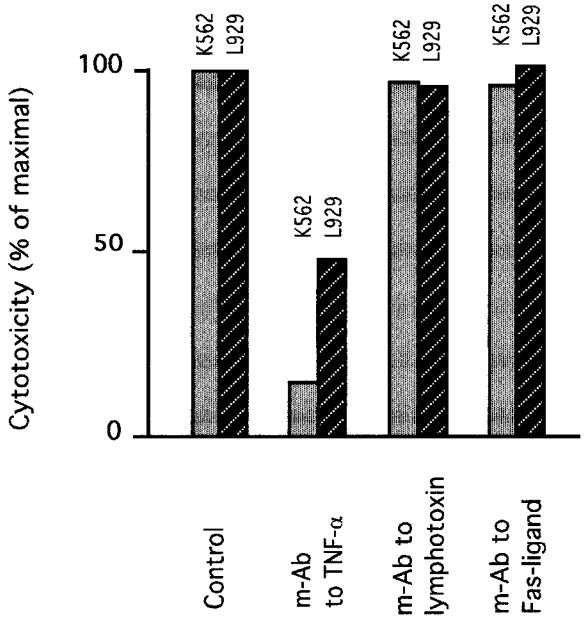

Fig. 4. Cytotoxic activity of membranes of K562 cells (gray columns) and L929 cells (black hatched columns) in the presence of antibodies against different cytotoxic factors. Cell membranes were isolated after co-cultivation of K562 and L929 cells for 5 h and assayed for cytotoxic activity versus fresh L929 and K562 cells, respectively, in the presence of monoclonal antibodies against TNF-α (dilution 1:500) or against lymphotoxin (dilution 1:500) or polyclonal antibodies against FAS-ligand (dilution 1:500). The cytotoxicity observed in control experiment (about 50% of dead cells after 24-h incubation of target cells with the cytotoxic membranes) was considered as 100% and all other values observed in experiments on immunosuppression were correspondingly normalized.

K562 cells are immunologically related to TNF-α. The TNF-related protein with apparent molecular mass of 53–55 kDa was also found in the medium along with the classical 17-kDa soluble TNF-α. Some small TNF-α-related proteins with apparent molecular masses of less then 14 kDa also accumulated in the medium upon co-cultivation of K562 and L929 cells. We interpret these small factors as products of degradation of proteins with higher molecular masses.

The Membrane-Bound and Soluble Cytotoxic Factors Expressed by K562 and L929 Cells Upon Co-Cultivation Trigger Apoptosis in Both K562 and L929 Cells

In order to examine the mechanism of the cytotoxic process triggered by soluble and membrane-bound factors expressed by L929 and K562 cells under conditions of co-cultivation, the morphology of target cells and the patterns of DNA fragmentation in these cells were studied. Extensive fragmentation of nuclear DNA into oligonucleosomal ladders was observed after 24-h incubation of either L929 cells with cytotoxic membranes of K562 cells or of K562 cells with cytotoxic membranes of L929 cells (Fig. 7A and data not shown). Furthermore, fragmentation of cell nuclei into smaller particles ("apoptotic bodies") was observed in both cultures after co-cultivation for 48 h (Fig. 7B). These observations suggest that both cytotoxic membranes of L929 or K562 cells and soluble factors released by these cells upon co-cultivation trigger apoptosis in target cells. The apoptotic mechanism of cell death correlates well with the presence of TNF-α and/or related cytotoxic factors in the medium and cell membranes, as well as with the existence of a long (20-h) time interval between the initial contact of target cells with the cytotoxic

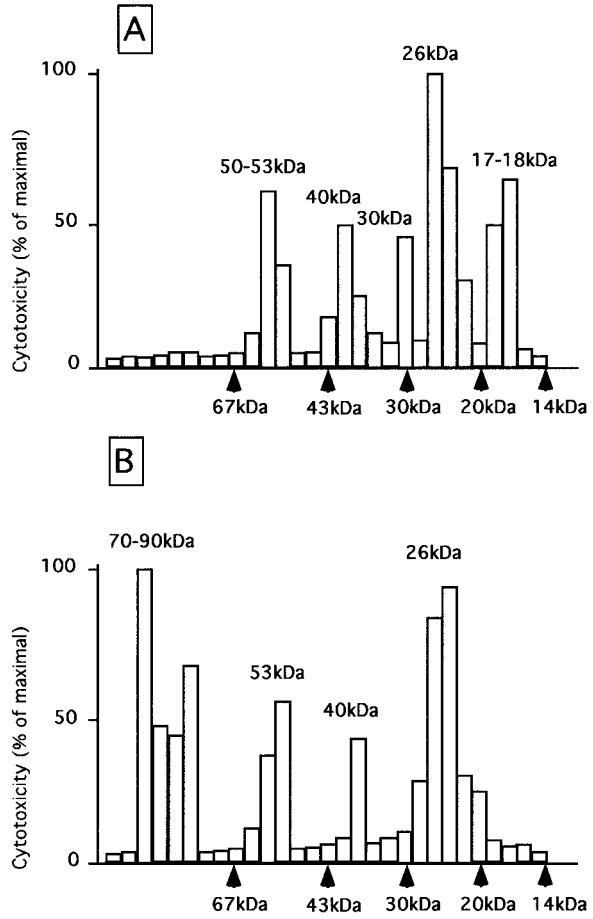

Fig. 5. Size fractionation by SDS-PAGE of membrane-bound cytotoxic factors expressed by K562 (**A**) and L929 (**B**) cells in the course of co-cultivation. Membranes were collected 10 h after the beginning of co-cultivation. Each gel was loaded with 20 μg of membrane protein. Proteins were eluted from consecutive sections of the gel and assayed for cytotoxic activity. For details, see Materials and Methods.

membranes or medium and the death of these cells.

DISCUSSION

The ability to activate expression of soluble and membrane-bound cytotoxic factors in response to contact with target cells is considered a characteristic feature of lymphoid cells. Our present results demonstrate that some cultured nonlymphoid cells possess the same property. Two of our observations seem to be of major importance. First, we have demonstrated that expression of cytotoxic factors by nonlymphoid cells is activated by direct physical contact with cells of a different type. The factors identifying cell types are exposed on cell membranes, as incubation of L929 cells with isolated membranes of K562 cells was sufficient to trigger production of soluble cytotoxic factors by L929 cells. Second, the majority of both membrane-bound (this paper) and soluble (this paper and also [Gnuchev et al., 1997] cytotoxic factors produced by nonlymphoid cells under conditions of co-cultivation are immunologically related to TNF-α. Recent evidence indicates that different tumor cells can kill tumor-infiltrating lymphocytes by expressing a membrane-bound cytotoxic factor: Fas (APO1/CD95) ligand [Bennett et al., 1998; Chouaib et al., 1997; Gratas et al., 1998; Hug, 1997; Rabinovich et al., 1998; Strand et al., 1996]. Cell lines derived from the above tumors also are characterized by permanent expression of Fas-ligand [Bennett et al., 1998]. We failed to detect this factor among the cytotoxic proteins expressed by K562 and L929 cells under conditions of co-cultivation. Hence, a distinct molecular mechanism is responsible for the incompatibility of these cells.

Some clones of cytolytic lymphocytes express membrane-bound TNF-α [Kinkhabwala et al., 1990; Monastra et al., 1996; Ratner and Clark, 1993] or another membrane-bound cytotoxic factor immunologically related to TNF-α, which has a molecular weight of 50–60 kDa [Liu et al., 1989]. Here we found that membrane-bound cytotoxic proteins expressed by K562 and L929 cells possessed the property of reacting with antibodies against TNF-α. Furthermore, a cytotoxic protein with apparent molecular mass of 53 kDa was detected in cytotoxic membranes of both K562 cells and L-929 cells. Cytotoxic proteins with other molecular masses (17–90 kDa) were also found in membranes of K562 and L929 cells under conditions of co-cultivation. Although we failed to detect the classical membrane-bound form of TNF-α (26 kDa) [Kriegler et al., 1988; Perez et al., 1990] among the radiolabeled membrane-bound proteins precipitated by TNF-α-specific antibodies, the cytotoxic activity of a 26-kDa protein was detected after electrophoretic separation of the membrane-bound cytotoxic proteins from either L929 or K562 cells. The apparent discrepancy between these results could be explained by a higher specific cytotoxic activity of the 26 kDa protein compared to other proteins recognized by the TNF-α-specific antibodies. The nature of the high-molecular-weight proteins recognized by TNF-α-specific

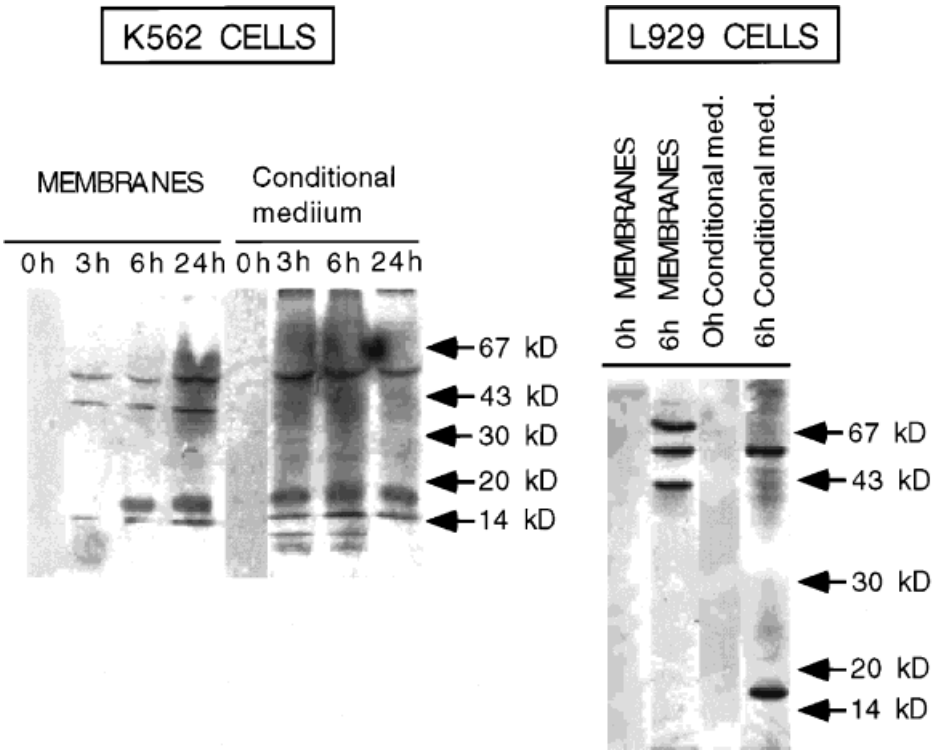

Fig. 6. Tumor necrosis factor (TNF)-related proteins in cytotoxic membranes of K562 and L929 cells and released into the medium during the course of co-cultivation for the indicated time. To discriminate the soluble TNF-related factors released by K562 and L929 cells under co-cultivation, two separate experiments were carried out with either K562 cells or L929 cells pre-incubated with ^{14}C-labeled amino acids. Proteins were then immunoprecipitated and separated by SDS-PAGE (see Materials and Methods). Note the accumulation in K562 cells of membrane-bound cytotoxic proteins during the course of co-cultivation.

antibodies remains to be studied. We can conclude that in response to contact with cells of another type, nonlymphoid cultured cells activate expression of membrane-bound cytotoxic factors that are immunologically related to TNF-α although their precise relation to TNF-α is not yet clear. The wide spectrum of molecular masses of these proteins might be due to cleavage of a single large precursor or to posttranslational modification of a single small precursor. In this connection, different size forms of TNF-α originating as a result of glycosylation of the 17-kDa TNF-α polypeptide have been described [Sherry et al., 1990]. TNF-α cross-reactive proteins with apparent molecular masses of 50, 70, and 120 kDa were previously found in chick embryo cells [Wride and Sanders, 1993)].

Isolation of clones of L-929 cells capable of expressing TNF-α has been reported previously [Rubin et al., 1986; Spriggs et al., 1988; Vanhaesebroeck et al., 1991]. However, these selected clones produced TNF-α either constantly or under permanent selection for TNF-α resistance. Hence, there was no question of fast activation of TNF-α expression in response to external stimuli such as contact with other cells. In our case, no detectable expression of either soluble or membrane-bound TNF-α by any of the cell lines tested was observed before cells of different kinds were allowed to contact each other. At 1 h after contact, soluble cytotoxic factors were released into the medium, and membrane-bound TNF-α and/or immunologically related proteins could be detected 3 h after cell contact. Clearly, this result can only be explained by activation of TNF-α expression in response to contact with cells of a different type. This, in turn, signifies that nonlymphoid cells possess receptors capable of discriminating cells of a different kind. Furthermore, nonlymphoid cells must also possess molecular mechanisms connecting these receptors with systems regulating expression of TNF-α and possibly of other currently uncharacterized cytotoxic proteins. In other

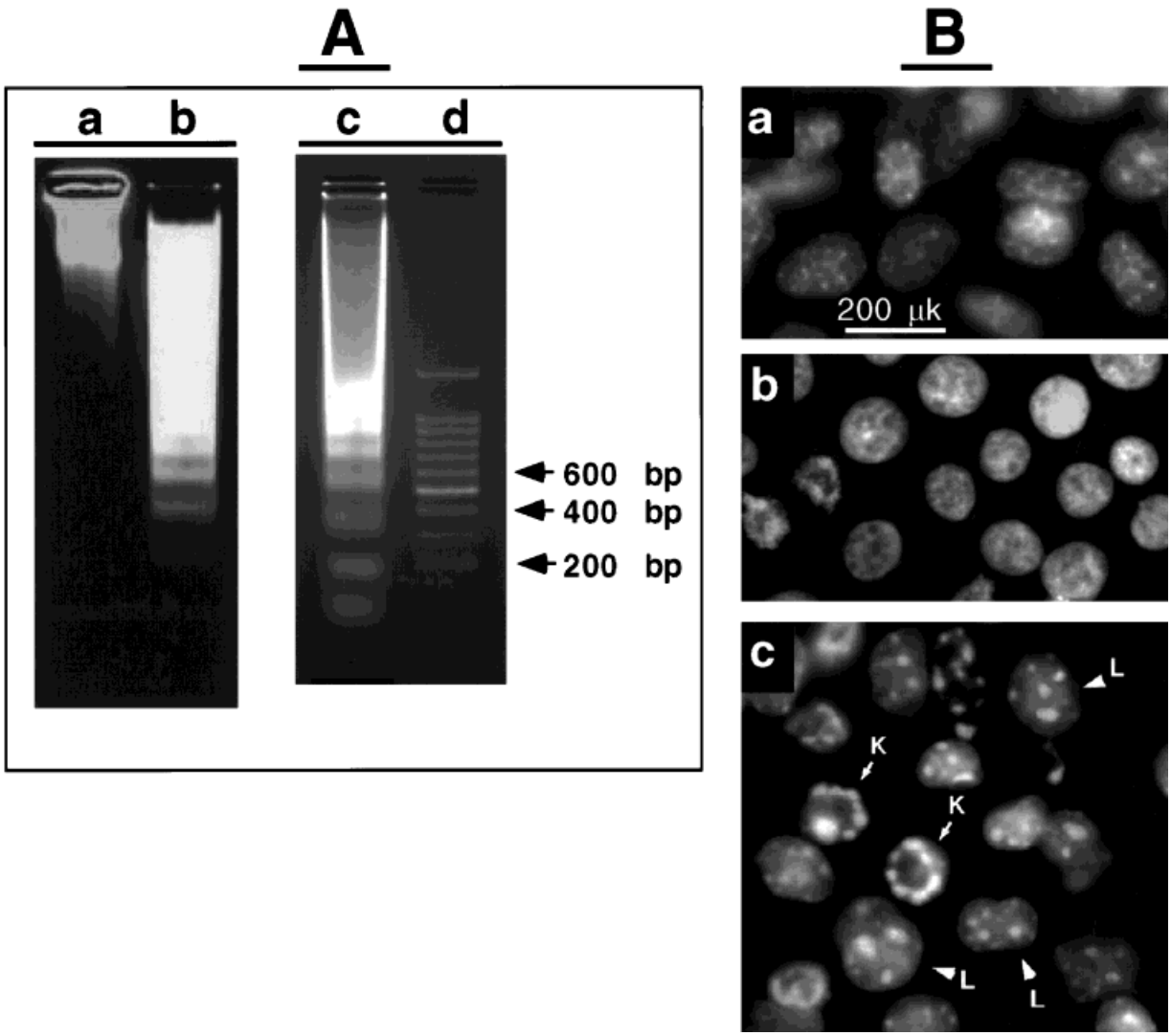

Fig. 7. Characteristics of apoptotic death of K562 and L929 cells upon their co-cultivation or incubation with cytotoxic membranes. **A:** Size distribution of DNA extracted from (Aa) control K562 cells (total DNA) and (Ab, Ac) from K562 cells incubated for 24 h with cytotoxic membranes of L929 cells. (Ab, total DNA; Ac, Triton X100 soluble DNA fragments; Ad, molecular-weights marker. **B:** DAPI staining of nuclei of (a) control L929 cells, (b) control K562 cells, and (c) L929 and K562 cells after co-cultivation for 48 h. Note apparition of large speckles ("apoptotic" nuclear bodies), especially spectacular in K562 cells (K, arrows), but also visible in L929 cells (L, arrowheads) along with the much smaller speckles also present in nuclei of control L929 cells.

words, our results suggest that individual non-lymphoid cells possess a molecular mechanism regulating cellular compatibility.

Cytolytic granules with enclosed perforin and certain proteolytic enzymes (granzymes) constitute the main weapon used by T lymphocytes to kill target cells at a distance (for a review, see [Trapani, 1998]. However, under certain conditions T lymphocytes may also excrete TNF-α and/or immunologically related proteins of higher molecular weight [Santis et al., 1992; Zychlinsky et al., 1990]. We never observed excretion of perforin by any of non-lymphoid cells tested in our experiments. The classical 17 kDa soluble form of the TNF-α seems to be the major, although not the sole, cytotoxic factor released into the medium by both K562 and L929 cells under conditions of co-cultivation [Gnuchev et al., 1997] (also this paper). We observed a certain similarity of the sizes of cytotoxic factors present in the medium and on cell membranes; the 53-kDa TNF-α-cross-

reactive protein was present in membranes of both K562 and L969 cells, as well as in the medium. Furthermore, there seems to be an equilibrium between soluble and membrane-bound cytotoxic factors. These observations suggest that soluble cytotoxic proteins may originate as a result of limited and site-specific proteolysis of membrane-bound cytotoxic factors, or by gradual release of membrane-bound factors into the medium.

To conclude, the main result of the present investigation is the demonstration of the ability of various nonlymphoid cultured cells to recognize and to kill cells of other types, using both membrane-bound and soluble cytotoxic factors including TNF-α and immunologically related proteins. The cytolytic processes mediated by nonlymphoid and lymphoid cells share some similarities (fast response, utilization of both membrane-bound and soluble cytotoxic factors) but differ significantly as far as the nature of the cytotoxic factors is concerned.

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