Target Cell-Induced Apoptosis of Interleukin-2-Activated Human Natural Killer Cells: Roles of Cell Surface Molecules and Intracellular Events

By Akira Yamamura, Kazuyuki Taga, Howard S. Mostowski, and Eda T. Bloom

We previously reported that natural killer (NK)-sensitive target cells, K562, kill interleukin-2–stimulated (lymphokine-activated killer [LAK]) but not unstimulated NK cells. We have now investigated the molecular basis of this phenomenon. Soluble monoclonal antibody (MoAb) to CD18 inhibited 75% of K562-induced DNA fragmentation and membrane disruption, whereas blocking MoAb to Fas partially inhibited only the DNA fragmentation. MoAbs to CD2, CD11a, CD11b, B7, or CD16 had limited or no effect on K562-induced death of LAK cells. Receptor ligation with either immobilized MoAb to CD18 or Fas induced membrane disruption and DNA degradation in LAK cells independently of K562, and MoAb to CD18, CD11a, or CD11b enhanced DNA fragmentation induced by anti-Fas. Fas-L–transfected Raji cells also killed LAK cells, but only if Fas-L expression was amplified. K562 cells rapidly triggered protein phosphorylation in LAK cells, and the tyrosine kinase inhibitor, Herbimycin A, inhibited DNA fragmentation and membrane disruption. Protease inhibitors strongly suppressed K562-mediated DNA fragmentation of LAK cells, but not membrane disruption. In conclusion, (1) K562-induced death of LAK cells involves primarily CD18, although other molecules, such as Fas, may also be involved; (2) K562-mediated apoptosis of LAK cells requires tyrosine phosphorylation and protease activity; (3) engagement of Fas by immobilized MoAb or Fas-L on target cells can also kill LAK cells; and (4) Fas-immobilized MoAb synergizes with coimmobilized MoAb to CD11a, CD11b, or CD18 for LAK cell killing. Activation-induced death of NK cells may represent a mechanism for NK cell regulation. This is a US government work. There are no restrictions on its use.

ACTIVATION-INDUCED apoptosis or programmed cell death, distinguished by certain morphologic characteristics and DNA disintegration, has been reported to regulate the function of antigen-stimulated T cells.1 Natural killer (NK) cells, in contrast, appear to recycle and function repeatedly after attacking and lysing target cells,2 and the fate of lymphokine-activated killer (LAK) cells after interaction with target cells has not been well studied. However, evidence exists that NK cells are indeed activated after interaction with target cells. For example, interaction with NK-sensitive target cells induces calcium influx and inositol phosphate generation3 and tyrosine phosphorylation4,5 in NK cells. Adhesion molecules, including members of the β2 integrin family, appear to be involved in at least some of the signaling pathways in the activation of NK cells. Sugie et al6 reported that K562 induces activation of protein tyrosine kinases (PTK) in human NK cells through the LFA-1 α and β subunits, and Umeheara et al7 reported phosphorylation of β2 integrin β chain (CD18) in NK cells in response to interleukin-2 (IL-2). Finally, we have recently reported that interaction with NK-sensitive target cells induces death by apoptosis in IL-2–activated NK or LAK cells, but not in native NK cells.8

Certain cell surface molecules, including Fas, have been implicated in the induction of apoptosis.9,10 Proteases have also been shown to induce apoptotic morphology11 and to be required for induction of activation-induced programmed cell death12,13 or apoptosis induced by cytolytic lymphocytes.14

We have now investigated the role of adhesion molecules in K562-induced death and DNA fragmentation of IL-2–activated NK cells, also known as LAK cells. We report that signaling through CD18 enables K562 to trigger membrane lysis and DNA fragmentation and that the response requires tyrosine phosphorylation and involves protease activities. We also identified the Fas–Fas-L interactions as another pathway resulting in death by apoptosis of LAK cells, but one that is probably less important in the K562-mediated effect.

MATERIALS AND METHODS

Reagents. Recombinant IL-2 (rIL-2) was provided by Amgen (Thousand Oaks, CA). Methyl-[3H]-thymidine, Na2[35Cr]O4, and 3P-orthophosphate were purchased from Dupont NEN (Boston, MA). Herbimycin A (HA), Leupetin, and phenylmethylsulfonyl fluoride (PMSF) were from Boehringer Mannheim (Indianapolis, IN). Antibodies and cDNAs. MoAbs to CD2 (TS2/18), CD5 (OKT-1), CD11a (TS1/22), CD11b (LM2/1), and CD18 (TS1/18) were produced from hybridomas (ATCC, Rockville, MD) as ascites in pristane-pretreated BALB/c mice and purified by precipitation in saturated (NH4)2SO4 and passage over Sepharose protein-G sepharose beads (Pharmacia, Piscataway, NJ). Purified antibodies to CD3 (OKT-3) and CD54 (ICAM-1) were obtained from Ortho Diagnostic Systems (Raritan, NJ). Purified antibody to B7-1, CD16, CD22 (Leu14), and fluorescence-tagged antibodies to CD3 (fluorescein isothiocyanate [FITC]), CD14 (FITC), CD16 (-FITC), CD20 (-FITC), and CD56 (-phycoerythrin [PE]) were obtained from Becton Dickinson (Mountain View, CA). Antibodies to B7-2 and tumor necrosis factor-α (TNF-α) were from Pharmingen (San Diego, CA) and Genzyme (Cambridge, MA), respectively. MoAbs (IgGl) to Fas antigen (M3 MoAb, which inhibits binding of Fas-L and induces apoptosis when immobilized, and M33 MoAb, which neither blocks ligand binding nor induces apoptosis11,12) and Fas-L cDNA were generously provided by Drs Mark Alderson and David Lynch (Immunix Research and Development Corp, Seattle, WA). The cDNA for glyceraldehyde-3-phosphate-dehydrogenase (GAPDH)13 was provided by Dr Dov Pluznik (Center for Biologies Evaluation Research, Bethesda, MD). Murine IgG1, IgG2a, and IgG2b were purchased from Sigma (St Louis, MO). In preliminary tests, antibodies were titrated for effect, and 10 μg/mL was selected for use in all subsequent experiments, unless otherwise indicated.

From the Division of Cellular and Gene Therapies (HFM-518) and the Division of Hematologic Products, Center for Biologies Evaluation Research, Food and Drug Administration, Bethesda, MD.

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Address reprint requests to Eda T. Bloom, PhD, Chief, Laboratory of Cellular Immunology (HFM-518), Division of Cellular and Gene Therapies, Center for Biologies Evaluation Research, Food and Drug Administration, 8800 Rockville Pike, Bethesda, MD 20892.

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Preparation of NK cells. NK cells were obtained as described previously with modification.17 Buffy coats, obtained from healthy donors, as well as the National Institutes of Health Blood Bank were centrifuged over ficoll-hypaque (LSM; Organon Teknika-Cappel, Durham, NC) to obtain mononuclear cells and then over 47% Percoll (Pharmacia) to deplete the high-density lymphocytes. Cells were then incubated on plastic dishes for 1 hour to deplete monocytes. The resulting NK-enriched population was incubated for 30 minutes on ice, with antibodies to CD5, CD22, and CD36 to further deplete T cells, B cells, and monocytes, respectively; incubated with goat antimouse IgG-coated magnetic beads (Advanced Magnetics Inc, Cambridge, MA) at 4°C for 30 minutes on ice (2 cycles); and exposed to a magnetic field. The unbound, negatively selected cells were stained with fluorescence-labeled antibodies to CD3 (T cells), CD14 (monocytes), CD20 (B cells), and CD16 and CD56 (NK cells) and fluorescence was assessed on a FACSscan (Becton Dickinson). NK cells (CD16+ and/or CD56+) were always 70% to 95% pure, as verified by flow cytometric analyses. These cells were preincubated with or without rIFN-γ (100 U/mL) for 60 hours in RPMI 1640 medium containing 10% heat-inactivated fetal calf serum (FCS), Hyclone, Logan, UT) and 50 μg/mL of Gentamicin (Biofluids, Rockville, MD) in 5% CO2 at 37°C.

Target cells. K562, an erythroleukemic cell line; U937, a histiocytic lymphoma cell line; and MOLT-4, established from a T-cell lymphoma, were used as NK-sensitive target cells. Daudi and Raji, established from B lymphomas, and UCLA-SO-M14 (M14), a hu- humane melanoma cell line,18 were used as NK-insensitive, LAK-sensitive cells. These cells were maintained in RPMI 1640 medium with 10% FCS and Gentamicin.

51Cr-release assays. The 51Cr-release assay is traditionally used to measure disruption of the plasma membrane.19 In a reverse cytoxicity assay,51Cr release from NK or LAK cells was measured to examine the lysis of NK or LAK cells induced by cells normally used as targets. After preculture with or without rIL-2 (100 U/mL) for 60 minutes in RPMI 1640 medium containing 10% heat-inactivated fetal calf serum (FCS), Hyclone, Logan, UT) and 50 μg/mL of Gentamicin (Biofluids, Rockville, MD) in 5% CO2 at 37°C.

Target cells. K562, an erythroleukemic cell line; U937, a histiocytic lymphoma cell line; and MOLT-4, established from a T-cell lymphoma, were used as NK-sensitive target cells. Daudi and Raji, established from B lymphomas, and UCLA-SO-M14 (M14), a human melanoma cell line,18 were used as NK-insensitive, LAK-sensitive cells. These cells were maintained in RPMI 1640 medium with 10% FCS and Gentamicin.

To determine if apoptosis of LAK cells induced by K562 is generalizable and therefore of potential biologic relevance, two additional NK-sensitive targets (U937 and MOLT-4) were compared with K562. The results showed a range of activities. U937 and MOLT-4 as well as K562 induced significant DNA fragmentation, whereas UCLA-SO-M14, Daudi, and

RESULTS

Apopotisis of LAK cells induced by K562 and other target cells. We previously reported that K562 cells induce lysis or membrane disruption (measured by 51Cr-release) and apoptosis (assessed by morphologic analyses and DNA retention) of IL-2–stimulated, but not native human NK cells.8 To determine if apoptosis of LAK cells induced by K562 is generalizable and therefore of potential biologic relevance, two additional NK-sensitive targets (U937 and MOLT-4) and three NK-insensitive (but LAK-sensitive) targets (UCLA-SO-M14, Daudi, and Raji) were compared with K562 for their ability to induce DNA fragmentation in IL-2–activated NK cells. The results showed a range of activities. U937 and MOLT-4 as well as K562 induced significant DNA fragmentation, whereas UCLA-SO-M14, Daudi, and
Raji exhibited decreased to no activity (Fig 1). None of the cells induced DNA fragmentation in native NK cells (data not shown), confirming our previous findings. These data suggest that molecules associated with NK and LAK sensitivity of target cells may be involved in target-cell-induced apoptosis of LAK cells.

Effects of antibodies to CD18, CD2, CD16, or Fas on K562-mediated induction of membrane disruption and DNA degradation in LAK cells. We examined the roles in K562-induced apoptosis of adhesion molecules, because they have been implicated in the IL-2-induced upregulation of NK lytic activity. In addition, we tested the involvement of Fas, because it triggers apoptosis in sensitive cells and is upregulated by IL-2 treatment of NK cells (from 10% ± 2% to 55% ± 11% in purified NK cells [85% to 90%] after 3 days of IL-2 treatment). As shown in Fig 2, 75% of both DNA fragmentation and membrane disruption was abrogated by anti-CD18, whereas MoAb to CD2 marginally reduced killing and blocking MoAb (M3, IgG1) to Fas inhibited 40% of DNA fragmentation but had a negligible effect on membrane disruption. Combinations of these antibodies did not inhibit K562-mediated killing beyond the effect of anti-CD18 alone, and antibodies to CD11a and CD11b also had no effect when combined, either in the presence or absence of K562 (data not shown). Although ligation of CD16 induces apoptosis of LAK cells and CD28/B7 interactions are involved in NK-like activities, neither antibodies to CD16 nor a combination of antibodies to B7-1 and B7-2 blocked the K562-induced apoptosis of LAK cells (Fig 2). These antibodies were functional because immobilized anti-CD16 lysed 15% of LAK cells and anti-B7-1 and -B7-2 completely blocked binding to CD28 (data not shown), in agreement with the results of previous reports. Because TNF-α kills cells by apoptosis and can be membrane-bound, the effect of neutralizing antibody to TNF-α (2 to 10 μg/mL) on K562-mediated killing of LAK cells was examined, but it failed to inhibit K562-mediated killing, suggesting that it is also not involved in the effect. These data suggest that, although target cells may induce membrane disruption of LAK cells and/or DNA disintegration through several cell surface receptors and pathways, including CD18, other adhesion molecules or Fas, CD18 was clearly the most dominant receptor among those tested.

That antibody to CD18, the common β chain of the β2 integrins, but not antibodies to the α chains of these integrins, LFA-1 (CD11a) and Mac-1 (CD11b), blocked the target-cell induced apoptosis of LAK cells further suggested that anti-CD18 inhibited apoptosis by preventing signaling rather than by steric interference. Antibodies immobilized on plastic (for use in the 3H-Tdr assay) or on Sepharose protein-G beads (for use in the 51Cr assay) were therefore tested directly for their ability to induce lysis and DNA degradation of LAK cells. The data showed that anti-CD18 induced DNA fragmentation and membrane disruption of LAK cells, but antibodies to CD11a, CD11b, or CD2 produced no effect beyond background (Fig 3), suggesting that signaling through CD18 is involved in K562-mediated killing of LAK cells.

Fas ligation can signal both DNA degradation and membrane disruption in LAK cells, although it has a paradoxical role in the K562-mediated effect. Because anti-Fas (M3) partially inhibited K562-induced DNA fragmentation but did not affect membrane disruption of LAK cells (Fig 2), and, paradoxically, Fas-L mRNA was below detectable limits in K562 by Northern blot analysis (Fig 4A), the role of Fas was investigated further by examining direct triggering by Fas-L and including antibodies to Fas in K562-mediated cytotoxicity assays. Raji cells, which have no apoptosis-
butyrate" (Fig 4A). Although untreated Fas-L-Raji induced Fas-L and expression was enhanced by treatment with Na butyrate in response to immobilized antibodies for measurement of 3H-TdR retention and plate-immobilized MoAb for assay of lysis. Isotype-matched IgGl was used as a control. Data are expressed as the mean ± SEM from three experiments. In soluble form, none of the antibodies had any effect on background 3H-TdR retention or 51Cr-release in the absence of K562.

inducing effect on LAK cells (Fig 1), were transfected with Fas-L and expression was enhanced by treatment with Na butyrate (Fig 4A). Although untreated Fas-L-Raji induced no detectable DNA fragmentation or membrane disruption of LAK cells, Na butyrate-treated cells markedly induced these effects (Fig 4B and C), suggesting that a threshold expression of Fas-L may be required for induction of apoptosis in LAK cells when the Fas-Fas-L pathway is the sole trigger for the killing. M3 (but not M33 control MoAb) dramatically inhibited DNA fragmentation and abrogated membrane disruption induced in LAK cells by butyrate-treated Fas-L-Raji, in contrast to those induced by K562, in which M3 partially inhibited the DNA fragmentation but had no effect on membrane disruption (Fig 4D and E). Also in contrast to K562, Fas-L-Raji cells induced lysis and DNA degradation in native NK cells, but to a reduced degree compared with LAK cells (data not shown). These data support the conclusion that Fas-L-Raji and K562 use different pathways for killing LAK cells.

In tests of receptor ligation by anti-Fas antibodies, plastic-immobilized M3, but not M33 control, induced both DNA fragmentation (45% ± 4%, by 3H-TdR retention) and membrane disruption (50% ± 3%, by 51Cr-release assay) of LAK cells. Finally, although M3 anti-Fas MoAb immobilized on Sepharose protein-G beads had only a marginal effect, when Fas was cotiled with CD18 or with CD11a or CD11b using antibodies coimmobilized on Sepharose protein-G beads, a synergistic induction of DNA fragmentation was observed (Fig 5). These data confirm that engagement of Fas can also induce apoptosis in LAK cells. They further suggest that a different mechanism(s) may be involved than that used when β2 integrin molecules are ligated and that coexpression of multiple membrane receptors may produce enhanced apoptosis of LAK cells.

PTK activation in K562-induced apoptosis of LAK cells. To understand the intracellular events after coculture of LAK cells with K562 cells, we first assessed whether protein kinases were activated. Whole cell lysates from 32P-orthophosphate-labeled LAK cells cocultured with K562 cells were resolved by SDS-PAGE and the results showed the induction of phosphoproteins of 42, 48, 50, and 90 kD within 1 minute (Fig 6). It is interesting that a phosphoprotein of about 92 kD disappeared, perhaps representing dephosphorylation and concomitant reduction in apparent molecular weight to 90 kD. We then precultured LAK cells with HA, a specific inhibitor of src family protein tyrosine kinases, at 0.3 μg/mL and 1 μg/mL in 0.1% dimethyl sulfoxide (DMSO) or DMSO alone (0.1%) for 20 hours to evaluate the contribution of this family of tyrosine kinases. After washing, LAK cells were radiolabeled and used in 51Cr-release and 3H-TdR retention assays. HA significantly reduced or abrogated membrane disruption and DNA degradation in LAK cells in a dose-dependent fashion, as compared with the DMSO control (Fig 7). These findings suggest that activation of src family protein tyrosine kinases is required for K562-mediated apoptosis of LAK cells.

Serine or cysteine proteases are involved in DNA fragmentation in apoptosis of LAK cells induced by K562. Because some apoptosis-inducing pathways have been reported to involve serine or cysteine protease activities,12,13 we examined the involvement of proteases in the K562-induced apoptosis of LAK cells. Leupeptin, an inhibitor of cysteine proteases or PMSF, an inhibitor of serine protease was added to the 4 hours of coculture of isotopically labeled LAK cells with K562 at concentrations of 200, 60, or 20 μmol/L. DNA fragmentation of LAK cells, as assessed by 3H-TdR retention, was significantly inhibited in a dose-dependent fashion by either leupeptin or PMSF, whereas, in contrast, leupeptin only marginally affected the 51Cr-release and PMSF had no effect (Table 1). These results indicate that the protease activity was required for the K562-mediated apoptosis of LAK cells.

DISCUSSION

Apoptosis has received a great deal of attention for its role in regulating the number of both immature and mature lymphocytes.9,38,39 Immature T cells undergo negative selection in the thymus through apoptosis,40 and activation-dependent apoptosis of differentiated antigen-specific CD4+ T cells regulates functional expression of antigen-specific mature T cells.1,41 In contrast to T cells, NK cells do not usually recognize targets cells in a strictly antigen-specific fashion, thereby presumably enabling a first line of defense quickly and without antigen presentation. Moreover, they appear to recycle their lytic activity after lysing sensitive target cells.2 However, similar to T cells, NK cells can be activated by target cells,4,6 and our earlier data have suggested that this activation may result in death of a proportion of IL-2-activated NK (LAK) cells, but not native NK cells, with the characteristics of apoptosis,8 a phenomenon that may be analogous to the activation-induced cell death of mature T

![Graph](https://example.com/graph.png)
TARGET CELL-INDUCED APOPTOSIS OF LAK CELLS

Fig 4. Effect of Fas-L transfection on ability of target cells to induce apoptosis of LAK cells. (A) Analysis by Northern blot showed low expression of Fas-L mRNA by purified peripheral T cells (lane 1) and by Fas-L-Raji (lane 2), dramatically increased expression by Fas-L-Raji after butyrate treatment (lane 3), and very low or undetectable Fas-L mRNA expression by Raji control transfectant (lane 4) or control transfectants treated with butyrate (lane 5) or K562 (lane 6). Probing the same membrane with the cDNA for GAPDH verified that differential RNA loading did not account for the increased Fas-L in lane 3. (B and C) Butyrate-treated Fas-L-Raji (■) and K562 (□), but not untreated Fas-L-Raji (□), control transfectant (△), or control transfectant treated with butyrate (▲) induced DNA degradation ([B] ³²P-TdR retention) or membrane disruption ([C] ¹¹Cr-release). Data are the percentages at titrated target:effector cell ratios (T:E; eg, K562:LAK or Fas-L-Raji:LAK) and are from one experiment representative of three. (D and E) M3 anti-Fas dramatically reduced DNA fragmentation ([D] ³²P-TdR retention; □ and membrane disruption ([E] ¹¹Cr-release; □) mediated by Fas-L-Raji in which expression of Fas-L was enhanced by sodium butyrate. It also reduced DNA fragmentation mediated by K562 (□), but had no effect on membrane disruption mediated by K562 (□). Values are expressed as the mean ± SD of quadruplicate samples. Data are from one experiment representative of experiments using LAK cells from two different subjects.

Our data showed that the ability of target cells to induce apoptosis in LAK cells was associated with their NK sensitivity in standard ¹¹Cr-release assays, suggesting that cell surface components may be important for the death by apoptosis of LAK cells induced by target cells. Because we and many others have shown that β₂ leukocyte integrins are...
involved in signaling pathways in NK cells and are activated in IL-2–stimulated NK cells, we examined the involvement of LFA-1 and Mac-1 in the converse phenomenon, K562-induced killing of LAK cells. Antibody to CD18, the common β chain of these integrins, strongly inhibited both lysis of LAK cells (as indicated by 51Cr-release) and DNA fragmentation (as indicated by 'H-TdR retention). Antibodies to CD11a and CD11b, two α chains used by the β2 integrins, had no effect. Together with the finding that immobilized antibodies to CD18 induced membrane disruption and DNA fragmentation in LAK cells directly, this suggested that anti-CD18 blocked signaling through CD18, rather than simply interfering through steric hinderance with the engagement of receptor by ligand on target cells. Our experiments did not encompass an exhaustive test of the involvement of all integrins or adhesion molecules; thus, it is possible that integrins may be a model for the involvement of other such adhesion molecules in the membrane disruption of LAK cells induced by target cells.

The involvement of Fas in K562-induced death by apoptosis of LAK cells is more difficult to resolve. Engagement of Fas by either plastic-immobilized antibody (M3) or butyrate-treated Fas-L-Raji cells transduced strong signals through Fas inducing membrane disruption and DNA degradation in LAK cells, and both the membrane disruption and DNA degradation induced by Fas-L-Raji were blocked by antibodies to Fas, showing that triggering through Fas clearly can transmit an apoptotic signal. In contrast, engagement of Fas by M3 immobilized on protein-G Sepharose beads or by unenhanced Fas-L-Raji induced only weak or undetectable apoptosis. However, when M3 was coinmobilized with anti-

Fig 5. Synergistic effect of Sepharose protein-G–immobilized antibodies to Fas (M3) and β2 integrins (MoAb to CD11a, CD11b, or CD18) in the induction of DNA fragmentation in LAK cells. Data are the percentage of DNA fragmentation measured by 'H-TdR retention and are the average ± SEM from experiments on LAK cells from five different donors. Isotype-matched IgG1 was used as a control. For the experiment shown here, Fas was expressed on 44% of LAK cells as determined by flow cytometric analysis. In these same experiments, K562 (ratio of 3:1) induced 13.2% ± 4.8% DNA fragmentation.

CD11a (LFA-1) or CD11b (Mac-1) on protein-G sepharose beads, a synergistic signal was transduced such that similar levels of LAK cell apoptosis were induced as by K562. Therefore, although anti-Fas failed to block K562-mediated membrane disruption and Fas-L mRNA was below detectable levels in K562 cells, we cannot exclude a role for Fas-mediated intracellular signaling in the K562 effect. The possibility of interaction between cell surface molecules such as integrins and Fas in the induction of apoptosis of LAK cells may explain why anti-Fas partially inhibited K562-induced DNA degradation in LAK cells, although we could detect no effect on membrane disruption.

Recently, Ortaldo et al12 and Azzoni et al33 reported that apoptosis of IL-2–activated NK cells could be induced by triggering through FcγRIII (CD16). We confirmed that DNA
degradation and membrane disruption of LAK cells was mediated by immobilized anti-CD16 MoAb; however, anti-CD16 did not inhibit 51Cr-release or the DNA fragmentation induced in LAK cells by K562 (data not shown), indicating CD16 is not involved in the apoptosis of LAK cells induced by K562. Antibody to ICAM-1 (CD54) blocked K562-induced DNA fragmentation, but immobilized anti–ICAM-1 itself had no effect on LAK cells (data not shown), suggesting that ICAM-1 is involved as ligand on K562 for the β2 integrins CD11a/CD18 (LFA-1) or CD11b/CD18 (Mac-1) on LAK cells. It is of further interest that soluble MoAb to CD11a or CD11b, in contrast to MoAb to CD18, only marginally blocked the K562-induced apoptosis of LAK cells, suggesting that heterodimers of CD18 with a CD1 la or CD1 lb are involved in ligand-binding for subsequent induction of apoptosis of LAK cells by K562, but that signalling through CD18 is required. These data also suggest that several surface receptors on target cells may be capable, under appropriate conditions, of inducing death by apoptosis in IL-2–activated NK cells.

Signaling through integrins induces activation of tyrosine kinases in NK cells,6,42 and K562 cells have been shown to activate LFA-1–associated PTKs in NK cells after coculture.5 We therefore investigated the involvement of PTK in the K562-induced apoptosis of LAK cells and found (1) that K562 induced newly phosphorylated proteins in LAK cells within 1 minute and (2) that apoptosis of LAK cells induced by K562 was abrogated by preculture of LAK cells with HA. These results suggested that activation of endogenous PTK in LAK cells is required for K562-induced apoptosis. However, from our data, we cannot exclude the additional participation of other PTK or of ser/thr kinases.

Because proteases can induce apoptotic morphology,11 are required for induction of activation-induced programmed cell death,12,13 and are important, if not required, for the induction of apoptosis induced by cytolytic cells using the granule exocytosis/perforin pathway,14,50-52 we investigated their involvement in the K562-induced apoptosis of LAK cells. In the current system, K562-mediated apoptosis of LAK cells was abrogated by cysteine- or serine-protease inhibitors in the 4 hours coculture, supporting the role of proteases in the induction of apoptosis. Voelkel-Johnson et al53 have reported that TNF-α–induced apoptosis involves protease activity. However, neutralizing antibody to TNF-α had no effect on the K562-induced DNA fragmentation, indicating that TNF-α is not involved in the K562-induced effect on LAK cells.

In summary, we have investigated the K562-induced death of IL-2–activated NK cells. This activation-associated cell death involves membrane disruption and DNA disintegration of LAK cells induced by NK-sensitive targets. The mechanism involves signaling through integrins and requires src family PTK and protease activities. Engagement of other cell surface molecules, such as Fas, may also trigger LAK cell death by apoptosis under the appropriate conditions. Killing of IL-2–activated NK cells exhibits similarities with other activation-induced apoptosis systems, suggesting that the pathways delineated here may be relevant to other forms of ligand-mediated apoptosis and may represent a mechanism for NK cell regulation.

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Table 1. Protease Activity Is Involved in the K562-Induced DNA Disintegration But Not Membrane Disruption of LAK Cells

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Concentration (μmol/L)</th>
<th>DNA Fragmentation (% H-TdR retention)</th>
<th>Cell Membrane Disruption (% 51Cr-release)</th>
</tr>
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<tr>
<td>Leupeptin</td>
<td>200</td>
<td>71 ± 14</td>
<td>16 ± 6</td>
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<td></td>
<td>60</td>
<td>59 ± 5</td>
<td>10 ± 4</td>
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<td>20</td>
<td>21 ± 11</td>
<td>14 ± 2</td>
</tr>
<tr>
<td>PMSF</td>
<td>200</td>
<td>40 ± 5</td>
<td>–2 ± 4</td>
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<tr>
<td></td>
<td>60</td>
<td>42 ± 4</td>
<td>–8 ± 2</td>
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<tr>
<td></td>
<td>20</td>
<td>23 ± 2</td>
<td>–2 ± 10</td>
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* The percentage of inhibition of each assay was calculated as 100 × (% DNA Fragmentation or % Lysis Without Protease Inhibitor – % DNA Fragmentation or % Lysis With Protease Inhibitor)/(% DNA Fragmentation or % Lysis Without Protease Inhibitor). Data are displayed as means of three experiments. Killing of LAK cells by K562 in the absence of inhibitors was 17.7 ± 1.2% lysis by 51Cr-release and 15.0% ± 3.2% DNA fragmentation by 3H-TdR retention assays (mean ± SEM for the 3 experiments).

Fig 7. HA inhibits K562-induced-DNA fragmentation (■; 3H-TdR retention) and LAK cell membrane disruption (□; 51Cr-release) of LAK cells. Killing of LAK cells by K562 in the absence of inhibitors was 18.8 ± 2.5% by 51Cr-release and 17.8 ± 4.5% by 3H-TdR retention. Data are from one experiment representative of two independent experiments, and values are expressed as the mean ± SD of triplicate samples.
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Target cell-induced apoptosis of interleukin-2-activated human natural killer cells: roles of cell surface molecules and intracellular events

A Yamauchi, K Taga, HS Mostowski and ET Bloom