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The role of apoptosis in antibody-dependent cellular cytotoxicity

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Abstract. Apoptosis in three lymphoma cell lines has been studied following cytotoxicity induced in vitro by normal human blood lymphocytes utilizing either natural killer (NK) or antibody-dependent cellular cytotoxic (ADCC) mechanisms. Guinea-pig L_2C leukaemic lymphocytes, but not the human cell lines Daudi and Jurkat, revealed a degree of time- and temperature-dependent apoptotic death upon simple culture in vitro. NK cytotoxicity at low effector:target ratios (E:T) induced both release of ^{51}Cr and apoptosis. However NK cytotoxicity at higher E : T, and ADCC at all E : T, increased the level of ^{51}Cr release while reducing the level of apoptosis. The findings were consistent with the apoptotic process being cut short by intervention of necrotic death. The same characteristics accompanied ADCC whether the effectors were recruited by Fcγ regions of antibody coating the targets, or by bispecific antibodies attaching one arm to the targets and the other to Fcγ receptors type III on effectors. This finding, and the high level of cytotoxicity elicited by the bispecific method, confirm the belief that NK cells, in addition to exerting NK cytotoxicity, represent the principal effectors for ADCC among blood mononuclear cells. Our results suggest that NK cells have both apoptotic and necrotic mechanisms available for killing their targets, but use only the latter for ADCC.

Key words: Apoptosis – DNA fragmentation – Cellular cytotoxicity – NK cells

Introduction

Two major modes of cell death are currently recognized in nucleated eukaryotic cells, necrosis and apoptosis [15, 26]. These differ both morphologically and biochemically, although there is some evidence that the dichotomy might not always be clear [13]. Apoptosis involves active participation by the target cell in sequential metabolic events, and because of this is often termed "programmed cell death." It has been observed during embryogenesis, metamorphosis, and normal tissue turnover, including the death of large numbers of thymocytes [27]. The immune system has been reported to use both mechanisms of killing against a variety of targets [4, 29].

Characteristics of apoptosis include the breaking of nuclear DNA into fragments that are multiples of subunits containing 180–200 base pairs [27]. This results from the activation of an endogenous Ca^{2+}/Mg^{2+}-dependent endonuclease, which cleaves the DNA between the nucleosomes [4]. The result of the apoptotic process is the formation of membrane-intact apoptotic bodies, which may contain segments of the nucleus and/or intact mitochondria. During the formation of these bodies new epitopes appear on the surface membrane that allow recognition and phagocytosis by macrophages or, in the case of some tumours, by adjacent tumour cells [7]. The engulfed bodies undergo secondary necrosis within the phagocyte. Under conditions in vitro these bodies may escape phagocytosis and remain clearly visible.

Cells attacked by complement do not exhibit apoptosis, their mode of death being necrotic [4]. Perforin, which is similar in structure to the C9 component of the membrane-attack complex of complement, can be found in the cytolytic granules of cytotoxic T lymphocytes (CTL) and natural killer (NK) cells, and also appears not to induce apoptosis [6, 30]. However, lymphotoxin and tumour necrosis factor α (TNFα) have been reported to induce apoptotic death in susceptible target cells [22], as has a protein found in NK cell granules [23]. There has been much debate on the extent to which each of these cytotoxic molecules is used by cytotoxic effector cells, and therefore which mode

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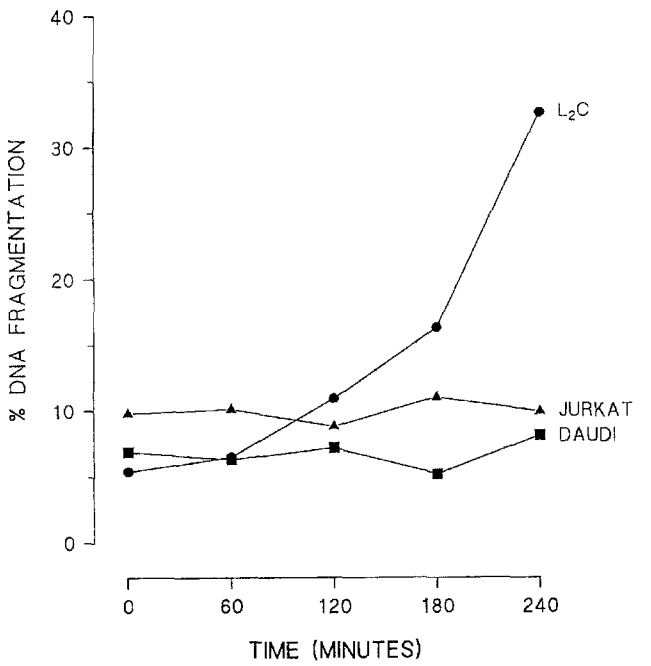

Fig. 1. Effect of in vitro culture on the percentage DNA fragmentation of L_2C, Daudi, and Jurkat target cells. Target cells were cultured at 5×10^6/ml in complete medium at 37° C for the indicated period. Following electrophoresis of ^{125}I-labelled DNA (1% agarose gel, 89 mM TRIS/borate, pH 8.4, 35 V, 15 h) the percentage DNA fragmentation was calculated by counting gel zones corresponding to intact and fragmented DNA

of cell death cytotoxic cells are apt to induce [2, 17]. CTL have been reported to induce both necrosis and apoptosis [31]. Both NK cells and their lymphokine-activated killer (LAK) derivatives have also been reported to induce apoptosis during antibody-independent killing [5]. However, in contrast to antibody-independent killing by CTL and NK cells, there is little information on the role of apoptosis during antibody-dependent cellular cytotoxicity (ADCC). One report has demonstrated that Chang liver targets undergo apoptosis during ADCC, but this has not been confirmed for other systems [24].

In this report we describe experiments that suggest that apoptosis is not the major mechanism of cell death in ADCC. We confirmed that antibody-independent NK killing can be accompanied by apoptosis in susceptible target cells, but at high effector-to-target ratios the degree of apoptosis declined. When antibody was available on the target-cell surfaces to recruit the NK cells for ADCC, the degree of cytotoxicity, as measured by release of ^{51}Cr, was greater; however, a decrease in apoptosis was detected.

Materials and methods

Antibodies. Hybridoma cell lines producing the monoclonal antibodies (mAb) 3G8 (anti-FcγRIII [9]), RJD 2A10 (anti-idiotype to the sIgM of L_2C guinea-pig leukaemic cells, produced in the laboratory [8]), and WR17 (anti-CD37 [21]) were expanded as ascitic tumours in pristane-primed Balb/c × CBA(F1) mice. Polyspecific antisera to human tonsil and L_2C cells were raised in rabbits using the protocol described by Stevenson et al. [25]. IgG from rabbit antisera and mouse ascitic fluid was purified by ammonium sulphate precipitation and ion-exchange chromatography as described by Elliott et al. [8]. F(ab′γ)$_2$ fragments of mAb were prepared by pepsin digestion at pH 4.1 according to the method of Lamoyi and Nisonoff [18], and passed through an immunosorbent column of sheep anti-(mouse Fcγ) to remove any possible contaminating IgG.

Bispecific F(ab′γ)$_2$ derivatives were prepared as described previously [10]. Briefly, F(ab′γ)$_2$ from the two chosen mAb were first reduced to provide Fab′γ with free hinge-region SH groups (Fab′γ-SH). The SH groups on one of the Fab′γ-SH were alkylated with excess *o*-phenylenedimaleimide (Sigma Chemicals, Poole, UK), providing free maleimide groups (Fab′γ-Mal). Finally the Fab′γ-SH and Fab′γ-Mal species were allowed to react at a ratio of 1 : 1 under conditions allowing cross-linking of SH and maleimide groups whilst avoiding oxidation of SH groups. The final products were reduced and alkylated, removing any homodimers and preventing further disulphide interchange, before fractionation on Ultrogel AcA44 (LKB).

Target cells. The human cell lines Daudi (B lymphoblastoid, Flow Laboratories [16]) and Jurkat were cultured in RPMI-1640 medium (Gibco, Paisley, UK) supplemented with L-glutamine (2 mM)n sodium pyruvate (1 mM), streptomycin and penicillin (100 IU/ml), and 25 mM HEPES plus 10% Myoclone (Gibco). Cells were removed and washed once before being resuspended at 10^7/ml in RPMI-1640 medium (supplemented as above) plus 10% heat-inactivated Myoclone (complete medium). L_2C leukaemic cells were isolated from leukaemic guinea-pigs with a white blood cell count of more than 50 000/μl. The blood was layered onto an equal volume of Lymphoprep (Nycomed, Oslo, Norway) and centrifuged at 800 *g* for 20 min. The lymphocyte band was removed from the interface was washed twice with RPMI-1640 medium.

Effector cells. Blood from normal volunteers, collected into preservative-free citrate, was prepared as for L_2C cells. Peripheral blood lymphocytes (PBL) isolated as the mononuclear cell fraction were resuspended at 10^7/ml in complete medium following washing in RPMI-1640 medium.

^{51}Cr-release cytotoxicity assay. A 1-ml sample of target cells at 10^7/ml in RPMI-1640 medium (without fetal calf serum) was incubated with 100 μl $Na_2{}^{51}CrO_4$ (Amersham, UK) for 30 min at 37° C, washed four times in RPMI-1640 medium and resuspended at 2×10^5/ml in complete medium. Cytotoxicity was measured by a standard ^{51}Cr-release assay. Each well of a 96-well, U-bottom, microculture plate (Nunc, Intermed, Denmark) received 50 μl antibody diluted with complete medium (or medium alone for NK activity), followed by 10^4 ^{51}Cr-labelled target cells. These were left to incubate for 15 min at 4° C before addition of $(1-20) \times 10^5$ effector cells (total volume 200 μl). Cell contact was initiated by centrifugation (100 *g*, 5 min) before incubation at 37° C (humidified, 5% CO_2) for 3.5 h. The cells were sedimented at 300 *g* for 5 min and 100-μl aliquots of supernatant removed for counting on a gamma scintillation counter (Rackgamma II, LKB) to assess the release of ^{51}Cr from target cells. All dilution points were assayed in triplicate and mean values determined. Percentage-specific ^{51}Cr release was calculated by the usual method [14], using 1% Nonidet P40 (NP40, BDH) detergent lysis to represent maximal release.

DNA fragmentation. The DNA of target cells was labelled with ^{125}I by incubation at 10^7/ml in RPMI-1640 medium containing 50 μCi/ml [5-^{125}I]iodo-2′-deoxyuridine (Amersham) for 30 min at 37° C, washed four times with RPMI-1640 medium (300 *g*, 5 min), and resuspended at 10^7/ml in complete medium. Cytotoxicity assays were performed as for ^{51}Cr-release assays except for the use of 5×10^4 target cells. Following the appropriate treatment of the target cells, the cell suspension was pelleted (500 *g*, 1 min).

Cell pellets were mixed with 20 μl 10 mM EDTA, 50 mM TRIS/HCl pH 8.0, 0.5% sodium dodecyl sarkosinate (Sigma), and 0.5 mg/ml proteinase K (Sigma), and incubated for 1 h at 50° C. A 10-μl sample of 0.5 mg/ml RNase A (Sigma) was added and the solution was incubated for a further 1 h at 50° C. The temperature was increased to 70° C for addition of 10 μl 10 mM EDTA pH 8.0, 1% low-melting-temperature agarose, 0.25% bromophenol blue, and 40% sucrose. Samples were

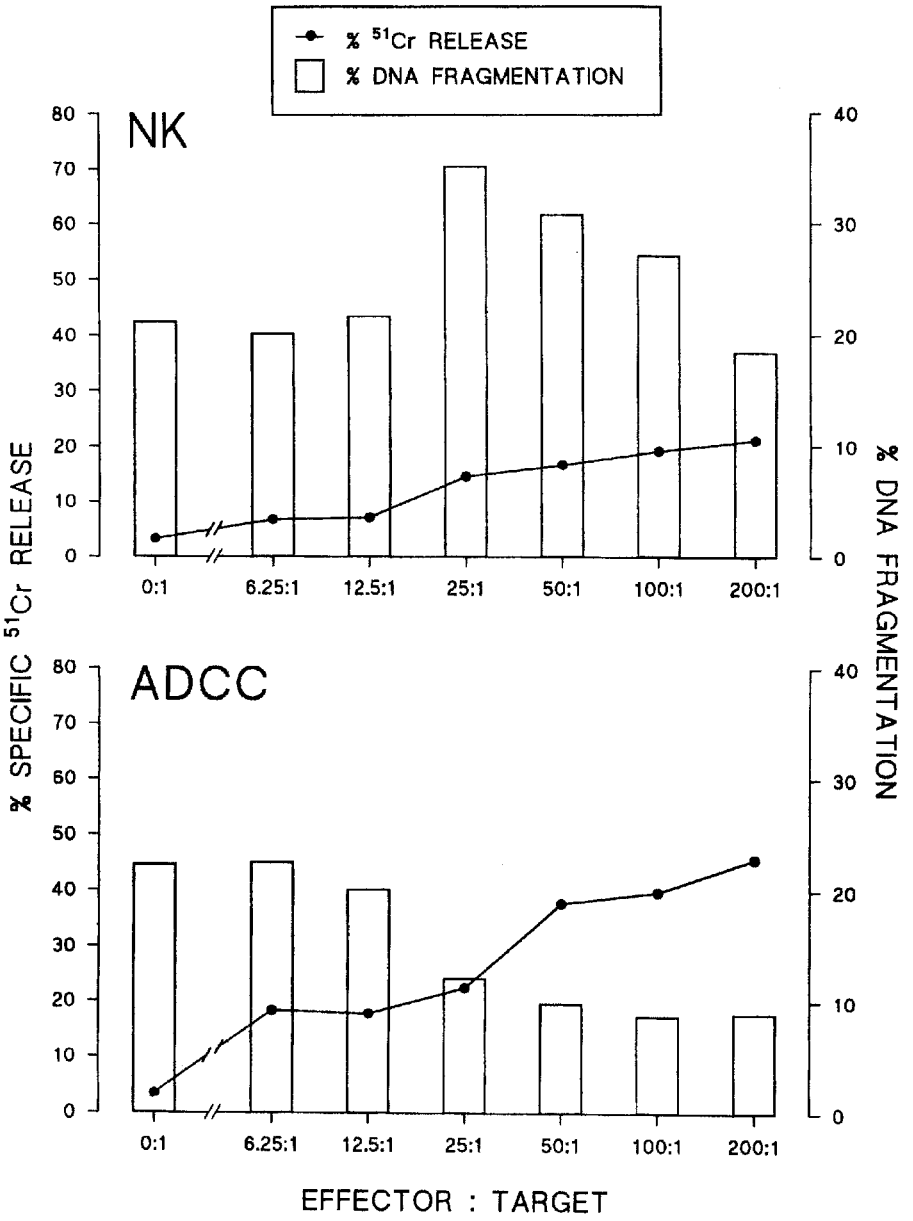

Fig. 2. Comparison of DNA fragmentation and ^{51}Cr release from L_2C target cells following natural killer cell (NK) killing and antibody-dependent cellular cytotoxicity (ADCC). ^{125}I- or ^{51}Cr-labelled L_2C cells were incubated with human peripheral blood lymphocytes (PBL) at the indicated ratio for 3.5 h with (ADCC) or without (NK) rabbit polyspecific anti-L_2C IgG at 1 μg/ml. Following electrophoresis of ^{125}I-labelled DNA (1% agarose gel, 89 mM TRIS/borate, pH 8.4, 35 V, 15 h) the percentage DNA fragmentation was calculated after cutting the gel into intact and fragmented DNA zones. The percentage specific ^{51}Cr release was calculated using L_2C cells without effectors for background ^{51}Cr release, and cells lysed with 1% NP40 for maximal release. Results for ^{51}Cr release are means of triplicates. Results are representative of four experiments using three different donors. The ranges are given in Table 1

loaded into dry wells of a 1% agarose gel and run at 30–40 V overnight in 89 mM boric acid, 89 mM TRIS (Sigma), 2 mM EDTA, pH 8.4. For samples where the DNA was not labelled with ^{125}I, ethidium bromide was added to the gel to 0.5 μg/ml prior to electrophoresis. Gels containing ethidium bromide were visualised under UV light. ^{125}I-labelled gels were dried at room temperature under vacuum and, where appropriate, were exposed to autoradiographic film (Hyperfilm-MP, Amersham) at –70° C. For calculation of the percentage DNA fragmentation dried gels were segmented into individual lanes, and each lane divided into high-molecular-mass and fragmented DNA sections (the DNA was visualised either on the autoradiograph or under UV light). The samples were counted for ^{125}I content using a Rackgamma II, and the percentage DNA fragmentation calculated as:

$$\frac{\text{fragmented DNA (cpm)}}{\text{high-molecular-mass DNA + fragmented DNA (cpm)}} \times 100\%$$

Results

Cultured target cells

Some fragmentation of the DNA of L_2C guinea-pig leukaemic cells was observed following culture in vitro at 37° C, yielding a "ladder" of fragments upon gel electrophoresis. The experiment depicted in Fig. 1 shows that after a 4-h culture at 37° C approximately 35% of the DNA of L_2C cells had fragmented; the range in different experiments was 19%–38% (six experiments). The DNA from Daudi and Jurkat target cells failed to undergo any significant fragmentation under identical culture conditions (Fig. 1, range Daudi 4%–10%, Jurkat 7%–13%, four experiments). Micrococcal nuclease digests of Daudi and Jurkat cells confirmed that DNA fragmentation could be detected in these cells. The nuclear morphology of L_2C cells cultured for 4 h at 37° C showed typical features of

Table 1. Effect of natural killer cell (NK) cytotoxicity and antibody-dependent cellular cytotoxicity (ADCC)[a] on the ^{51}Cr release and DNA fragmentation of L_2C, Jurkat, and Daudi target cells

Target cell	Specific ^{51}Cr release[b] (%)		DNA fragmentation[c] (%)	
	NK	ADCC	NK	ADCC
L_2C	15 (10–21)[d]	23 (19–37)	35 (23–45)	12 (6–18)
Jurkat	12 (6–16)	30 (15–36)	38 (25–38)	11 (7–18)
Daudi	4 (0–4)	53 (31–53)	13 (9–13)	11 (10–15)

[a] ^{125}I- or ^{51}Cr-labelled target cells were incubated with human peripheral blood lymphocytes (E:T, 25:1) for 3.5 h with (ADCC) or without (NK) 1 μg/ml rabbit anti-L_2C or anti-(human tonsil cells)

[b] Percentage specific ^{51}Cr release was calculated using target cells without effectors as background ^{51}Cr release, and 1% NP40 lysis as maximal release. Results for ^{51}Cr release are represented as means of triplicates

[c] Following electrophoresis of ^{125}I-labelled DNA (1% agarose gel, 89 mM TRIS/borate, pH 8.4, 35 V, 15 h) the percentage DNA fragmentation was calculated by cutting the gel into intact and fragmented DNA zones

[d] Ranges from at least three experiments are given in parentheses

apoptotic cell death, including cellular condensation and nuclear fragmentation. This was not observed with cells cultured at 4° C, which maintained a normal morphology. Daudi and Jurkat target cells failed to show any abnormal nuclear morphology when cultured at 37° C under identical conditions.

Effect of NK cytotoxicity and ADCC on apoptosis of target cells

Addition of human PBL to the in vitro culture of L_2C target cells resulted in a degree of antibody-independent NK cytotoxicity as measured by the release of ^{51}Cr from the target cells. The specific ^{51}Cr release was shown to increase with an increasing effector:target ratio (E:T), resulting in 21% ^{51}Cr release at an E:T of 200:1 (Fig. 2). Measurement of the percentage DNA fragmentation under identical conditions indicated that at higher E:T the percentage DNA fragmentation declined whilst the release of ^{51}Cr continued to increase. Each cytotoxicity result shown is for a single donor, though a panel of five different donors was used, and each experiment was repeated at least three times. Variation in ^{51}Cr release and DNA fragmentation between different donors was significant, though the same pattern was always observed.

Addition of the polyspecific rabbit anti-L_2C antibody to L_2C cells, and of human PBL as effectors, resulted in ADCC of the targets. The release of ^{51}Cr was observed to be much greater than that induced by NK cytotoxicity at the same E:T (Fig. 2). Again the release of ^{51}Cr increased with an increasing E:T. However, the percentage DNA fragmentation was shown to decrease with an increasing E:T, so that at the higher E:T DNA fragmentation was lower than the spontaneous level in the absence of cellular effectors. Therefore, ADCC at the higher E:T induced greater release of ^{51}Cr but inhibited or cut short the spontaneous apoptosis of the target cells. At an E:T of 25:1, target cells subjected to NK cytotoxicity showed morphological changes of apoptosis but no such morphology was seen to accompany ADCC.

Similar results were obtained for the Jurkat cell line: NK cytotoxicity was accompanied by ^{51}Cr release and DNA fragmentation at lower E:T, but DNA fragmentation declined at higher E:T. ADCC induced higher ^{51}Cr release but less DNA fragmentation (Table 1). The induction of apoptosis by NK cytotoxicity but not by ADCC was confirmed by a study of the nuclear morphology. Daudi target cells were shown to be more resistant to NK cytotoxicity, with only 10% ^{51}Cr release at an E:T of 200:1. Measurement of the DNA fragmentation showed no significant increase with increasing E:T. ADCC increased the release of ^{51}Cr but did not alter the percentage DNA fragmentation (Table 1). The absence of apoptosis in the Daudi targets following NK cytotoxicity and ADCC was confirmed by study of the nuclear morphology.

The experiments described above used a single antibody concentration whilst varying the E:T. To confirm that the effects observed were due to differences in cytotoxic mechanisms, and not to the variable E:T directly (say from feeder effects), ADCC experiments were performed using a single E:T (25:1) whilst the antibody concentration was varied. The results confirmed those described above: increasing the antibody concentration increased the release of ^{51}Cr from the L_2C target cells, but inhibited the spontaneous DNA fragmentation (Fig. 3). Initial experiments had determined that the antibody alone did not significantly affect the ^{51}Cr release or percentage DNA fragmentation.

The polyspecific antibodies used for studies of ADCC were directed against multiple molecules on the target cells and may have recruited more than one type of FcR-bearing effector cell for cytotoxicity. In order to study a more precise system bispecific $F(ab'\gamma)_2$ antibodies were used, recruiting NK cells via FcγRIII to tumour targets via sIgM on L_2C and CD37 on Daudi cells [11]. Use of these bispecific $F(ab'\gamma)_2$ antibodies resulted in redirected cellular cytotoxicity (RCC) as measured by the release of ^{51}Cr from target cells. The ^{51}Cr release from L_2C targets increased at higher antibody concentrations (Fig. 3). Measurement of the percentage DNA fragmentation revealed a similar pattern to that induced by ADCC, with a reduction in DNA fragmentation as the ^{51}Cr release increased. The extent of ^{51}Cr release was greater (60%) than that induced by ADCC (45%), as was the inhibition of spontaneous DNA fragmentation: this was reduced to just 5% at the highest concentration of antibody, compared with 10% for ADCC and a spontaneous level of 31%. Although the extent of DNA fragmentation induced by ADCC and RCC is significantly different, a comparison at the same level of ^{51}Cr release reveals a similar level of DNA fragmentation. A similar pattern of results was observed with Daudi target cells.

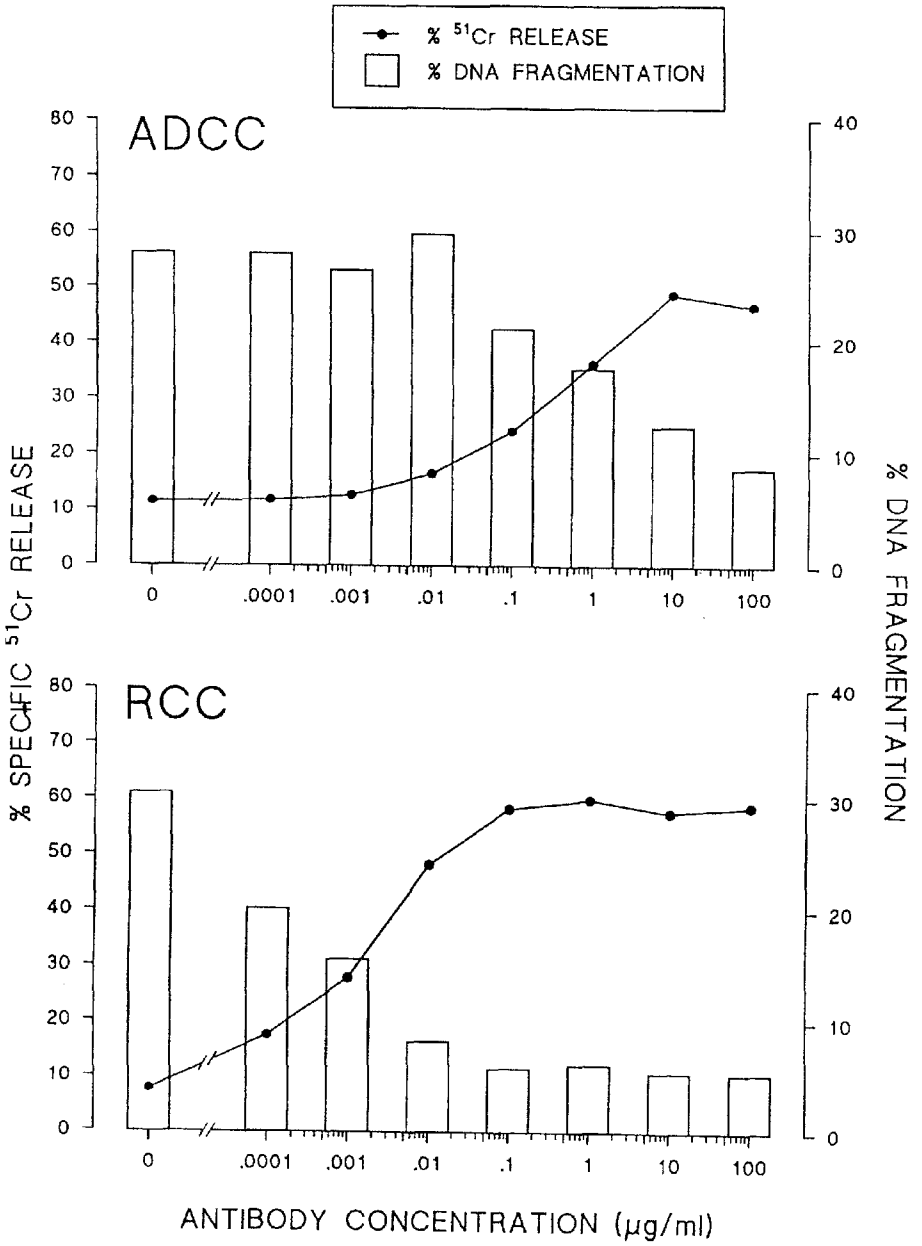

Fig. 3. Comparison of DNA fragmentation and ^{51}Cr release from L_2C target cells following ADCC and redirected cellular cytotoxicity (RCC). ^{125}I- or ^{51}Cr-labelled L_2C cells were incubated with human PBL (PBL : L_2C, 25 : 1) for 3.5 h in the presence of either rabbit anti-L_2C IgG (ADCC), or bispecific F(ab′γ)$_2$ anti-idiotype × anti-FcγRIII (RCC). Following electrophoresis of ^{125}I-labelled DNA (1% agarose gel, 89 mM TRIS/borate, pH 8.4, 35 V, 15 h) the percentage DNA fragmentation was calculated after cutting the gel into intact and fragmented DNA zones. The percentage specific ^{51}Cr release was calculated using L_2C cells without effectors for background ^{51}Cr release, and 1% NP40 lysis for maximal release. Results for ^{51}Cr release are means of triplicates. Results are representative of three experiments using two different donors. Ranges at 1 μg/ml: ^{51}Cr release 19%–37% (ADCC), 40%–65% (RCC); DNA fragmentation 6%–18% (ADCC), 3%–12% (RCC)

Discussion

L_2C guinea-pig leukaemic target cells, when isolated from blood and cultured in vitro, were shown to undergo time- and temperature-dependent (37° C) apoptosis. This was detected by the appearance of a ladder of DNA fragments upon electrophoresis as well as condensation and fragmentation of the nucleus, both features being typical of apoptotic cell death [4, 15]. The dependence upon culture at 37° C is consistent with reports that apoptosis has an essential component of active self-destruction, with a requirement for macromolecular synthesis [28]. Spontaneous apoptosis has been reported also in certain human lymphoid leukaemias cultured in vitro, including at least some cases of B-cell chronic lymphocytic leukaemia (B-CLL) [3] and T cell acute lymphoblastic leukaemia [1]. In contrast the Jurkat and Daudi target cell lines did not exhibit significant apoptotic death in culture, presumably because they, in contrast to L_2C cells, have been selected for growth in vitro.

The role of apoptosis in cell-mediated cytotoxicity has remained controversial [2]. Various reports have suggested that engagement of NK cells with NK-susceptible targets, or of CTL with antigen-specific targets, results in cytotoxicity due to apoptosis in the target cells [12, 19]. Results from NK cytotoxicity of L_2C and Jurkat targets in this study suggest that, at low E : T, apoptotic cell death parallels the release of ^{51}Cr. Although the ^{51}Cr release was very low a large degree of DNA fragmentation was observed at lower E : T (35% at 25 : 1). These results therefore confirm that NK cells can induce apoptosis in susceptible targets. However, at high E : T (>25 : 1) the level of DNA fragmentation declined whilst the degree of ^{51}Cr release continued to increase, suggesting that necrotic mechanisms were dominating. These results are consistent with observations made by Duke et al. [5], who studied NK and CTL killing

and observed a similar decline in apoptosis induced by NK cells at high E:T, although this did not occur for CTL.

Addition of antibodies against target cells in the presence of the effector cell population resulted in antibody-dependent cellular cytotoxicity (ADCC). The recruitment of effector cells by antibody resulted in cytotoxicity for all target cells studied, and the levels of ^{51}Cr release, were greater than those induced by NK cytotoxicity. However, there was no increase in the level of DNA fragmentation accompanying the ADCC. Indeed, ADCC of L_2C target cells resulted in a significant inhibition of the spontaneous DNA fragmentation expected over the same period of time in the absence of any effector mechanism. The same concurrence of high levels of ^{51}Cr release and low levels of apoptosis was seen in ADCC whether the E:T or the antibody concentration was varied. Whether apoptosis was inhibited or merely cut short is not apparent.

Because ADCC could involve any FcγR-bearing effector cells, and not merely NK cells, and because the antibodies used were polyspecific, the observed ADCC could be the result of a complex interaction among numerous cell types and surface molecules. Therefore a more precise analysis was carried out to take advantage of the fact that only NK cells among the blood mononuclear cells bear FcγRIII. Bispecific F(ab′γ)$_2$ antibodies linked NK cells (via anti-FcγRIII) to target cells (via anti-idiotype or anti-CD37) to induce RCC. These studies confirmed that apoptosis is not a feature of cytotoxicity induced by FcγRIII-dependent mechanisms. It is of interest that RCC yielded more impressive ^{51}Cr release and suppression of nuclear fragmentation than did ADCC: a significant factor here could be a higher-affinity interaction of FcγRIII with antibody sites (in RCC) than with Fc regions (in ADCC).

We conclude from the above experiments that NK cells at low E:T can induce antibody-independent cytotoxicity in susceptible targets by processes that include apoptosis. At high E:T, and when effector cells are recruited by antibody, the degree of cytotoxicity increases and is accompanied by diminished apoptosis. To explain these results one must asume that NK cells have at least two cytotoxic mechanisms available, with at least one involving apoptosis. Different situations could lead to the dominance of one or other of these mechanisms, the balance being likely to depend upon the type of effector cell, the method of recruitment (particularly the surface receptors engaged), and the susceptibility of the target. Any of these factors could have led to our results differing from those of Stacey et al. [24], who observed apoptosis during ADCC. Cellular mechanisms that could account for cytotoxicity without apoptosis include the utilization of perforin [6, 30]. The induction of apoptosis could result from imbalances of the internal signals in the target cell [20] and/or an effector-cell-derived molecule such as tumour necrosis factor or lymphotoxin [22]. Because all of the above studies have been carried out in vitro, and on a limited range of target cells, further work is required to assess the extent to which the findings are mimicked in vivo.

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References

1. Baxter GD, Collins RJ, Harmon BV, Kumar S, Prentice RL, Smith PJ, Lavin MF (1989) Cell death by apoptosis in acute leukaemia. J Pathol 158: 123–129
2. Berke G (1991) Lymphocyte-triggered internal target disintegration. Immunol Today 12: 396–399
3. Collins RJ, Verschuer LA, Harmon BV, Prentice RL, Pope JH, Kerr JF (1989) Spontaneous programmed cell death (apoptosis) of B-chronic lymphocytic leukaemia cells following their culture in vitro. Br J Haematol 71: 343–350
4. Duke RC, Chervenak R, Cohen JJ (1983) Endogenous endonuclease-induced DNA fragmentation: an early event in cell-mediated cytolysis. Proc Natl Acad Sci USA 80: 6361–6365
5. Duke RC, Cohen JJ, Chervanek R (1986) Differences in target cell DNA fragmentation induced by mouse cytotoxic T lymphocytes and natural killer cells. J Immunol 137: 1442–1447
6. Duke RC, Persechini PM, Chang S, Liu CC, Cohen JJ, Young JD (1989) Purified perforin induces target cell lysis but not DNA fragmentation. J Exp Med 170: 1451–1456
7. Duvall E, Wyllie AH, Morris RG (1985) Macrophage recognition of cells undergoing programmed cell death (apoptosis). Immunol 56: 351–358
8. Elliott TJ, Glennie MJ, McBride HM, Stevenson GT (1987) Analysis of the interaction of antibodies with immunoglobulin idiotypes on neoplastic B lymphocytes; implications for immunotherapy. J Immunol 138: 981–988
9. Fleit HB, Wright SD, Unkeless JC (1982) Human Fc gamma receptor distribution and structure. Proc Natl Acad Sci USA 79: 3275–3280
10. Glennie MJ, McBride HM, Worth AT, Stevenson GT (1987) Preparation and performance of bispecific F(ab′γ)$_2$ antibody containing thioether-linked Fab′γ fragments. J Immunol 139: 2367–2375
11. Greenman J, Hogg NM, Nikoletti SM, Slade CJ, Stevenson GT, Glennie MJ (1992) Comparative efficiencies of bispecific F(ab′γ)2 and chimeric mouse/human IgG antibodies in recruiting cellular effectors for cytotoxicity via Fcγ receptors. Cancer Immunol Immunother 34: 361–369
12. Gromkowski SH, Brown TC, Cerutti PA, Cerottini JC (1986) DNA of human Raji target cells is damaged upon lymphocyte-mediated lysis. J Immunol 136: 752–756
13. Ishigami T, Kim KM, Horiguchi Y, Higaki Y, Hata D, Heike T, Katamura K, Mayumi M, Mikawa H (1992) Anti-IgM antibody-induced cell death in a human B-lymphoma cell line, B104, represents a novel programmed cell death. J Immunol 148: 360–368
14. Karpovsky B, Titus JA, Stephany DA, Segal DM (1984) Production of target-specific effector cells using hetero-cross-linked aggregates containing anti-target cell and anti-Fcγ receptor antibodies. J Exp Med 160: 1686–1701
15. Kerr JFR, Wyllie AH, Currie AR (1972) Apoptosis: a basic biological phenomenon with wide-ranging implications for tissue kinetics. Br J Cancer 26: 239–257
16. Klein E, Klein G, Nadkarni JS, Nadkarni JJ, Wigzell H, Clifford P (1968) Surface IgM-kappa specificity on a Burkitt lymphoma cell line in vivo and in derived culture lines. Cancer Res 28: 1300–1310
17. Krahenbuhl O, Tschopp J (1991) Perforin-induced pore formation. Immunol Today 12: 399–403
18. Lamoyi E, Nisonoff A (1983) Preparation of F(ab′)2 fragments from mouse IgG of various subclasses. J Immunol Methods 56: 235–243
19. Liu Y, Mullbacher A, Waring P (1989) Natural killer cells and cytotoxic T cells induce DNA fragmentation in both human and murine target cells in vitro. Scand J Immunol 30: 31–37
20. McConkey DJ, Chow SC, Orrenius S, Jondal M (1990) NK cell-induced cytotoxicity is dependent on a Ca^{2+} increase in the target. FASEB J 4: 2661–2664
21. Moore K, Cooper SA, Jones DB (1987) Use of the monoclonal antibody WR17 identifying the CD37 gp40-45 Kd antigen complex in the diagnosis of B-lymphoid malignancy. J Pathol 152: 13–21
22. Robaye B, Mosselmans R, Fiers W, Dumont JE, Galand P (1991) Tumour necrosis factor induces apoptosis (programmed cell death) in normal endothelial cells in vitro. Am J Pathol 138: 447–453

23. Shi L, Kraut RP, Aebersold R, Greenberg AH (1992) A natural killer cell granule protein that induces DNA fragmentation and apoptosis. J Exp Med 175: 553–556
24. Stacey NH, Bishop CJ, Halliday JW, Cooksley WGE, Powell LW, Kerr JFR (1985) Apoptosis as the mode of cell death in antibody-dependent lymphocytotoxicity. J Cell Sci 74: 169–179
25. Stevenson GT, Eady RP, Hough DW, Jurd RD, Stevenson FK (1975) Surface immunoglobulin of guinea-pig leukaemic lymphocytes. Immunology 28: 807–820
26. Willams GT (1991) Programmed cell death: apoptosis and oncogenesis. Cell 65: 1097–1098
27. Wyllie AH, Kerr JFR, Currie AR (1980) Cell death: the significance of apoptosis. Int Rev Cytol 68: 251–306
28. Wyllie AH, Morris RG, Smith AL, Dunlop D (1984) Chromatin cleavage in apoptosis: association with condensed chromatin morphology and dependence on macromolecular synthesis. J Pathol 142: 67–77
29. Young JD-E (1989) Killing of target cells by lymphocytes: a mechanistic view. Physiol Rev 69: 250–314
30. Young JD-E, Cohn ZA, Podack ER (1986) The ninth component of complement and the pore-forming protein (perforin 1) from cytotoxic T cells: structural, immunological and functional similarities. Science 233: 184–190
31. Zychlinsky A, Zheng LM, Liu C, Young JD (1991) Cytolytic lymphocytes induce both apoptosis and necrosis in target cells. J Immunol 146: 393–400