Virchows Arch (1998) 433:131–134

ORIGINAL ARTICLE

Koichi Ohshima · Kae Shimazaki · Junji Suzumiya
Motomu Kanda · Midori Kumagawa
Masahiro Kikuchi

Apoptosis of cytotoxic T-cells in histiocytic necrotizing lymphadenitis

Received: 4 November 1997 / Accepted: 9 March 1998

Abstract Cell death is necrotic or apoptotic. In histiocytic necrotizing lymphadenitis (HNL), apoptosis is the main form of cell death, resulting in the creation of nuclear debris that is one of the characteristic features of HNL. To investigate the cell type of apoptotic cells, 12 cases of HNL were analyzed using the immunohistochemical staining for TIA-1, a cytotoxic granule of either cytotoxic T or NK cells. One quarter to over half of all apoptotic cells were positive for TIA-1, and some of the nuclear debris was also positive. The necrotic lesions of HNL were found to consist of nuclear debris, apoptotic cells, histiocytes and lymphocytes. The lymphocytes were mainly CD8-positive T-cells or CD4-positive cells, while B- and NK cells were only rarely observed. The number of TIA-1-positive lymphocytes was more closely related to the number of CD8-positive cells than to the number of CD4 cells. In double staining, the TIA-1 positive lymphocytes were mainly CD8 positive, but rarely CD4 positive. In HNL, then, CD8-positive cytotoxic T-cells are likely to undergo apoptosis.

Key words TIA-1 · CTL · Apoptosis · Histiocytic necrotizing lymphadenitis

Introduction

Cell death is necrosis and apoptosis [10]. Morphologically, apoptotic cells show shrinkage of the cell volume and condensation of both the cytoplasm and nuclear chromatin; however, apoptotic cells are usually immediately removed by phagocytic cells [9].

Histiocytic necrotizing lymphadenitis (HNL) is a well-established non-neoplastic disease entity of lymph nodes and is known to have a self-limiting clinical course. Morphologically, only nodes are affected by the disease, and these are characterized by a proliferation of transformed lymphocytes and histiocytes with apoptotic cells and nuclear debris [2]. We have previously reported that the majority of proliferating cells are CD8-positive T-cells [6], and according to double staining, immunological staining and in situ apoptosis detection methods, most apoptotic cells have been found to be T lymphocytes [4]. It seems that the main pathological characteristics of HNL consist in apoptosis and a proliferation of T-lymphocytes, especially cytotoxic T-cells.

Cytotoxic lymphocytes, including both T-cells and natural killer (NK) cells, are characterized by the inclusion of cytoplasmic granules that fuse with the plasma membrane following target cell recognition. One of these granules is TIA-1 (recently renamed GMP-17 [5], and to confirm that apoptosis is the mode of death of cytotoxic T-cells, we performed immunological staining for TIA-1.

K. Ohshima (✉) · K. Shimazaki · J. Suzumiya · M. Kanda
M. Kumagawa · Masahiro Kikuchi
Department of Pathology, School of Medicine,
Fukuoka University, Nanakuma 7-45-1,
Jonan-ku, Fukuoka 814-01, Japan
Fax: (+81)-92-861-7300

Materials and methods

Twelve cases of HNL obtained from the lymph node files in the Department of Pathology, Fukuoka University School of Medicine were examined. Specimens were each divided into two parts: paraffin-embedded sections for light microscopy were fixed in buffered formalin, while the fresh specimens were kept in liquid nitrogen until examined.

Fresh tissue specimens for immunohistochemical study were embedded in OCT compound and kept in liquid nitrogen until examination. Serial cryostat sections were prepared for immunohistochemical staining using the so-called labelled streptavidin-biotin (LSAB) method. To determine the types of cells in the affected foci, the following were used; CD4 for helper/inducer T cells (Becton-Dickinson, Mountain View, Calif.), CD8 for suppressor/cytotoxic T cells, CD19 and CD20 for B cells (Becton-Dickinson), CD68 for histiocytes (Dakopatts, Glostrup, Denmark) and CD56 for NK cells (Becton-Dickinson). Antibodies to TIA-1 for cytotoxic T or NK cells (Coulter; Hialeah, Fla.) were also used.

Frozen sections were used for the immunological staining for CD4, CD8, CD19, CD20, CD68, CD56 and TIA-1. Paraffin-embedded sections were used for the immunological staining of CD3 for T cells, CD20 for B cells (Dakopatts) and TIA-1.

Double staining of TIA-1 and CD8, or TIA-1 and CD4 was performed, using previously reported methods [6]. Frozen sections were used for double staining. Briefly, the first reaction products, which were brown in colour, were produced by incubation with di-

aminobenzidine (DAB), while the next product was recognized to be red in colour by new fuchsin after an alkaline phosphatase reaction.

Results

Table 1 summarizes the clinical and immunohistochemical findings in the 12 HNL cases. There were 8 female patients, while the other 4 were male. The median age was 22 years. All patients presented with lymphadenopathy of the neck, and all of them recovered from the disease within 3 months.

Histologically, all specimens biopsied showed only part of the lymph nodes to be involved. These areas were composed of histiocytes and transformed lymphocytes, which were accompanied by small lymphocytes and nuclear debris. In the lesions, we frequently observed apoptosis, but necrosis was rarely seen. The main distinguishing features of the apoptotic cells were a loss of cell volume and chromatin condensation.

In the areas involved, we counted the number of TIA-1-positive apoptotic cells in paraffin-embedded sections. We also examined the TIA-1, CD8, CD4, CD56 or CD19 positivity of the lymphocytes in the involved areas in frozen sections.

On immunohistochemical staining, the cell components of the affected necrotizing areas were found to be composed mainly of CD4-, CD8- and CD68-positive cells. Other cells, such as CD56-positive NK cells, were very rarely found. CD19-positive B cells were also very rare. CD68-positive cells were confirmed as histiocytes. In almost all cases, the number of CD8-positive lymphocytes was either larger than or the same as that of CD4-positive lymphocytes. The number of TIA-1-positive lymphocytes was thus found to be more closely related to CD8-positive than to CD4-positive lymphocytes.

A quarter to two-thirds of the apoptotic cells were positive for TIA-1; some of the nuclear debris was also positive.

In double staining, most of the TIA-1-positive lymphocytes were CD8 positive, but it was rare for them to be CD4 positive.

Discussion

HNL is characterized by a proliferation of transformed T-lymphocytes with many histiocytes and a large amount of nuclear debris [2, 6]. The main type of cell death is considered to be apoptosis, and not necrosis. Using the double-staining method of immunostaining and in situ labelling of nuclear DNA fragments, the apoptotic cells were identified as T lymphocytes [8]. Only a few of these cells were CD8 positive (suppressor/ cytotoxic T cells), while the number of CD4 positive cells was even smaller (helper/ inducer T cells) [4].

We reported previously that the proliferating cells in HNL were CD8-positive cells. In spite of proliferative activity of CD8 cells and the lack of such proliferation in CD4 cells, the CD8 to CD4 ratio did not increase very much during this period, suggesting that there must be extensive cell death of CD8-positive cells elsewhere in the lymph node [6].

Cytotoxic T lymphocytes (CTLs) and NK cells are characterized by their cytoplasmic granules that fuse with the plasma membrane following target cell recognition. One of these granules is TIA-1, which has recently been renamed GMP-17 [5].

GMP-17 has been proven to be a useful reagent for the identification of cytotoxic lymphocytes at sites of tissue destruction during both graft-versus-host disease [7] and renal allograft rejection [3]. In addition, a close correlation has been found between the expression of GMP-17 and the expression of perforin and granzyme B in NK cells and CTLs [1, 3], suggesting that GMP-17 may play a part in lymphocyte-mediated cytotoxicity and induce apoptosis.

In the present study, about half the apoptotic cells were positive for TIA-1, and some of nuclear debris was also positive. The necrotic lesions of HNL consisted of nuclear debris, apoptotic cells, histiocytes and lymphocytes. The lymphocytes were mainly CD8-positive or CD4-positive T-cells, while B and NK cells were few in number. The number of TIA-1-positive lymphocytes was closely related to the number of CD8-positive, rather than CD4-positive, cells. In double staining, the TIA-1-positive lymphocytes were mainly CD8 positive, but rarely CD4 positive. These findings support the theory

Table 1 Immunological findings in apoptotic cells and lymphocytes in the necrotizing lesions

Case No	Age	Sex	Duration	Apoptotic cells[a]	Lymphocytes (%)				
				TIA-1 (/100)	TIA-1	CD8	CD56	CD4	Double[b]
1	21	F	1 week	56	40	50	5	30	CD8[c]
2	26	F	2 weeks	45	30	50	2	15	CD8
3	22	M	2 weeks	43	20	40	<1	30	CD8
4	15	F	2 weeks	64	50	70	5	10	CD8
5	13	F	2 weeks	24	40	50	2	30	CD8
6	35	F	3 weeks	72	30	40	2	20	CD8
7	11	M	4 weeks	32	40	50	2	20	CD8
8	24	F	5 weeks	11	50	80	5	15	CD8
9	23	F	6 weeks	56	40	60	2	30	CD8
10	19	M	6 weeks	22	20	40	<1	40	CD8
11	22	M	7 weeks	26	30	30	1	20	CD8
12	32	F	7 weeks	38	10	15	2	40	CD8

[a] Number of TIA-1-positive apoptotic cells is indicated as the number of TIA-1-positive apoptotic cells in 100 apoptotic cells
[b] Double staining of TIA-1 and CD8, or TIA-1 and CD4
[c] TIA-1-positive lymphocytes are mainly CD8 positive

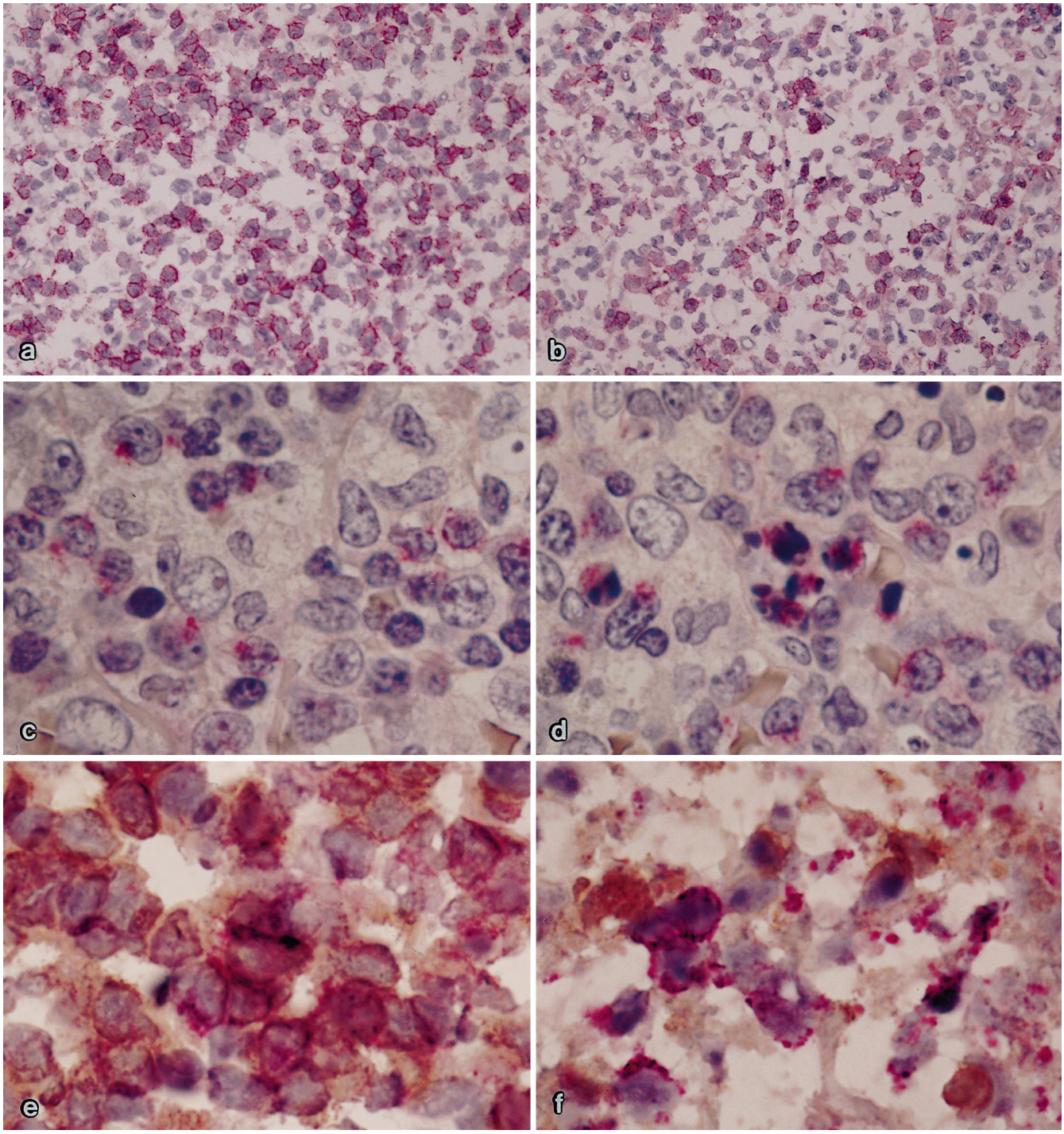

Fig. 1a–f Immunological findings. The cell components of the affected necrotizing areas were mainly composed of CD4-, CD8- and CD68-positive cells. The number of CD8 positive lymphocytes (**a**) was larger than or the same as that of CD4 positive lymphocytes (**b**). Apoptotic cells were positive for TIA-1, as was nuclear debris. TIA-1 positive apoptotic cells were also phagocyted by histiocytes (**c, d**). In double staining, the TIA-1-positive lymphocytes (*red*) were mainly CD8 positive (*brown*; **e**), but rarely CD4-positive (*brown*; **f**)

that in HNL, CD8-positive cytotoxic T-cells are the cell population observed to undergo apoptosis.

Acknowledgment This work was supported in part by Grants-in-Aid for the Encouragement of Young Scientists from the Ministry of Education, Science and Culture, Japan.

References

1. Cesano A, Visonneau S, Clark SC, Santoli D (1993) Cellular and molecular mechanisms of activation of MHC nonrestricted cytotoxic cells by IL-12. J Immunol 151:2943–2957

2. Kikuchi M (1972) Lymphadenitis showing focal reticulum cell hyperplasia with nuclear debris and phagocytes: a clinicopathological study (in Japanese) Acta Hematol Jpn 35:379–380
3. Lipman M, Stevens A, Strom T (1994) Heightened intragraft CTL gene expression in acutely rejecting renal allografts. J Immunol 152:5120–5127
4. Liu Q, Ohshima K, Shinohara T, Kikuchi M (1995) Apoptosis in histiocytic necrotizing lymphadenitis. Pathol Int 45:729–734
5. Medley QG, Kedersha N, O'Brien S, Tian Q, Schlossman SF, Streuli M, Anderson P (1996) Characterization of GMP-17, a granule membrane of natural killer cells following target cell recognition. Proc Natl Acad Sci USA 93:685–689
6. Ohshima K, Kikuchi M, Sumiyoshi Y, Masuda Y, Mohtai H, Eguchi F, Takeshita M (1991) Proliferating cells in histiocytic necrotizing lymphadenitis. Virchows Arch [B] 61:97–100
7. Sale G, Anderson P, Browne M, Myerson M (1992) Evidence of cytotoxic T-cell destruction of epidermal cells in human graft-vs-host disease. Immunohistology with monoclonal antibody TIA-1. Arch Pathol Lab Med 116:622–625
8. Takakuwa T, Ohnuma S, Koike M, Hoshikawa M, Koizumi H (1996) Involvement of cell-mediated killing in apoptosis in histiocytic necrotizing lymphadenitis (Kikuchi-Fujimoto disease). Hitopathology 28:41–48
9. Vaux DL (1993) Toward an understanding of the molecular mechanisms of physiological cell death. Proc. Natl Acad Sci USA 90:786–789
10. Wyllie AH (1981) Cell death: a new classification separating apoptosis from necrosis. In: Bowen ID, Lockshin RA (eds.) Cell death in biology and pathology. Chapman Hall, London, pp 9–34