Caspase-8 deficiency in T cells leads to a lethal lymphoinfiltrative immune disorder

Leonardo Salmena and Razqallah Hakem

Ontario Cancer Institute and Department of Medical Biophysics, University of Toronto, Toronto, Ontario M5G 2C1, Canada

Caspase-8 is best known for its role in mediating cell death through death receptors such as CD95 (Fas/Apo1) (1–3). Recent evidence supports a new paradigm that suggests that caspase-8 also has nonapoptotic functions in the transduction of signals via the T cell receptor, B cell receptor, and Toll-like receptors (4–7). Despite extensive characterization of the biochemical and cellular functions of caspase-8, its physiological role is largely unknown. A recent study reported that humans with a germline point mutation of Caspase-8 manifested lymphoedema, splenomegaly—conditions normally associated with autoimmunity (5). Somewhat paradoxically, these patients were also concluded to be immunodeficient and succumbed frequently to microbial infections as a result of their inability to activate T, B, and NK cells (5). Thus, loss of caspase-8 leads to a complex immune condition manifesting features of immunodeficiency and autoimmunity.

Because caspase-8 deletion is associated with embryonic lethality in mice (7–9), we used the loxp/Cre recombinase system to generate casp8−/− mice (6). In keeping with its role as an effector of apoptosis, casp8−/− T cells were refractory to cell death induced by CD95. However, caspase-8 deficiency was also associated with T cell lymphopenia, defective activation-induced T cell proliferation, and defective T cell responses to viral infection. tcas8−/− mice were concluded to be immunodeficient, which recapitulated the immunodeficiency identified in humans with Caspase-8 deficiency (5, 6).

We now report that tcas8−/− mice developed an age-dependent lethal lymphoproliferative and lymphoinfiltrative immune disorder. With age, tcas8−/− mice developed lymphoedema, splenomegaly, and accumulated nonclonal T cell infiltrates in the lungs, liver, and kidneys accompanied by tissue damage. Furthermore, T cells isolated from old tcas8−/− mice were in a perpetual state of activation, which could account for the observed pathological phenotypes. This study uncovers novel physiological functions for caspase-8 in immune regulation and function.

RESULTS AND DISCUSSION

In this study, we monitored a cohort of aging casp8−/− mice. Mice were considered “old” after 8 wk of age and were designated...
“Otcasp8^−/−” for “Old-tcasp8^−/−.” Otcasp8^−/− mice were visibly smaller, often appeared weak, and weighed significantly less than their casp8^fl/fl control littermates (Fig. 1 A). The survival of Otcasp8^−/− mice was reduced compared with control littermates; Otcasp8^−/− mice perished at an average age of 51.7 wk with lethality observed as early as 14 wk, whereas control mice lived, on average, for >100 wk (Fig. 1 B). Furthermore, a predominant feature manifested in Otcasp8^−/− mice was chronic splenomegaly and/or lymphoadenopathy (Fig. 1 C). Based on these observations, we reasoned that the lethality observed in Otcasp8^−/− mice may be due to defective homeostasis leading to a prominent immune disorder.

To investigate this disorder, lymphoid and nonlymphoid organs were dissected from Otcasp8^−/− mice and analyzed. Gross observation of thymi from control and Otcasp8^−/− mice showed no difference in size at all ages (Fig. S1 A), available at http://www.jem.org/cgi/content/full/jem.20050683/DC1). Thymocyte subpopulations and surface expression of the CD4, CD8, CD44, CD25, CD69 and casp8fl/fl(Tcasp8^−/−) mice with controls (Fig. 2 A and Table S1, available at http://www.jem.org/cgi/content/full/jem.20050683/DC1); this is consistent with the observations made in young casp8^−/− mice (Ycasp8^−/−) (6). Surprisingly, the peripheral T cell lymphopenia reported in Ycasp8^−/− mice (6) was no longer apparent in Otcasp8^−/− mice (Fig. 2 B). Lymphoproliferation in Otcasp8^−/− mice was initially identified by splenomegaly and lymphoadenopathy and confirmed by increased total lymphocyte counts (Fig. 2 B). Furthermore, the age-dependent expansion of total lymphocyte numbers was more pronounced in Otcasp8^−/− mice compared with littermate controls (Fig. 2 B). These data, together with FACS (Becton Dickinson) analysis of splenocytes and LN cells, shows that the B cell to T cell ratio does not vary with age, yet lymphoid hyperplasia is apparent in Otcasp8^−/− mice. Therefore, tcasp8^−/− mice show two distinct phenotypes: peripheral T cell lymphopenia early in life and in older mice. Although the decreased T cell to B cell ratio is maintained, caspase-8

Figure 1. Decreased weight and decreased viability in old tcasp8^−/− mice. (A) Representative mice demonstrate relative sizes of old casp8^fl/fl; LckCre (Otcasp8^−/−) mice compared with casp8^fl/fl control mice. Old tcasp8^−/− (n = 5, n = 14) weighed significantly less than their casp8^fl/fl control littermates (n = 5, n = 10). All mice were >24 wk old. Error bars represent the mean ± SEM as described in Materials and methods; *, P = 0.05. (B) Kaplan–Meier analysis represents the percent survival of control (n = 18) and tcasp8^−/− (n = 15) cohort mice versus age in weeks. (C) Representative LN and spleens demonstrate lymphoadenopathy and splenomegaly in old tcasp8^−/− mice. An asterisk indicates statistical significance.

Figure 2. Lymphoproliferation in old tcasp8^−/− mice. (A) Representative flow cytometric analysis identified the proportion of T cells (Thy1.2^+^) versus B cells (B220^+^) and the proportion of CD4^+^ (Thy1.2^+^CD4^+^) versus CD8^+^ (Thy1.2^+^CD8^+^) T cells in control, tcasp8^−/−, and lpr mice. Numbers represent percentage of cells per quadrant. (B) Total lymphocyte, total B cell, and total T cell counts were evaluated in the LNs (top left) and spleen (top right). Total lymphocyte counts in the LNs and spleen were plotted against mouse age (bottom left and right, respectively). A line of best-fit was plotted on each graph. Error bars represent the mean ± SEM as described in Materials and methods.
deficiency is associated with a balanced expansion of both T and B lymphocyte numbers in the periphery. The accumulation of B cells, despite the fact that caspase-8 is deficient only in T cells, suggests that expansion of B cell populations are secondary to and dependent on changes in mutant T cells—implying that caspase-8 is required for regulating lymphocyte homeostasis, possibly through control of autocrine T cell signals and signaling to B cells.

Examination of nonlymphoid organs via histology and immunohistochemistry identified unusual T cell infiltration in the liver, lungs, and kidneys of Otcasp8+/−/− mice compared with control mice (Fig. 3; and Fig. S2 and Fig. S3 A, available at http://www.jem.org/cgi/content/full/jem.20050683/DC1). Livers from 30-wk-old Otcasp8+/−/− mice displayed T cell accumulation as focal perivascular infiltrates (Fig. 3 A, iv and vii–viii, and Fig. S2 A). Similarly, lungs of Otcasp8+/−/− mice contained obvious interstitial peribronchiovascular T cell infiltration (Fig. 3 A, vi; and Fig. S2). Kidneys from Otcasp8+/−/− mice also manifested focal interstitial and periglomerular T cell infiltration (Fig. 3 A, v; and Fig. S2). Increased infiltration of T cells to the liver, kidneys, and lung of Otcasp8+/−/− mice was confirmed via FACS analysis (Fig. 3 B). T cell infiltration was observed as early as 20 wk of age and progressed as Otcasp8+/−/− mice grew older (Fig. 3 C). At approximately 1 yr of age, disrupted lung tissue organization was associated with much more wide-
spread and abundant T cell infiltration (Fig. 3 C, iv–vi) compared with controls (Fig. 3 C, i–iii). Abnormal T cell infiltrates were absent in other nonlymphoid organs, including the brain, heart, pancreas, and stomach (Fig. S2). T cell infiltrates were not observed in control mice, and minimal B cell presence was detected in the nonlymphoid organs of control or Otcasp8−/− mice (Fig. 3 A, i–iii and iv; and Fig. S2). To address the self-reactivity of casp8−/− T cells, we measured the serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) as a measure of T cell–induced damage to hepatocytes. No increases in serum levels of ALT and AST were detected in Otcasp8−/− mice, suggesting that either the levels of tissue damage are low and undetectable or that no tissue damage is occurring (unpublished data). With respect to the clonality of infiltrating T cells, detection of CD4+ and CD8+ T cells in lung infiltrates and various Vβ chains of the TCR on circulating T cells demonstrates the polyclonal nature of this immune disorder (Fig. S3, A and B).

To determine the proliferative status of infiltrating and circulating lymphocytes, we performed in vivo 5-bromo-2′-deoxyuridine (BrdU) staining in Otcasp8−/− and control mice. We observed that pulmonary T cell infiltrates in Otcasp8−/− mice contained actively proliferating T cells as determined immunohistochemically with anti-BrdU and anti-Ki67 antibodies (Fig. 4 A). Examination of freshly isolated T cells from Otcasp8−/− mice via flow cytometry revealed a greater proportion of BrdU+ cells in CD4+, CD8+, and B220+ lymphocytes (Fig. 4, B and C). Furthermore, we observed an up-regulation of the cell surface activation markers CD69, CD44, CD25, and CD95 on both CD4+ and CD8+ T cells derived from Otcasp8−/− compared with control mice (Fig. 4 D). Accordingly, decreased detection of the T cell memory marker CD62L on Otcasp8−/− T cells indicated a previous T cell activation event (Fig. 4 D). Otcasp8−/− T cells express the same cell surface markers as antigen-activated T cells in contrast to homeostatically proliferating T cells—which, as shown in a recent publication, do not show this cell surface marker profile (10). Infiltrating T cells from the livers of Otcasp8−/− mice also displayed increased activation marker expression (unpublished data). Similar to mice with T cell–specific ablation of CD95, Otcasp8−/− mice older than 6 mo of age consistently displayed increased expression of activation markers on circulating T cells, and no such activation was observed in littermate control mice (11). By contrast, no indicators of activation were identified on B cells isolated from Otcasp8−/− mice (Fig. S4, available at http://www.jem.org/cgi/content/full/jem.20050683/DC1). These data suggest that Otcasp8−/−–derived T cells are activated in vivo. Because Otcasp8−/−–derived T cells are clearly proliferating in vivo, we investigated whether these T cells may have overcome the proliferation defect observed in Ytasp8−/− mice (6). Similar to Ytasp8−/−–derived T cells, Otcasp8−/−–derived T cells had defective in vitro proliferative responses to various stimuli (anti-CD3, anti-CD3, and CD28; anti-CD3 and IL2) relative to controls (Fig. S5, available at http://www.jem.org/cgi/content/full/jem.20050683/DC1). Furthermore, activated Otcasp8−/−–derived T cells were resistant to CD95L–induced apoptosis as expected (unpublished data). Therefore, in vitro experiments with casp8−/− T cells derived from old mice show that the defective proliferation and inhibition of CD95-mediated death are independent of age. In contrast, the increased expression of activation markers and BrdU labeling show that circulating and infiltrating Otcasp8−/− T cells are indeed activated and proliferate in vivo. Although currently unclear, we propose that casp8−/− T cells accumulate in vivo with age, perhaps because of their inability to be eliminated by CD95–induced apoptosis and/or activation-induced cell death. Furthermore, casp8−/− T cells may accumulate due to a proliferative process related to their activation status. It appears that the increased population of activated T cells in Otcasp8−/− mice may be a result of a phenomenon that includes both lymphoproliferation (as determined by BrdU labeling and Ki67 staining) and lymphoaccumulation (due to the impaired CD95 death pathway). Overall, we show that Otcasp8−/− mice manifest an age-dependent T cell infiltration that progresses with age and may ultimately be responsible for lethality of Otcasp8−/− mice by disrupting the function of vital organs such as the liver, lungs, and kidneys.

Because caspase-8 is located directly downstream of CD95 in the death receptor pathway, we reasoned that deletion of caspase-8 could lead to an autoimmune lymphoproliferative syndrome (ALPS)-like disease as observed in lpr and gld mice (12). ALPS is a childhood disorder caused by mutations in the genes encoding CD95, CD95 ligand, and caspase-10 (12–16). Characterized by chronic lymphoproliferation, ALPS patients also display splenomegaly, lymphoadenopathy, and lupus-like phenotypes including increased circulating immunoglobulins (IgGs) and the presence of autoimmune antibodies (14, 17). ALPS is uniquely defined by resistance to CD95–induced apoptosis and accumulation of unusual double-negative CD3+B220+CD4−CD8− T cells (14, 18). Through comparison with lpr mice, we found that Otcasp8−/− mice do not accumulate double-negative T cells (Fig. 2 A). Furthermore, increased (IgGs) and the presence of antinuclear antibodies were not observed in Otcasp8−/− mice of various ages (Fig. 5, A and B). In lpr disease, immune complexes are found deposited in glomeruli leading to glomerulonephritis (19, 20); no accumulation of immune complexes was observed in the glomeruli of Otcasp8−/− mice (Fig. 5 C). These findings suggest that the immune disorder observed in Otcasp8−/− mice is distinct from the ALPS phenotype observed in lpr mice.

The phenotype of IL-2 and IL-2RB–deficient mice is similar to tasp8−/− mice in that young mice show impaired proliferation and effector functions, and as these mice get older they show massive enlargement of LNs, spleen, and gut-associated lymphoid tissue due to polyclonal expansion of T and B cells (21–24). It has been shown that IL-2 and IL-2RB are necessary for maintenance of CD4+CD25+ regulatory T...
cells. When analyzed in \( \text{Otcasp8}^{−/−} \) mice, the proportion of regulatory T cells was similar to that of littermate control mice (unpublished data).

Controlled regulation of cell death is essential for lymphocyte development and function, and disruption of these processes may predispose for immune disorders (1, 25, 26). That the CD95-mediated death receptor pathway plays an important role in the elimination of activated and/or potentially autoreactive lymphocytes is underscored in humans where disruption of CD95 signaling increases the risk for ALPS (27).

We have identified the association of caspase-8 deficiency and T cell–dependent lymphoproliferation of T and B cell populations manifested as lymphoadenopathy and splenomegaly. In mice, a finding that underscores the importance of the multifunctional role of caspase-8 in apoptosis, cell growth, and immune homeostasis. We propose a model that argues that caspase-8 in T cells is required for the maintenance of lymphocyte homeostasis. When absent in T cells, abnormal lymphocyte homeostasis emerges, producing T cell lymphopenia in young mice, and as mice age, B cell and T cell compartments expand, producing lymphoproliferation and a lethal T cell infiltrating disorder.

**MATERIALS AND METHODS**

**Animals.** \( \text{tasp8}^{−/−} \) (caspase 8\( ^{95} \)) \( \text{Lck-Cre} \) mice were generated previously (6). All mice were of a mixed 129J/C57BL/6 genetic background. \( \text{lpr} \) mice were of a C57BL/6 genetic background. PCR genotyping of \( \text{tasp8}^{−/−} \) mice was performed with primer 5’-CCAGGAAAAAGATTGTGACT-3’ and primer 5’-GGCCCTTCTGAGTACTGTCACCTGT-3’. Lck-Cre was identified using primers specific for the \( \alpha e \) reoviridae gene: 5’-TCCCGAT-TATCTTCTATATCTTCAAG-3’ and primer 5’-GCTCGACCAGTTTAGTTACCC-3’.

**Flow cytometry.** Single-cell suspensions prepared from the thymus, spleen, and LN s were stained with the indicated antibodies conjugated to allophycocyanin, phycoerythrin, fluorescein, perCP, or biotin and streptavidin-PerCP (BD Biosciences) at 4°C in PBS + 10% FCS (GIBCO BRL). Lymphocytes were analyzed via flow cytometry (FACSCalibur; BD Biosciences) with CellQuest software (Applied Biosystems).

**Serology.** Tail blood was collected in Vacutainer tubes (Becton Dickinson), centrifuged at 14,000 g for 1 min at 4°C to clear sera, and frozen. Serum immunoglobulin (IgG) levels were determined via ELISA according to the manufacturer’s instructions (Southern Biotechnology). Anti-dsDNA serum levels were also detected via ELISA (Alpha Diagnostic). Serum ALT and AST levels were measured by the Toronto Medical Laboratories.

**Proliferation and cytotoxicity experiments.** For in vivo proliferation analysis, 40-wk-old mice were injected intraperitoneally with 0.6 mg of BrdU (Sigma-Aldrich) in 200 μL of PBS twice daily for 3 d. Lymphocytes were isolated from the spleen and LN s, appropriate cell surface markers were stained as described above, and BrDU incorporation was revealed with a BrdU-Flow kit (BD Biosciences). In vitro proliferation analysis and activation-induced cell death experiments were performed as described previously (6).

**Histology, immunohistochemistry, and immunofluorescence.** Organs were fixed in buffered formalin, processed for paraffin-embedded sectioning, and stained with hematoxylin-eosin (Fisher). For immunohistochemistry, paraffin sections were incubated with anti-B220 (BD Biosciences) and/or anti-CD3 (DakoCytomation), anti-CD4 (Serotec) and anti-CD8 (Serotec) antibodies. Anti-rat horseradish peroxidase (DakoCytomation) and anti-rabbit alkaline phosphatase antibodies (DakoCytomation) revealed B220 and CD3 double labeling, respectively. Single antibody immunohistochemistry staining was revealed with horseradish peroxidase. Immunoreactivities were revealed via incubation in diaminobenzidine and p-nitrophenylphosphate. In vivo BrDU labeling was revealed via immunohistochemistry using an HRP-linked anti-BrdU antibody (Jackson Immunoresearch). Identification of immune complexes in kidney and other tissues was revealed using a Cy3-conjugated anti-mouse IgG antibody (Jackson Immunoresearch Laboratories).

**Statistical analysis.** Data are expressed as the mean ± SEM. \( p \)-values were determined using the Student’s \( t \) test. Values of \( p \leq 0.05 \) were considered significant.

**Online supplemental material.** Fig. S1 shows that thymocyte development is not affected in \( \text{tasp8}^{−/−} \) mice. Fig. S2 shows no increased T cell infiltration was observed in nonlymphoid organs, including the brain, heart,
pancreas, and stomach. Fig. S3 shows polyclonal T cell infiltration in old tasp8/−/− mice. Fig. S4 shows the expression levels of B cell activation markers are not affected in Otcasp8−/−/− mice. Fig. S5 shows in vitro activation-induced proliferation in Otcasp8−/−/−-derived T cells. Table S1 shows percentage of T and B cells in the spleen and LNs in control and Otcasp8−/−/− mice. Online supplemental material is available at http://www.jem.org/cgi/content/full/jem.20050683/DC1.

The authors thank all members of the Hakem lab and L. Bernt for assistance in isolating lymphocytes from nonlymphoid organs. We thank W.C. Yeh, M. Woo, A. Hakem, L.Tamblyn, and J. Abraham for critically reviewing the manuscript; and H. Su, O. Sanchez, C. McKerlie, and M. Post for helpful discussions.

L. Salmena was supported by a Canadian Institutes of Health Research (CIHR) doctoral award. This study was funded by grants from the CIHR (MOP 36537) and the Terry Fox Foundation (TFPP 12000) to R. Hakem. R. Hakem is supported by a doctoral award. This study was funded by grants from the CIHR (MOP 36537) and the Terry Fox Foundation (TFPP 12000) to R. Hakem. R. Hakem is supported by a salary award from the CIHR.

The authors have no conflicting financial interests.

Submitted: 5 April 2005
Accepted: 3 August 2005

REFERENCES

1. Green, D.R., N. Dronin, and M. Pinksoski. 2003. Activation-induced cell death in T cells. ImmunoL. Rev. 193:70–81.
2. Boldin, M.P., T.M. Goncharov, Y.V. Golitsev, and D. Wallach. 1996. Involvement of MACH, a novel MORT1/FADD-interacting protease, in Fas/APO-1- and TNF receptor-induced cell death. Cell. 85:803–815.
3. Muzio, M., A.M. Chinnaiyan, F.C. Kischkel, K. O'Rourke, A. shevchenko, J. Ni, C. Scaffidi, J.D. Bretz, M. Zhang, R. Gentz, et al. 1996. Flice, a novel FADD-homologous ICE/CED-3-like protease, is recruited to the CD95 (Fas/APO-1) death-inducing signaling complex. Cell. 85:817–827.
4. Su, H., N. Bidere, L. Zheng, A. Cubre, K. Sakai, J. Dale, L. Salmena, R. Hakem, S. Straus, and M. Lenardo. 2005. Requirement for caspase-8 in NF-kappaB activation by antigen receptor. Science. 307:1465–1468.
5. Chun, H.J., L. Zheng, M. Ahnbad, J. Wang, C.K. Speirs, R.M. Siegel, J.K. Dale, J. Puck, J. Davis, C.G. Hall, et al. 2002. Plenotropic defects in lymphocyte activation caused by caspase-8 mutations lead to human immunodeficiency. Nature. 419:395–399.
6. Salmena, L., B. Lemmers, A. Hakem, E. Matsyiak-Zablocki, K. Murakami, P.Y. Au, D.M. Berry, L. Tamblyn, A. Shehabeldin, E. Mign, et al. 2003. Essential role for caspase 8 in T-cell homeostasis and T-cell-mediated immunity. Genes Dev. 17:883–895.
7. Kang, T.B., T. Ben-Moshe, E.E. Varfolomeev, Y. Peswzer-Jung, N. Yoge, A. Jurewicz, W. Waisman, O. Bremner, R. Haifner, E. Gustafsson, et al. 2004. Caspase-8 serves both apoptotic and nonapoptotic roles. J. Immunol. 173:2976–2984.
8. Varfolomeev, E.E., M. Schuchmann, V. Lurta, N. Chinnukulaksh, J.S. Beckmann, I.L. Mett, D. Rebrikov, V.M. Brodienski, O.C. Kemper, O. Kollet, et al. 1998. Targeted disruption of the mouse Caspase 8 gene ablates cell death induction by the TNF receptors, Fas/APO1, and DR3 and is lethal prenatally. Immunity. 9:267–276.
9. Sakamaki, K., T. Inoue, M. Asano, K. Sudo, H. Kazama, J. Sakagami, S. Sakada, M. Otsuki, S. Nakamura, S. Toyokuni, et al. 2002. Ex vivo whole-embryo culture of caspase-8-deficient embryos normalize their aberrant phenotypes in the developing neural tube and heart. Cell Death Differ. 9:1196–1206.
10. King, C., A. Illic, K. Kocheh, and N. Savetnick. 2004. Homeostatic expansion of T cells during immune insufficiency generates autoimmunity. Cell. 117:265–277.
11. Hao, Z., B. Hampel, H. Yagita, and K. Rajewsky. 2004. T cell-specific ablation of Fas leads to Fas ligand-mediated lymphocyte depletion and inflammatory pulmonary fibrosis. J. Exp. Med. 199:1355–1365.
12. Nagata, S., and T. Suda. 1995. Fas and Fasligand: lpr and gld muta-
tions. Immunol. Today. 16:39–43.
13. Fisher, G.H., F.J. Rosenberg, S.E. Straus, J.K. Dale, L.A. Middleton, A.Y. Lin, W. Strober, M.J. Lenardo, and J.M. Puck. 1995. Dominant interfering Fas gene mutations impair apoptosis in a human autoimmune lymphoproliferative syndrome. Cell. 81:935–946.
14. Straus, S.E., M. Sneller, M.J. Lenardo, J.M. Puck, and W. Strober. 1999. An inherited disorder of lymphocyte apoptosis: the autoimmune lymphoproliferative syndrome. Ann. Intern. Med. 130:591–601.
15. Wang, L., Z. Zheng, A. Loboto, F.K. Chan, J. Dale, M. Sneller, X. Yao, J.M. Puck, S.E. Straus, and M.J. Lenardo. 1999. Inherited human Caspase 8 mutations underlie defective lymphocyte and dendritic cell apoptosis in autoimmune lymphoproliferative syndrome type II. Cell. 98:47–58.
16. Cohen, P.L., and R.A. Eisenberg. 1991. Lpr and gld: single gene models of systemic autoimmunity and lymphoproliferative disease. Annu. Rev. Immunol. 9:243–269.
17. Sneller, M.C., J.K. Dale, and S.E. Straus. 2003. Autoimmune lymphoproliferative syndrome. Corr. Optis. Bliesmatul. 15:417–421.
18. Fleisher, T.A., J.M. Puck, W. Strober, J.K. Dale, M.J. Lenardo, R.M. Siegel, S.E. Straus, and J.J. Bleeing. 2001. The autoimmune lymphoproliferative syndrome. A disorder of human lymphocyte apoptosis. Clin. Rev. Allergy Immunol. 20:109–120.
19. Fukuyama, H., M. Adachi, S. Suematsu, K. Mowa, T. Suda, N. Yoshida, and S. Nagata. 1998. Transgenic expression of Fas in T cells blocks lymphoproliferation but not autoimmune disease in MRL-lpr mice. J. Immunol. 160:3805–3811.
20. Fukuyama, H., M. Adachi, S. Suematsu, K. Mowa, T. Suda, N. Yoshida, and S. Nagata. 2002. Requirement of Fas expression in B cells for tolerance induction. Eur. J. Immunol. 32:223–230.
21. Willerford, D.M., J. Chen, J.A. Ferry, L. Davidson, A. Ma, and F.W. Alt. 1995. Interleukin-2 receptor alpha chain regulates the size and content of the peripheral lymphoid compartment. Immunity. 3:521–530.
22. Schorle, H., T. Holtschke, T. Hunig, A. Schimpl, and I. Horak. 1991. Development and function of T cells in mice rendered interleukin-2 deficient by gene targeting. Nature. 352:621–624.
23. Sadlack, B., H. Merz, H. Schorle, A. Schimpl, A.C. Feller, and I. Horak. 1993. Ulcerative colitis-like disease in mice with a disrupted interleukin-2 gene. Cell. 75:253–261.
24. Nelson, B.H. 2004. IL-2, regulatory T cells, and tolerance. J. Immunol. 172:3983–3998.
25. Lenardo, M., K.M. Chan, F. Horung, H. McFarland, R. Siegel, J. Wang, and L. Zheng. 1999. Mature T lymphocyte apoptosis–immune regulation in a dynamic and unpredictable antigenic environment. Annu. Rev. Immunol. 17:221–253.
26. Rathmell, J.C., and C.B. Thompson. 2002. Pathways of apoptosis in lymphocyte development, homeostasis, and disease. Cell. 109(Suppl): S197–107.
27. Nagata, S. 1999. Fas ligand-induced apoptosis. Annu. Rev. Genet. 33:29–55.