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

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Caspase-8 is best known for its cell death function via death receptors. Recent evidence indicates that caspase-8 also has nonapoptotic functions. Caspase-8 deficiency is associated with pathologies that are unexpected for a proapoptotic molecule, such as abrogation of activation-induced lymphocyte proliferation, perturbed immune homeostasis, and immunodeficiency. In this study, we report the long-term physiological consequences of T cell–specific deletion of caspase-8 (*tcasp8*$^{-/-}$). We show that *tcasp8*$^{-/-}$ mice develop an age-dependent lethal lymphoproliferative and lymphoinfiltrative immune disorder characterized by lymphoadenopathy, splenomegaly, and accumulation of T cell infiltrates in the lungs, liver, and kidneys. Peripheral *casp8*$^{-/-}$ T cells manifest activation marker up-regulation and are proliferating in the absence of any infection or stimulation. We also provide evidence suggesting that this immune disorder is different from the autoimmune lymphoproliferative syndrome. Interestingly, the condition described in *tcasp8*$^{-/-}$ mice manifests features consistent with the disorder described in humans with *Caspase-8* deficiency. These findings suggest that *tcasp8*$^{-/-}$ mice may serve as an animal model to evaluate Caspase-8–deficient patient prognosis and therapy. Overall, our study uncovers novel in vivo functions for caspase-8 in immune regulation.

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Caspase-8, an aspartate-specific cysteine protease, 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 lymphoadenopathy and 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 *tcasp8*$^{-/-}$ 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. *tcasp8*$^{-/-}$ mice were concluded to be immunodeficient, which recapitulated the immunodeficiency identified in humans with *Caspase-8* mutation (5, 6).

We now report that *tcasp8*$^{-/-}$ mice developed an age-dependent lethal lymphoproliferative and lymphoinfiltrative immune disorder. With age, *tcasp8*$^{-/-}$ mice developed lymphoadenopathy, splenomegaly, and accumulated nonclonal T cell infiltrates in the lungs, liver, and kidneys accompanied by tissue damage. Furthermore, T cells isolated from old *tcasp8*$^{-/-}$ 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 *tcasp8*$^{-/-}$ mice. Mice were considered "old" after 8 wk of age and were designated

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"*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 CD95 were similar in *Otcasp8*$^{-/-}$ and control littermates (Fig. S1 B). In contrast, analysis of peripheral lymphocyte populations from spleen and LNs identified a decreased B-to-T cell ratio and a decreased proportion of CD8$^+$ T cells relative to CD4$^+$ T cells in *Otcasp8*$^{-/-}$ mice compared 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 *tcasp8*$^{-/-}$ mice (*Ytcasp8*$^{-/-}$) (6). Surprisingly, the peripheral T cell lymphopenia reported in *Ytcasp8*$^{-/-}$ 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

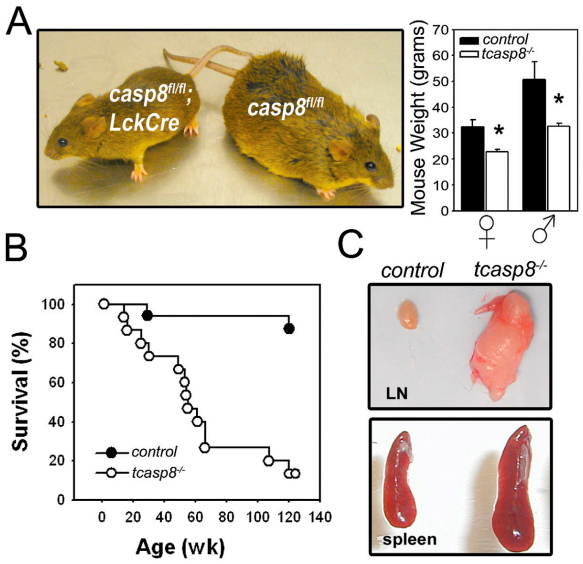

Figure 1. Decreased weight and decreased viability in old *tcasp8*$^{-/-}$ mice. (A) Representative mice demonstrate relative sizes of old *casp8*$^{fl/fl}$; LckCre (*Otcasp8*$^{-/-}$) mice compared with *casp8*$^{fl/fl}$ control mice. Old *tcasp8*$^{-/-}$ (n_{male} = 5, n_{female} = 14) weighed significantly less than their *casp8*$^{fl/fl}$ control littermates (n_{male} = 5, n_{female} = 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.

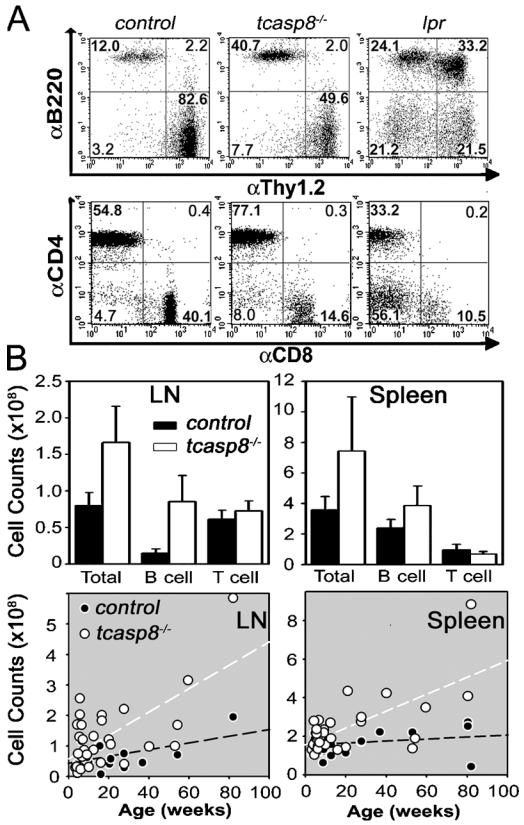

Figure 2. Lymphoproliferation in old *tcasp8*$^{-/-}$ mice. (A) Representative flow cytometric analysis identified the proportion of T cells (Thy1.2$^+$) versus B cells (B220$^+$) and the proportion of CD4$^+$ (Thy1.2$^+$CD4$^+$) versus CD8$^+$ (Thy1.2$^+$CD8$^+$) T cells in control, *tcasp8*$^{-/-}$, and *lpr* mice. Numbers represent percentage of cells per quandrant. (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.

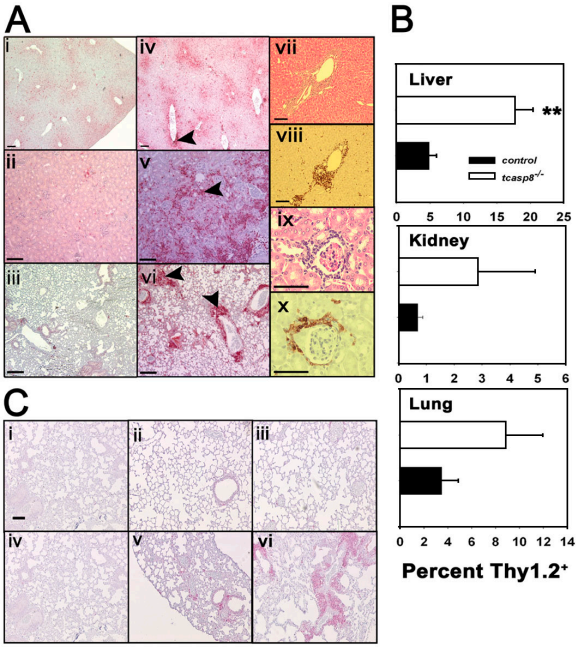

Figure 3. Age-dependent multitissue T cell infiltration in old *tcasp8*$^{-/-}$ mice. (A) Liver (i, iv), kidney (ii, v), and lung (iii, vi) sections from 30-wk-old mice were immunohistochemically stained with anti-B220 antibodies (to reveal B cells) and anti-CD3 antibodies (to reveal T cells). Consecutive liver and kidney sections stained with hematoxylin-eosin (vii, ix) and anti-CD3 (viii, x) demonstrate focal interstitial T cell accumulation in perivascular (vii, viii) or periglomerular (ix, x) areas, respectively. (B) Single cell suspensions were prepared from the liver, kidneys, and lungs, and T cell infiltrates were quantitated via flow cytometry. This analysis was repeated in a minimum of three mice per genotype. Error bars represent the mean ± SEM as described in Materials and methods. **, P = 0.00486. (C) Analysis of lung tissue from 6-wk-old (i, iv), 20-wk-old (ii, v), and 50-wk-old (iii, vi) control (i–iii) and *tcasp8*$^{-/-}$ (iv–vi) mice with anti-CD3 antibodies demonstrates an age-dependent increase in T cell infiltration. Lung tissues from control mice appear relatively normal at all ages (i–iii), whereas abnormal tissue is observed in the lung sections of 50-wk-old *tcasp8*$^{-/-}$ mice (vi). Black arrowheads indicate areas of focal T cell accumulation. Bars indicate 0.1 mm.

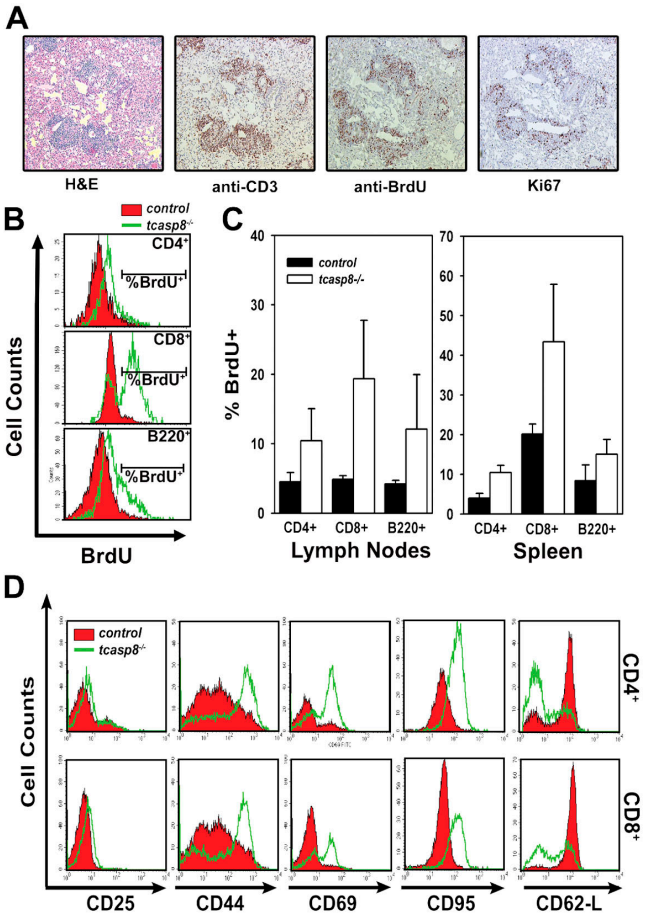

Figure 4. Infiltrating and circulating T cells from old *tcasp8*$^{-/-}$ mice are proliferating and activated. In vivo BrdU incorporation was determined after injecting BrdU into mice. (A) Pulmonary T cell infiltrates in old *tcasp8*$^{-/-}$ mice contained actively proliferating T cells as determined immunohistochemically via BrdU staining and Ki67 labeling. (B) Examination of circulating T cells via flow cytometry revealed a greater proportion of BrdU^{+} cells in each of the CD4^{+}, CD8^{+}, and B220^{+} lymphocyte compartments of old *tcasp8*$^{-/-}$ mice. (C) BrdU incorporation in CD4^{+}, CD8^{+}, and B220^{+} lymphocytes was quantitated ($n = 3$) for each genotype. Error bars represent the mean ± SEM as described in Materials and methods. (D) Representative flow cytometry analysis of activation markers in peripheral T cells isolated from lymphoid organs isolated from old *tcasp8*$^{-/-}$ mice and littermate controls. Cell surface expression of the activation markers CD69, CD44, CD25, and CD95 and the T cell memory marker CD62L on both CD4^{+} and CD8^{+} subsets of T cells are shown.

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 ix–x; 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 *Ytcasp8*$^{-/-}$ mice (6). Similar to *Ytcasp8*$^{-/-}$-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-2Rβ–deficient mice is similar to *tcasp8*$^{-/-}$ 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-2Rβ are necessary for maintenance of CD4$^+$CD25$^+$ regulatory T

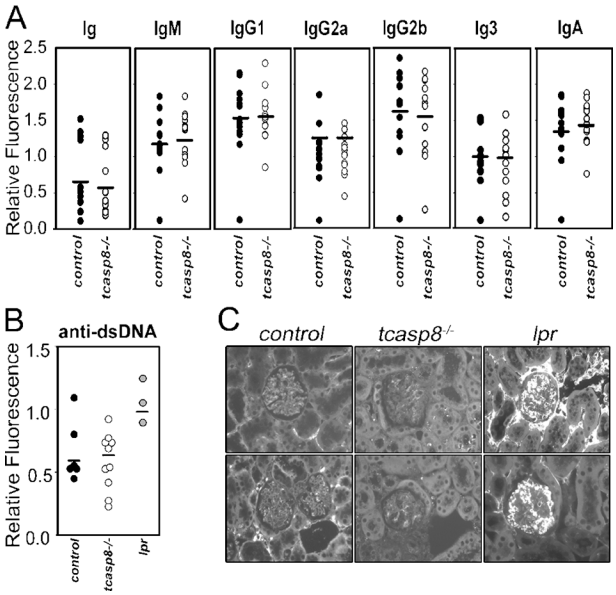

Figure 5. Old *tcasp8*$^{-/-}$ mice do not manifest systemic lupus erythematosus–like autoimmune phenotypes. (A) Levels of circulating IgG clonotypes and (B) anti-dsDNA antibodies were analyzed via ELISA in tail blood samples. Horizontal bars represent the mean. (C) Representative glomeruli from 30-wk-old control and *tcasp8*$^{-/-}$ mice showing no accumulation of immune complexes compared with *lpr* mice manifesting high levels of glomerular immune complex accumulation.

cells. When analyzed in *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 addition, we have uncovered nonclonal infiltration of T cells into the liver, lungs, and kidneys that became more severe with age and was likely to contribute to the increased lethality of *Otcasp8*$^{-/-}$ mice. Finally, *Otcasp8*$^{-/-}$ mice develop an age-dependent disease that is distinct from ALPS (or *lpr* in mice) and may represent a new form of immune disorder. Interestingly, the condition described in *Otcasp8*$^{-/-}$ mice manifests features consistent with the disorder described in humans with *Caspase-8* mutation (5). These findings suggest that *tcasp8*$^{-/-}$ mice may serve as an animal model to evaluate *Caspase-8* mutant patient prognosis and therapy.

The absence of caspase-8 in T lymphocytes alone is sufficient to provoke an age-dependent and lethal immune disorder 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. *tcasp8*$^{-/-}$ (*caspase 8*$^{fl/fl}$; Lck-Cre) mice were generated previously (6). All mice were of a mixed 129J/C57BL/6 genetic background. *Lpr* mice were of a C57BL/6 genetic background. PCR genotyping of *tcasp8*$^{-/-}$ mice was performed with primer 5′-CCAGGAAAAGATTTGTGTACT-3′ and primer 5′-GGCCTTCCTGAGTACTGTCACCTGT-3′. *Lck-Cre* was identified using primers specific for the *cre recombinase* gene: 5′-TCGCGATTATCTTCTATATCTTCAG-3′ and primer 5′-GCTCGACCAGTTTAGTTACCC-3′. All experiments were performed in compliance with the guidelines of the Ontario Cancer Institute Animal Care Committee.

Flow cytometry. Single-cell suspensions prepared from the thymus, spleen, and LNs 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 LNs, 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 diamine benzidine 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 old *tcasp8*$^{-/-}$ 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 *tcasp8*$^{-/-}$ 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.

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