CD45 regulates apoptosis in peripheral T lymphocytes

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Abstract

Programmed cell death (apoptosis) is a key mechanism for regulating lymphocyte numbers. Murine lymph node lymphocytes cultured in vitro without added stimuli show significant levels of apoptosis over 24 h, detectable by staining with Annexin V. CD4 and CD8 T lymphocytes from transgenic (Tg) mice expressing single CD45RABC or CD45RO isoforms show increased apoptosis and the extent of apoptosis is inversely correlated with the level of CD45 expression. CD45 Tg cells exhibit phosphatidyl serine translocation and DNA oligonucleosome formation, and can be partially rescued from apoptosis by culture in caspase inhibitors or common γ-chain-binding cytokines. We conclude that CD45 is an important regulator of spontaneous apoptosis in T lymphocytes and this mechanism may contribute to the disease associations reported for individuals expressing CD45 variant alleles.

Introduction

Programmed cell death (PCD) or apoptosis is an important regulatory process in the immune system. Not only do many lymphocytes die at the termination of the acute phase of an immune response, so that few of the clonally expanded cells enter the memory pool, but also dysregulation of PCD contributes to the development of autoimmunity and neoplasia (1, 2). The induction of apoptosis is usually an active process, accompanied by distinct morphological and biochemical events, although these vary in different cell types and also depend on the apoptosis-inducing stimulus (3). In contrast to necrosis in which cell swelling and disruption of the cell membrane and cellular organelles are prominent (4), during apoptosis, cells condense into membrane-bound fragments that are rapidly phagocytosed by neighboring cells, with little inflammation (4, 5). Classically, apoptosis involves activation of caspases and DNA fragmentation, detectable as DNA ‘laddering’, but the morphological features of apoptosis can occur without DNA fragmentation (6) and caspase-independent pathways of apoptosis are well described (7).

In T lymphocytes, two major mechanisms have been described. Activated T cells become sensitive to signals transmitted through Fas, which lead to the activation of caspase-8 and subsequently caspase-3, followed by apoptosis [activation-induced cell death (AICD)]. While AICD is dependent on TCR signaling, activated T cell autonomous cell death (ACAD) or death by neglect occurs at the termination of immune responses and is thought to involve members of the Bcl-2 family of proteins which control the integrity of the mitochondrial membrane. Loss of mitochondrial integrity leads to release of factors that can directly induce apoptosis without caspase activation, although caspases may become activated and accelerate apoptosis (8, 9).

Regulation of apoptosis is clearly complex and depends on both activating and survival signals. Apart from molecules that directly deliver activating or death signals, other molecules regulate the process, including the leucocyte common antigen CD45. CD45 has been shown to be essential for normal signaling through the TCR and also to modulate signaling through cytokine receptors (10–12). Since both these pathways are important in survival or death of lymphocytes, it might be expected that CD45 would influence apoptosis and this is indeed the case since antibodies to CD45 have been consistently demonstrated to cause apoptosis of T and B lymphocytes (6). Galectin-1, which binds to CD45, can also induce apoptosis in cell lines, activated T cells and thymocytes (13). Interestingly, although phosphatidyl serine externalization, cell condensation and mitochondrial transmembrane potential changes occur after CD45 ligation, DNA fragmentation does not (6, 14). Activation of caspases does not appear to be a feature of CD45-induced apoptosis, so it is perhaps not surprising that DNA degradation does not occur. Finally, induction of apoptosis through CD45 ligation is unusual in that it does not appear to require the intracellular phosphatase domain of CD45 (15), although others authors, using
phosphatase and kinase inhibitors, disagree with this conclusion (6).

We have generated several lines of CD45 transgenic (Tg) mice expressing single high-molecular weight, CD45RABC, or low-molecular-weight, CD45RO, isoforms at different levels. We show here that T lymphocytes from these Tg animals survive poorly in vitro, that the cell death has the hallmarks of apoptosis and that the extent of apoptosis is inversely related to the level of expression of CD45. These data imply that CD45 plays an important role in regulating apoptosis mediated through a caspase-dependent pathway terminating in DNA fragmentation.

Materials and methods

Mice
CD45RABC and CD45RO Tg mice on the CD45−/− background have been described previously (16). They were bred under specific pathogen-free conditions at the Institute for Animal Health (IAH). All experiments were submitted for ethical review to the IAH ethical review committee and carried out fully in accordance with UK Home Office Regulations.

Flow cytometric analysis
The following reagents and antibodies were used to stain lymph node (LN) lymphocyte suspensions: Annexin V–FITC and mAbs CD3–PE (145-2C11), CD4–PE (L3T4), CD8–PE (53-6.7) and CD44–allophycocyanin (IM7) (all from BD Biosciences, Oxford, UK). Isotype-matched mAbs were used as controls. Apoptosis was detected by Annexin V staining. Briefly, cells were first stained for surface markers using antibodies. After washing with the binding buffer for Annexin V (BD Biosciences), cells were stained with Annexin V–FITC for 15 min at room temperature. After washing with binding buffer, 100,000 events per sample were collected on a FACSCalibur flow cytometer (Becton Dickinson, Mountain View, CA, USA) and analyzed using WinMDI software.

Cell culture
Murine peripheral lymph nodes (PLNs) (inguinal, axillary, superficial cervical, mesenteric, lumbar, pancreatic, sacral and popliteal) were harvested from 8-week-old mice and cultured in RPMI with 10% fetal bovine serum. To block spontaneous apoptosis, caspase family inhibitor (fluoromethylketone) Z-VAD(OMe)-FMK, caspase-3 inhibitor (aldehyde) Ac-DEVD-CHO, caspase-1 inhibitor (aldehyde) Ac-YVAD-CHO or FMK-negative control (all from ALEXIS, Axxora Ltd, UK) were added to the overnight culture at final concentrations of 50 μM. For the cytokine rescue assay, human recombinant IL-2 (R&D, Abingdon, UK), IL-7 (Biosource, CA, USA) or IL-15 (R&D) were added to the overnight culture at final concentrations of 10 ng ml−1.

Cell purification and RNA isolation
PLNs were harvested from 8-week-old male CD45+/+,
CD45RABC+ and CD45RO+ mice. For microarray analysis, T cells were purified first by negative selection using M-450 sheep anti-rat IgG and M-450 sheep anti-mouse IgG Dynabeads (DYNAL Biotech, UK) with mAbs against mouse B220 (RA6B2) and Class II (TIB120) (gifts from Dr A. LeBon, Edward Jenner Institute). The negatively selected T cells were then stained with CD3–FITC (BD Biosciences) and a CD3+ fraction sorted using a MoFlo cytometer (Cytomation, Fort Collins, CO, USA). The sorted cells were lysed and homogenized in Trizol reagent (Invitrogen, Paisley, UK).

Microarray hybridization and data analysis
Microarray hybridization was performed by the Department of Molecular Haematology, Institute of Child Health, London, UK. Briefly, the RNA was purified using a QiAmp RNeasy kit. cDNA was synthesized using a commercial cDNA synthesis system (Roche, East Sussex, UK) and purified using the GeneChip Sample Cleanup Module (Affymetrix) before the in vitro transcription reaction. Biotin-labeled cRNA was synthesized using the Enzo BioArray High Yield RNA transcript labeling kit. The cRNA was purified using the QiAmp GeneChip Sample Cleanup Module and the quality and quantity were assessed on the Agilent 2100 Bioanalyzer. Fifteen micrograms of fragmented cRNA was hybridized to the murine U74Av array for 16 h at 45°C. After hybridization, the arrays were stained with streptavidin PE (SAPE) followed by a biotinylated anti-streptavidin antibody and then stained again with SAPE to amplify the signal. The arrays were scanned on the GeneArray scanner (Affymetrix GeneChip® Scanner 3000). Scanned arrays were visualized and the image was analyzed using Affymetrix Microarray Suite 5.0.

Microarray data analysis was conducted using Genespring 7.2 (Silicon Genetics, Redwood City, CA, USA) software. Data were normalized to the 50th percentile per chip and to median per gene. Data from the four replicates within each group of mice were averaged. The data were first filtered to include only genes that had a detection call of present in at least 3 out of 12 samples. Genes with fold change higher than two were then selected. The selected gene list was subjected to one-way analysis of variance (ANOVA) in Genespring with a P-value cut-off of 0.05.

Real-time quantitative PCR
Total RNA was isolated using Trizol reagent (Invitrogen). The probes and primers were designed using Primer Express software. PCR was carried out as a one-step reaction using the reverse transcriptase qPCR kit (Eurogentec, Seraing, Belgium). Probes were obtained from Sigma–Genosys, labeled 5′ with the reporter fluorophore 6-carboxy-fluorescein and 3′ with the reporter fluorophore 6-carboxy-N,N′,N,N′-tetramethylrhodamine. Cycling was carried out on an ABI prism 7700 Sequence Detector (Applied Biosystems, Warrington, UK) as follows: cDNA synthesis for 30 min at 48°C, hot GoldStar activation for 10 min at 95°C followed by 40 cycles of 15 s at 95°C and 1 min at 60°C. Quantification of RNA was based on the cycle number (Ct) at which the change in the level of fluorescence from the reporter dye passed a significance threshold. Standard curves were generated using 10-fold dilutions from 10−1 to 10−5 of total RNA extracted from the CD3+ cells of the group of mice in which the gene of interest is expressed. To correct for differences in RNA between samples and for the efficiency of the reactions, the following equation was used,
where \( m \) is the slope of the standard curve and DF is the difference factor:

\[
\frac{(40 - C_t) \times m_{\text{genex}}}{m_{28S} \times \text{DF}}.
\]

The DF was calculated by dividing the mean of the 28S \( C_t \) for the samples by the mean 28S \( C_t \) for all samples. Fold changes were calculated as the difference in corrected \( C_t \) values to the log of base 2.

**DNA isolation and gel electrophoresis**

Cells were re-suspended at a concentration of \( 4 \times 10^6 \) cells ml\(^{-1}\) in lysis buffer (100 mM NaCl, 10 mM Tris pH 8, 25 mM EDTA, 0.5% SDS) with 200 \( \mu \)g ml\(^{-1}\) proteinase K and 200 \( \mu \)g ml\(^{-1}\) ribonuclease A and incubated at 55°C overnight. The precipitated DNA was re-suspended in TE buffer and 4 \( \mu \)g loaded on 2% agarose gel and the DNA visualized after staining with 5 \( \mu \)g ml\(^{-1}\) ethidium bromide.

**Results**

**CD45 Tg mice exhibit increased spontaneous apoptosis inversely correlated with the level of expression of surface CD45**

CD45 Tg mice expressing single CD45RABC or CD45RO isoforms as transgenes on a CD45\(^{-/-}\) background have been described previously (16). Several lines have been produced expressing varying levels of the transgenes, from barely detectable in CD45RO\(^{10}\) mice to approximately five times less than the total CD45 expressed in CD45\(^{+/+}\) mice in the CD45RABC\(^{hi}\) and CD45RO\(^{hi}\) Tg lines. Since CD45 is known to influence both signaling through the TCR and apoptosis, these data prompted us to examine whether the Tg lines showed increased apoptosis.

PLN cells (~50% Tcells) were cultured in vitro for varying periods of time and phosphatidyl serine membrane translocation was assessed by Annexin V staining. Figure 1(A and B) shows that both CD4 and CD8 T cells of CD45ABC\(^{hi}\) and CD45RO\(^{hi}\) Tg mice, expressing equivalent levels of the transgenes (see Fig. 3A), show increased spontaneous apoptosis compared with CD45\(^{+/+}\) cells at time 0 and 3 h. In contrast, Tg B cells show only a marginal increase (Fig. 1C).

Since the majority of T cells of these CD45 Tg mice shows an activated or memory phenotype with increased expression of CD44 (16), we considered whether the increased spontaneous apoptosis might be due to the presence of more activated cells. Figure 2 indicates that this is not the case, since when apoptosis is assessed in cells of CD45\(^{+/+}\) and Tg mice expressing equally high levels of CD44, the Tg cells still show increased apoptosis compared with CD45\(^{+/+}\).

The existence of Tg mice with CD45 expression levels varying over nearly three logs provided an opportunity to test how the level of expression of CD45 affects spontaneous apoptosis. Figure 3(A) illustrates staining of lymphocytes from CD45\(^{+/+}\), CD45RABC\(^{hi}\), CD45RO\(^{hi}\), CD45RO\(^{lo}\) and CD45\(^{-/-}\) mice with an antibody to all isoforms of CD45 and Fig. 3(B and C) spontaneous apoptosis of LN CD4 and CD8 T cells from the same mice (except for CD45RABC\(^{lo}\)). There is a clear inverse correlation between the amount of CD45 expressed at the cell surface and the extent of apoptosis ex vivo and following culture. However, it is still possible that differences in the combinations of isoforms expressed contribute to the extent of apoptosis, since wild-type CD45\(^{+/+}\) mice express multiple isoforms, while the CD45RO Tg mice express only a single isoform.

**Mechanisms of apoptosis in CD45 Tg mice**

A classical marker of apoptotic cell death is the formation of oligonucleosomes, which can be detected as DNA ladders. CD45\(^{+/+}\) and Tg LN cells were cultured overnight in medium and following culture. However, it is still possible that differences in the combinations of isoforms expressed contribute to the extent of apoptosis, since wild-type CD45\(^{+/+}\) mice express multiple isoforms, while the CD45RO Tg mice express only a single isoform.

**Fig. 1.** Annexin staining of LN lymphocyte. Lymphocytes from PLNs and mesenteric LNs of individual CD45\(^{+/+}\), CD45RABC\(^{hi}\) and CD45RO\(^{hi}\) Tg mice were cultured directly ex vivo (T0) or after culture for 3 h at 37°C. Cells were stained with Annexin V–FITC and CD4–PE (A), CD8–PE (B) or IgM–PE (C). Means and standard deviations for nine mice are shown in (A) and (B) and four mice in (C).
ACAD, in which loss of mitochondrial integrity leads to the release of factors that can directly induce apoptosis in a caspase-independent manner, although caspases may also be activated to accelerate apoptosis (17, 18). Partial rescue from ACAD can be achieved with several cytokines including IL-2, -6, -7 and -15 (17). We therefore tested the effects of some of these factors on apoptosis of CD45 Tg cells.

Figure 5 shows the results of experiments in which LN cells were cultured overnight with or without added cytokines. Both CD4 and CD8 CD45+/+ cells showed improved survival in the presence of IL-7 or a mixture of cytokines, although the effect on CD4 cells was small. However, CD45RABChi and CD45ROhi CD4 cells showed no response to the cytokines, while Tg CD8 T cells showed greatly improved survival at 24 h. These data imply that although altered CD45 expression affects survival of both CD4 and CD8 T cells, the mechanism may not be identical in both subsets.

We also examined the effects of caspase-1, caspase-3 and pan-caspase inhibitors on T cell survival. Although the magnitude of the effect of the inhibitors on survival of CD45+/+ cells was small, CD45RABChi and CD45ROhi CD4 cells showed no response to the cytokines, while Tg CD8 T cells showed greatly improved survival at 24 h. These data imply that although altered CD45 expression affects survival of both CD4 and CD8 T cells, the mechanism may not be identical in both subsets.

We also examined the effects of caspase-1, caspase-3 and pan-caspase inhibitors on T cell survival. Although the magnitude of the effect of the inhibitors on survival of CD45+/+

**Fig. 2.** Increased apoptosis is not due to activation of Tg cells. LN lymphocytes of CD45+/+, CD45RABChi and CD45ROhi mice were stained directly ex vivo (T0) or after culture for 3 h at 37°C. Cells were stained for CD44, CD4 or CD8 and Annexin V. The percentage of Annexin V-stained cells that expressed equally high levels of CD44 are shown for CD4 (A) and CD8 (B) T cells. Means and standard deviations for four mice are shown.

**Fig. 3.** Spontaneous apoptosis correlates with the level of expression of CD45. (A) Levels of CD45 expression in lymphocytes from CD45+/+, CD45RABChi, CD45ROhi, CD45ROlo and CD45−/− mice detected with an mAb against all CD45 isoforms. Filled histogram CD45+/+, dashed lines CD45ROhi and CD45RABChi, gray line CD45ROlo and solid black line CD45−/−. (B) Lymphocytes from some of the same mouse strains were stained directly ex vivo (T0) or after 3 h culture, with Annexin V–FITC and CD4–PE (B) and Annexin V–FITC and CD8–PE (C). Means and standard deviations for three mice are shown.

**Fig. 4.** DNA laddering of CD45 Tg lymphocytes. LN cells of CD45+/+, CD45RABChi and CD45ROhi mice were lysed and DNA was extracted either ex vivo (T0) or after 24 h culture. DNA was electrophoresed on agarose gels and visualized by ethidium bromide staining. This is representative of three experiments.
T cells varied from experiment to experiment (Fig. 6A and B), all three inhibitors increased survival, with the pan-caspase inhibitor showing the greatest effect. The inhibitors showed similar effects on CD45RABChi and CD45ROhi T cells, although the magnitude of the effect was always less than that on CD45+/+ cells. These results indicate that the CD45 Tg cells are dying by classical apoptosis resulting in DNA laddering and death is caspase dependent.

**Gene expression in CD45 Tg T cells**

We purified CD3+ T cells from LNs of CD45+/+, CD45RABChi and CD45ROhi mice and compared gene expression in the three mouse strains using Affymetrix U74av murine arrays. Microarray data analysis was performed using Genespring 7.2. Genes with >2-fold differences between CD45+/+ and CD45RABChi or CD45ROhi mice were subjected to one-way ANOVA in Genespring with a cut-off P-value of 0.05. Among these are several genes involved in apoptosis which are shown in Table 1. We used real-time quantitative PCR for four of these genes to confirm that there were indeed differences in expression between the mouse strains (Table 2).

### Table 1. Genes involved in apoptosis detected by microarray analysis

<table>
<thead>
<tr>
<th>Gene</th>
<th>CD45RABChi</th>
<th>CD45ROhi</th>
<th>Molecular function</th>
<th>Biological process (references)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Annexin A1</td>
<td>4.157</td>
<td>1.811</td>
<td>Binds to cellular membranes</td>
<td>Induction of apoptosis (41)</td>
</tr>
<tr>
<td>Bcl-2-binding component 3</td>
<td>nd</td>
<td>2.361</td>
<td>Protein binding</td>
<td>Induction of apoptosis (42)</td>
</tr>
<tr>
<td>Bim (Bcl-2111)</td>
<td>1.596</td>
<td>2.643</td>
<td>Forms heterodimers with Bcl-2</td>
<td>Apoptosis facilitator (43)</td>
</tr>
<tr>
<td>Caspase-4</td>
<td>5.488</td>
<td>1.901</td>
<td>Protease</td>
<td>Execution of apoptosis (44)</td>
</tr>
<tr>
<td>Cathepsin E</td>
<td>19.26</td>
<td>15.58</td>
<td>Proteinase</td>
<td>Induction of apoptosis (45)</td>
</tr>
<tr>
<td>Caspase-4</td>
<td>2.775</td>
<td>nd</td>
<td>Protease</td>
<td>Execution of apoptosis (46)</td>
</tr>
<tr>
<td>IFN-activated gene 204 (Ifi16)</td>
<td>16.92</td>
<td>18.36</td>
<td>Member of PYRIN superfamily</td>
<td>Induction of apoptosis (47)</td>
</tr>
<tr>
<td>RAD 21 homolog</td>
<td>1.771</td>
<td>2.131</td>
<td>Component of cohesin complex</td>
<td>Execution of apoptosis (48)</td>
</tr>
<tr>
<td>(Schizosaccharomyces pombe)</td>
<td>2.457</td>
<td>2.042</td>
<td>Member of TNF family</td>
<td>T cell cytotoxicity (49)</td>
</tr>
<tr>
<td>Proviral integration site 2</td>
<td>2.037</td>
<td>1.49</td>
<td>Protein kinase</td>
<td>Anti-apoptosis (50)</td>
</tr>
</tbody>
</table>

A/Fold change of genes showing higher expression in CD45RABChi or CD45ROhi versus CD45+/+ mice. B/Fold change of genes showing higher expression in CD45+/+ mice versus CD45RABChi or CD45ROhi mice. TNF, tumor necrosis factor.
Tcells in CD45 would regulate apoptosis and indeed the lack of peripheral protecting against apoptosis, it might be expected that CD45 show increased responses to cytokines (21). Because both molecule may act as a negative regulator, since CD45 in signaling through cytokine receptors, though here the comprehensive data indicate that the molecule is also involved particularly Lck, which leads to their activation (10, 12). Less a dominant effect is the dephosphorylation of Src kinases, CD45 may have negative regulatory roles in signaling, plays an important role in signaling through the TCR. Although a role in signaling and there is now abundant evidence that it tyrosine phosphatase indicated that it was likely to play demonstration that CD45 was a cell-surface-associated surface expression of CD45 exhibit SCID (19, 20). The normal lymphocyte function and mice and humans lacking CD45 has long been known to be an important molecule for Discussion

CD45 has long been known to be an important molecule for normal lymphocyte function and mice and humans lacking surface expression of CD45 exhibit SCID (19, 20). The demonstration that CD45 was a cell-surface-associated tyrosine phosphatase indicated that it was likely to play a role in signaling and there is now abundant evidence that it plays an important role in signaling through the TCR. Although CD45 may have negative regulatory roles in signaling, a dominant effect is the dephosphorylation of Src kinases, particularly Lck, which leads to their activation (10, 12). Less comprehensive data indicate that the molecule is also involved in signaling through cytokine receptors, though here the molecule may act as a negative regulator, since CD45−/− mice show increased responses to cytokines (21). Because both the TCR and cytokine receptors play roles in initiating or protecting against apoptosis, it might be expected that CD45 would regulate apoptosis and indeed the lack of peripheral T cells in CD45−/− mice has been ascribed to altered signaling and increased apoptosis during thymic selection (22–24). However, because the CD45−/− mice have very few peripheral T cells, the role of CD45 in regulating apoptosis initiated during peripheral T cell activation or neglect has received less attention.

Instead, several authors have demonstrated that ligation of CD45 can initiate cell death in a variety of CD45-positive cell types. Some of these studies have used galectin-1 as a ligand and it remains unclear whether all the effects demonstrated relate to ligation of CD45, since galectin-1 binds to a number of cell-surface molecules (14, 25). However, the use of mAbs provides unequivocal evidence that cross-linking of CD45, alone or with activating signals, can initiate cell death (6). The mechanisms underlying this phenomenon have been investigated and reveal unusual features. First, it appears that the phosphatase activity is not required since a CD45 mutant molecule, with a ‘dead’ phosphatase domain, can mediate the effect (15). Second, the apoptotic process, although showing hallmarks of apoptosis such as DNA condensation and phosphatidyl serine translocation, does not result in DNA fragmentation into oligonucleosomes (laddering). Activation of caspases is also not a prominent feature of CD45-ligation-induced apoptosis (6, 14). In contrast, the CD45RABC/− and CD45RO/− Tg cells described here, which express ~5-fold less CD45 than CD45+/−, undergo a process of apoptosis that results in DNA laddering, is caspase dependent and can be partially prevented by exposure to cytokines. So far the mechanisms underlying the apoptotic process that occur in CD45−/− and Tg cells appear identical except in the proportion of cells that are dying. Our data therefore show that CD45 is an important regulator of apoptosis initiated by withdrawal of stimuli, in T but not B cells. Furthermore, the level of expression is an important determinant of the extent of apoptosis.

The single-isoform Tg mice described here are useful models to study the effects of gross alterations in CD45 expression and our experiments indicate that the level of expression of CD45 is important for regulation of apoptosis, although the expression of more than one isoform, as in CD45+/− mice, may also play a role. The importance of the total amount of CD45 in immunopathology has been shown in Fas-deficient gld/gld mice in which inactivation of one CD45 allele prevents development of lymphoproliferation and autoimmunity by increasing apoptosis (26). Furthermore, decreased CD45 expression or phosphatase activity in human systemic lupus erythematosus has also been reported (27, 28). Although in these situations the underlying mechanisms associating altered CD45 expression with disease have not been clearly delineated, there is abundant evidence that altered susceptibility to apoptosis, for example in mice with genetically manipulated genes of the Bcl-2 family or other survival genes, is associated with altered susceptibility to autoimmunity (29).

Finally, polymorphisms of human CD45 that alter the alternative splicing of the molecule and therefore the combinations of isoforms expressed have been described (30–33). Two of these, exon 4 C77G and exon 6 A138G, have been shown to be associated with infectious and autoimmune diseases in epidemiological studies (34–38). Alterations in the phenotype and function of T cells have been described in individuals carrying the variant alleles (38, 39). Although altered susceptibility to apoptosis has not been reported in variant individuals, we have constructed mouse models for C77G and A138G, expressing either a CD45RABChi or CD45RO/− transgene and a normally splicing CD45 allele. T cells of these mice show altered apoptosis (40). Taken together, these data suggest that as well as possible effects on signaling through antigen-specific and cytokine receptors, another mechanism underlying the disease associations of the CD45 variants is altered regulation of lymphocyte apoptosis.

Table 2. Taqman real-time PCR analysis of expression of caspase-1, Annexin A1, IFN-activated gene 204 (Ilf16) and FasL (Tnfsf6)

<table>
<thead>
<tr>
<th></th>
<th>CD45RABC/−</th>
<th>CD45RO/−</th>
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<tbody>
<tr>
<td>Caspase-1</td>
<td>3.0</td>
<td>2.3</td>
</tr>
<tr>
<td>Annexin A1</td>
<td>1.5</td>
<td>1.4</td>
</tr>
<tr>
<td>Ilf16</td>
<td>1.5</td>
<td>1.2</td>
</tr>
<tr>
<td>FasL</td>
<td>1.2</td>
<td>1.3</td>
</tr>
</tbody>
</table>

Fold change in CD45RABC/− or CD45RO/− versus CD45+/+ mice.

The genes identified by microarray analysis are involved in apoptosis, including proteinases, death receptors, caspases and Bcl-2 family members and are increased in CD45Tg cells. In contrast, the anti-apoptotic gene, proviral integration site 2, is expressed at a higher level in CD45+/+ cells.

**Abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>ACAD</td>
<td>T cell autonomous cell death</td>
</tr>
<tr>
<td>AICD</td>
<td>activation-induced cell death</td>
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<tr>
<td>ANOVA</td>
<td>analysis of variance</td>
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<tr>
<td>IAH</td>
<td>Institute for Animal Health</td>
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<tr>
<td>LN</td>
<td>lymph node</td>
</tr>
<tr>
<td>PCD</td>
<td>programmed cell death</td>
</tr>
<tr>
<td>PLN</td>
<td>peripheral lymph node</td>
</tr>
<tr>
<td>SAPE</td>
<td>streptavidin PE</td>
</tr>
<tr>
<td>Tg</td>
<td>transgenic</td>
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