Fas (CD95)-independent regulation of immune responses by antigen-specific CD4~CD8~ T cells

Soo-Jeet Teh, Jan P. Dutz, Bruce Motyka and Hung-Sia Teh
Department of Microbiology and Immunology, University of British Columbia, Vancouver, Canada V6T 1Z3

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Abstract

Antigen-activated T cells of the CD4~CD8~ and the CD4~CD8~ phenotype are susceptible to antigen receptor-stimulated cell death. This form of apoptotic cell death has been shown to be dependent on the expression of the Fas (CD95) antigen and can occur via an autocrine mechanism involving the concomitant up-regulation of Fas and its ligand on activated T cells. Mutations in genes encoding Fas (Ipr) and the Fas ligand (gld) contribute to the development of an autoimmune syndrome similar to systemic lupus erythematosus in mice. These observations led to the suggestion that the Fas signaling pathway is an important regulator of immune responses in vivo. Here we evaluated the importance of the Fas pathway in regulating immune responses by male antigen-specific CD4~CD8~ T cells. We found that the in vivo elimination of male antigen-activated cells was independent of Fas expression by these cells. However, the elimination of these activated cells was inhibited by the transgenic expression of Bcl-2, a protein that inhibits multiple forms of apoptotic cell death. The transgenic Bcl-2 protein also inhibited the death of male antigen-activated cells following IL-2 deprivation. Cell death resulting from IL-2 deprivation occurred efficiently in male antigen-activated Fas~ cells. We propose that the rapid deletion of male antigen-activated Fas~ cells in vivo is due to limiting amounts of IL-2 that are available in the microenvironment of the activated cells at the peak of the response.

In contrast to naive T cells, activated T cells are susceptible to TCR-induced cell death. This has been reported for antigen- or anti-CD3 activated T cells of the CD4~CD8~ or CD4~ CD8~ phenotype (1–5). Interestingly, this form of TCR-induced apoptotic cell death is shown to be dependent on the expression of the Fas (CD95) antigen by activated T cells (2–5). The Fas antigen and its ligand are encoded by the Ipr and gld genes respectively (6). Importantly, mice with homozygous mutations for the Ipr or gld gene developed an autoimmune syndrome similar to systemic lupus erythematosus (6). Furthermore, the expression of Fas and its ligand is upregulated on activated T cells and TCR-induced cell death of activated cells can occur via an autocrine mechanism (7–9) leading to the proposal that the Fas signaling pathway is an important regulator of immune response in vivo (6,10). In support of this model is the recent observation that the peripheral deletion of CD4 cells by antigenic peptide was dependent on Fas expression by the activated CD4 cells (4). The Fas signaling pathway is also required for the in vivo deletion of self-reactive B cells by CD4 T cells (11). In this study we examine the requirement for Fas expression in the regulation of immune responses in vivo by CD8 cells expressing a male antigen-specific TCR.

CD4~CD8~ T cells expressing a transgenic TCR specific for the male antigen presented by D~ class I MHC molecules are positively selected in female H-2d H-Y TCR transgenic mice (12,13). CD4~CD8~ T cells expressing this transgenic TCR but lacking Fas expression were obtained by backcrossing the Ipr mutation from B6-lpr/lpr mice onto H-Y TCR transgenic mice. Positive and negative selection of the H-Y TCR occur normally in lpr/lpr mutant mice (14,15). It has also been shown that the male-specific CD8 cells proliferate vigorously in the spleen and lymph nodes of male, but not female, B6-nu/nu mice (16). This proliferation peaks at day 5 and declines rapidly thereafter. As this decline has been correlated with the appearance of apoptotic cells (17), it is attractive to speculate that the elimination of the activated male-specific cells occurs by an activation-induced cell death.

Correspondence to: H.-S. Teh
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Fig. 1. Fas-independent regulation of in vivo immune responses by male antigen-specific CD8 cells. Female or male B6-nu/nu mice were injected i.v. with $1 \times 10^7$ lymph node (brachial, auxiliary, inguinal and mesenteric) cells from 8- to 10-week-old female H-2b H-Y, H-Y/pr-lpr or H-Y/bcl-2 transgenic mice. On day 5 (A) and day 9 (B) two recipient mice from each group were killed and the frequency of male-specific cells in the lymph nodes (brachial, auxiliary inguinal, and mesenteric) of these mice was determined by three-color staining with FITC-labeled anti-CD8, phycoerythrin-labeled anti-CD4 and biotinylated T3.70 mAb followed by Streptavidin-TriColor. The T3.70 mAb is specific for the transgenic TCR $\alpha$ chain (12). The stained cells were analyzed with the FACScan flow cytometer using Lysys II software. A total of 25,000 events were analyzed. It was previously shown that peripheral T cells with the CD8$^+$ T3.70$^+$ phenotype were specific for the male antigen (29,30). Since the frequency of CD8$^+$T3.70$^+$ cells in the lymph nodes of the donor mice was not identical, the recipient nu/nu mice received slightly different numbers of CD8$^+$T3.70$^+$ cells at the beginning of the experiment. In the experiment shown each male and female nu/nu mouse received 2.2X10$^5$ H-Y, 4.5X10$^5$ H-Y/pr-lpr or 7.5X10$^5$ H-Y/bcl-2 CD8$^+$T3.70$^+$ cells. In another experiment the nu/nu recipient mice received 1.8, 3.5 or 2.9X10$^5$ CD8$^+$T3.70$^+$ cells from H-Y, H-Y/pr-lpr and H-Y/bcl-2 mice respectively. Similar results and conclusions were reached in that experiment. A total of three experiments were performed with similar results. The x-axis denotes T3.70 fluorescence and y-axis denotes CD8 fluorescence. The numbers indicate the percentages of CD8$^+$T3.70$^+$ cells in the lymph nodes of recipient mice.
but not female, nu/nu recipient mice on day 5, regardless of whether the inoculum came from female H-Y TCR, H-Y TCR/lpr/lpr or H-Y TCR/bcl-2 transgenic mice. As previously reported (16), there was a dramatic decline on day 9 in the frequency of male-specific cells in the lymph nodes of male nu/nu recipients which received male-specific precursor cells from H-Y TCR transgenic mice (Fig. 1B). This decline was prevented by the transgenic Bcl-2 protein, suggesting that it is due to a cell death mechanism which is inhibitable by Bcl-2. Surprisingly, this decline was also observed in male nu/nu recipients which received male-specific precursor cells from H-Y TCR/lpr/lpr transgenic mice. Since CD8+ T cells are known to give rise to B220+CD4+CD8- T cells in lpr mutant mice (20-22) it is conceivable that the reduction in CD8+ T3.70+ cells observed for the transferred H-Y/lpr cells on day 9 may be due to a phenotypic conversion of CD8+ T3.70+ cells into CD8-T3.70+ cells. We tested this possibility by determining the frequency of CD8+ T3.70+ and CD8-T3.70+ cells on days 5 and 9. It was found that the lymph nodes of nu/nu male recipients of H-Y/lpr cells contained 6.7% of CD8+ T3.70+ and 2.1% of CD8-T3.70+ cells on day 5 versus 0.7% of CD8+ T3.70+ and 2.9% of CD8-T3.70+ cells on day 9. Thus, it is unlikely that the decrease in the frequency of CD8+ T3.70+ cells observed on day 9 is the result of phenotypic conversion of CD8+ into CD8-. Rather these results indicate that the Fas signaling pathway is not essential for the elimination of activated male-specific cells between day 5 and day 9 of the response. Similar results were observed in the spleens of nu/nu recipient mice. A quantitation of the recovery of male-specific CD8 cells in the lymph nodes and spleens of male and female nu/nu recipient mice is shown in Fig. 2. Male-specific cells from H-Y TCR transgenic mice proliferated slightly more efficiently than male-specific cells from H-Y TCR/lpr/lpr transgenic mice. This is consistent with a role for the Fas-signaling pathway in potentiating activation signals from the TCR signaling pathway (23). The results also confirm a decline in the anti-male response between day 5 and day 9 that was inhibited by the transgenic Bcl-2 protein but not by the lack of Fas expression by male-specific cells.

The expression of Fas and TCR by male antigen-activated CD8 cells from H-Y, H-Y/lpr-lpr and H-Y/bcl-2 mice is shown in Fig. 3(A). Male antigen-activated cells from all three types of mice expressed the same level of the transgenic TCR β chain. In contrast, only activated cells from H-Y and H-Y/bcl-2 but not H-Y/lpr-lpr mice expressed high levels of the Fas antigen (Fig. 3A). We also determined whether the absence of Fas expression or the presence of the transgenic Bcl-2 protein in these cells protected them from TCR-induced cell death. As shown in Fig. 3(B), male antigen-activated Fas+ CD8 cells were highly susceptible to killing by immobilized anti-TCR antibody; Bcl-2 did not protect Fas+ cells from TCR-induced cell death. This killing was TCR-specific since immobilized anti-Thy-1 antibody did not induce killing of these activated cells (data not shown and 24). By contrast, male antigen-activated Fas- CD8 cells were resistant to killing by anti-TCR antibody (Fig. 3B). The lack of protection of Fas- cells from killing by stimulation with immobilized anti-TCR antibody appears to be at variance with the in vivo protection observed with the transgenic Bcl-2 protein. This discrepancy is likely due to the manner by which the male antigen-activated cells were stimulated. Stimulation with anti-TCR antibodies is independent of the affinity of the TCR for its physiological ligand or the density of the antigen-ligand present on antigen presenting cells. We propose that the transgenic Bcl-2 protein is sufficient to protect the male antigen-stimulated cells from a weak stimulus under physio-
Fas-independent immune regulation in vivo

(A) H-Y  H-Y/lpr/lpr  H-Y/bcl-2

Fas expression

TCRβ expression

(B) H-Y  H-Y/lpr/lpr  H-Y/bcl-2

- F23.1

+ F23.1
logical conditions but not by a strong stimulus such as stimulation with immobilized anti-TCR antibodies. We also determined whether the lack of Fas expression or the presence of the transgenic Bcl-2 protein protected male antigen-activated cells from cell death resulting from IL-2 deprivation. The percent specific killing as a result of IL-2 deprivation in these three populations were: 51% (H-Y), 75% (H-Y/lpr-lpr) and 19% (H-Y/bcl-2). These results indicate that cell death resulting from IL-2 deprivation was partially inhibited by the transgenic Bcl-2 protein but not by the lack of Fas expression. We consistently observed that H-Y/lpr-lpr cells were more sensitive to this form of cell death than H-Y cells. The reason for the greater sensitivity of Fas+ cells to cell death induced by IL-2 deprivation remains to be investigated. Importantly, these results indicate that Fas and Bcl-2 protects male antigen-activated cells from cell death by distinct mechanisms.

Our results are in contrast to those reported recently for CD4 cells expressing the 2B4 TCR specific for pigeon cytochrome c (PCC) peptide presented by Eκ class II molecules (4). In that study, administration of PCC peptide into 2B4 TCR transgenic mice caused the peripheral deletion of PCC peptide-specific CD4 cells and this deletion was dependent on Fas expression by the PCC peptide-specific CD4 cells. There are several differences in that study that
may account for the difference with regard to the importance of the Fas-signaling pathway in regulating immune responses in vivo: (i) the use of CD4 as opposed to CD8 cells, (ii) potential differences in the affinity of the TCR for their respective ligands, (iii) the administration of large doses of PCC peptide as opposed to a natural cell surface antigen and (iv) differences in the genetic background of the TCR transgenic mice, i.e., MRL-lpr/lpr as opposed to B6-lpr/lpr. It is unlikely that the observed difference is due to the use of CD8 as opposed to CD4 cells since both CD4 and CD8 cells are susceptible to Fas-dependent TCR-stimulated cell death (2). Further experiments are required to evaluate possibilities (ii) and (iii). With regard to the last possibility, it is important to note that the lpr/lpr mutation is responsible for accelerating the autoimmune disease in MRL mice rather than inducing it (6,25).

This study also provides an explanation for Fas-independent regulation of the anti-male response by CD4+CD8− T cells under in vivo conditions. Previous studies have shown that this particular male-specific response is dependent on CD4 T helper cells and the availability of IL-2 (17,26). As shown in Fig. 3(C), male antigen-activated Fas+ cells are highly susceptible to cell death following IL-2 deprivation. We propose that the rapid deletion of male antigen-activated Fas+ cells in vivo is due to limiting amounts of IL-2 that are available in the microenvironment of the activated cells at the peak of the response. The observed increased survival of the activated male-specific cells in the presence of the transgenic Bcl-2 protein is consistent with our observation that this transgenic protein inhibited cell death resulting from IL-2 deprivation (Fig. 3C). Another study also showed that overexpression of Bcl-2 can prolong the survival of IL-2-dependent T cells following IL-2 deprivation (27).

A recent study investigated the relative contribution of the Fas and tumor necrosis factor (TNF) receptor signaling pathways in inducing the death of activated CD4+ and CD8+ cells after stimulation with immobilized anti-CD3e mAb (28). The CD4+ and CD8+ cells were activated with concanavalin A for 48 h. It was found that whereas the death of most CD4+ T cells is Fas ligand mediated, the death of CD8+ T cells was primarily mediated by TNF. Our results are inconsistent with these conclusions since we found that cell death induced by stimulation of male antigen-activated CD8+ cells with immobilized anti-TCR antibodies was dependent on Fas expression (Fig. 3B) and insensitive to killing by exogenous TNF (data not shown). The CD8+ cells used in our studies were activated with male antigen for 5 days. It remains to be determined whether the manner by which the CD8+ cells were activated contributed to the differences observed in these studies. However, regardless of these differences, the important conclusion is that in addition to Fas ligand-mediated killing, other distinct molecular mechanisms can contribute to the autoregulatory apoptosis of antigen-activated CD8+ cells.

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Abbreviations

PCC     pigeon cytochrome c
TNF    tumor necrosis factor

References

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