IL-4 influences the differentiation and the susceptibility to activation-induced cell death of human naive CD8\(^{+}\) T cells

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Abstract

It is now well established that the cytokine environment influences the activation, differentiation, proliferation and death of T lymphocytes during the primary response to antigen. Using an in vitro model, we investigated the influence of IL-4, added at the onset of TCR stimulation, on phenotypic and functional markers of naive CD8\(^{+}\) T cell activation including the up-regulation of activation markers, proliferation as well as the susceptibility to activation-induced cell death (AICD). We report that IL-4, unlike IL-2 added at the onset of repeated TCR stimulation of naive CD8\(^{+}\) T cells prevents AICD, in part due to its ability to maintain the level of the survival-related protein Bcl-2. Moreover, TCR-triggered activation of naive CD8\(^{+}\) T cells in the presence of IL-4 leads to the development of a CD8\(^{+}\) T cell subset that proliferates normally, but which fails to exhibit characteristic activation parameters such as the up-regulation of CD25 and Granzyme B. Taken together, these results demonstrate that exposure to IL-4 during primary activation influences CD8\(^{+}\) T cell differentiation by inducing the development of a sub-population of AICD-resistant, proliferation-competent cells that do not show some of the typical features of CD8\(^{+}\) T cell activation.

Introduction

Optimal T lymphocyte activation is subsequent to the orchestrated interaction of several parameters including the strength and duration of TCR stimulation as well as the presence of co-stimulatory signals (1). Moreover, the cytokine environment during T cell activation influences T cell proliferation, differentiation and survival (2, 3). The influence of cytokines is illustrated by the polarization of recently activated CD4\(^{+}\) T cells into Th1 or Th2 effector cells in the presence of specific cytokines, namely IL-12, IL-18 or IFN-\(\gamma\) for Th1 and IL-4, -5, -6, -10 and -13 for Th2 differentiation (4).

IL-4 is a multifunctional cytokine involved in the regulation of immune responses. Initially recognized for its ability to support the growth and differentiation of B lymphocytes (5, 6), IL-4 is now known to have pleiotropic effects on different cells of the immune system (7, 8). It is well described that IL-4 induces de novo expression of type 2 cytokines in CD8\(^{+}\) T cells, but the evidence for an effect on the development of cytolytic properties is conflicting. IL-4 has been reported to enhance, reduce or have no effect on the ability of CD8\(^{+}\) T cells to exert cytotoxic functions and to mediate antigen clearance in various models (9–15). Since antigen clearance in these models might be dependent on different immune functions and because IL-4 may affect various cell types involved in the immune response, the direct effect of IL-4 on CD8\(^{+}\) T cell function is difficult to assess in vivo. Additionally, little is known about the effect of IL-4 on other parameters associated with T cell activation. Morris and colleagues have reported that IL-4 could suppress CD4\(^{+}\) T cell activation in mice, as demonstrated by inhibition of CD25 and cytokine gene expression (16). In contrast, a recent in vivo study showed that IL-4 is required for the development of a protective CD8\(^{+}\) T cell response against malaria (17).

Several reports have shown that IL-4 is endowed with anti-apoptotic properties. For example, it has been demonstrated that IL-4 prevents cell death by neglect of resting T cells in mice (18) and of naive B lymphocytes in humans (19, 20). In
addition, it can also promote B cell survival following Ig crosslinking (21) and renders activated B cells insensitive to apoptosis mediated by Fas ligation (22).

In this report, we demonstrate that IL-4 prevents activation-induced cell death (AICD) in purified naive CD8+ T cells in vitro. Addition of IL-4 during TCR stimulation led to the sustained expression of the survival-related protein Bcl-2 by CD8+ T cells which may explain, at least in part, their resistance to AICD. Interestingly, we show that CD3 cross-linking in the presence of IL-4 promotes the development of a CD8+ T cell sub-population that proliferates normally but which does not up-regulate CD25 expression and fails to express high levels of Granzyme B (GrB). Overall, our results strongly suggest that IL-4 directly affects the fate of naive CD8+ T cells following TCR-mediated stimulation.

Methods

Reagents and antibodies
IL-2 was obtained from the National Institutes of Health AIDS Research and Reference Reagent Program. Recombinant IL-7 and IL-13 were purchased from BioSource (Camarillo, CA, USA). Recombinant IL-4 and IL-15 were obtained from Sigma–Aldrich (St Louis, MO, USA) and PeproTech Inc. (Rocky Hill, NJ, USA), respectively. The anti-CD3 antibody was purified from the supernatant of the OKT3 hybridoma (American Type Culture Collection). The anti-CD28, anti-Fas and anti-HLA-DR mAbs were obtained from BD PharMingen (San Diego, CA, USA). The mouse anti-human CD8, CD25, CD27, CD45RA, CD45RO, CD62L, CCR7, OX40 and GrB antibodies were purchased from Becton Dickinson (San Jose, CA, USA).

Isolation, culture and activation of naive CD8+ T cells
PBMCs from healthy blood donors were isolated by Ficoll–HyPaque (Pharmacia, Uppsala, Sweden) density gradient. PBMCs were stained with Cy-chrome-labeled anti-CD8 and HyPaque (Pharmacia, Uppsala, Sweden) density gradient. PBMCs from healthy blood donors were isolated by Ficoll–HyPaque (Pharmacia, Uppsala, Sweden) density gradient}

IL-4 prevents AICD of CD8+ T cells

IL-4 protects naive CD8+ T cells from AICD

Results

IL-4 prevents AICD of CD8+ T cells

To determine whether IL-4 affects the susceptibility of CD8+ T cells to undergo AICD, we developed an in vitro AICD model.
driven by repeated TCR stimulation of naive CD8+ T cells. Naive CD8+ T cells were isolated by cell sorting from fresh PBMCs according to the expression of CD8 and CD45RA. Additional flow cytometry analyses showed that a large fraction of this sub-population expressed CD62L (87%), CD27 (92%) and CD28 (95%). Sorted cells expressed neither CD25 nor CD45RO (data not shown), further confirming the naive phenotype of these cells. Based on this phenotype, these cells meet the criteria of resting naive T cells (24, 25). Naive CD8+ T cells were initially activated using plate-coated anti-CD3 and anti-CD28 antibodies in the presence of IL-2 alone or IL-2 in combination with IL-4. After 3 days of culture, cells were re-stimulated for an additional 48 h using coated anti-CD3. The proportion of cells that underwent AICD was quantified based on Annexin-V–FITC and PI labeling. Figure 1(A) and (B) show that, in the presence of IL-2 alone, ~30% of the cell population underwent apoptosis after the second TCR stimulation. Interestingly, when IL-4 was added to the culture media, the percentage of apoptotic cells consistently dropped below 12% (n = 5). Moreover, it is worth noticing that the proportion of CD8+ T cell undergoing spontaneous apoptosis or passive cell death (i.e. in the absence of re-stimulation) is also 2-fold lower in the presence of IL-2 and IL-4 as compared with IL-2 alone. These results indicate that IL-4, added at the

Fig. 1. Susceptibility of naive CD8+ T cells to AICD. (A) Naive CD8+ T cells were stimulated in vitro using coated anti-CD3 (2 µg ml^-1) and anti-CD28 (1 µg ml^-1). IL-2 (100 U ml^-1) and IL-4 (500 U ml^-1) were added at the beginning of cell culture. After 5 days of stimulation, cells were re-stimulated for 48 h using plate-coated anti-CD3 (2 µg ml^-1). (B) The proportion of apoptotic cells within the total population was quantified based on Annexin-V–FITC and propidium iodide (PI) labeling. Both early (Annexin-V+, PI-) and late (Annexin-V+, PI+) apoptotic cells were taken into account to calculate the proportion of dead cells. The mean ± SD of two independent experiments is shown.
IL-4 interferes with naive CD8$^+$ T cell activation and differentiation

Activation of naive T cells is characterized by changes in surface expression of several molecules such as CD45RA, CD25 (the alpha chain of the high affinity receptor for IL-2) or Fas/CD95 (26, 27). To determine the impact of IL-4 on phenotypic changes associated to T cell activation, naive CD8$^+$ T cells were activated using anti-CD3 + anti-CD28 antibodies in the presence of IL-2 alone or in combination with IL-4. After 3 days of stimulation, the expression of activation markers was assessed by flow cytometry. For these experiments, naive CD8$^+$ T cells were co-cultured with irradiated autologous PBMCs to ensure the provision of optimal co-stimulatory signals. As irradiated PBMCs die rapidly in culture, they can be excluded from the analysis by gating on the live lymphocyte population, based on forward- and side-scatter profiles. As shown in Fig. 2A (n = 3), stimulation of naive CD8$^+$ T cells in the presence of IL-2 led to up-regulation of CD25 and Fas expression and to the down-regulation of CD45RA expression. However, addition of exogenous IL-4 to the TCR trigger led to a significantly reduced number of activated cells that had up-regulated CD25 and Fas, while a higher proportion of cells maintained CD45RA expression. The extent to which IL-4 modulates the frequency of cell expressing these markers varied from one donor to another but typically ranged from 20 to 60% for CD25, 20 to 50% for CD45RA and from 20 to 30% for Fas. The proliferation of naive CD8$^+$ T cells after TCR triggering was measured using $[^{3}H]$TdR incorporation. Results presented in Fig. 2B show that IL-4 treatment slightly decreases anti-CD3-induced proliferation of the bulk naive CD8$^+$ T cell population. However, it is impossible to determine using this assay if the diminished $[^{3}H]$TdR incorporation is due to a weak inhibition of the proliferation of the whole T cell population or to a selective block in the proliferation of a given cell sub-population. Taken together, these observations suggest that the presence of IL-4 at the time of TCR stimulation affects several parameters of naive CD8$^+$ T cell activation.

IL-4 promotes the development of a sub-population that proliferates but exhibits a non-activated phenotype following TCR stimulation

The IL-4-induced partial inhibition of normal CD8$^+$ T cell activation can result either from the defective TCR engagement after CD3 cross-linking or from a defect in the acquisition of specific features of T cell activation (such as up- or down-regulation of specific markers), without affecting others such as cell proliferation. To address this issue, we simultaneously monitored the proliferation and the acquisition of the activation marker CD25 by naive CD8$^+$ T cells stimulated in the presence or absence of exogenous IL-4. CFSE-labeled naive CD8$^+$ T cells were activated for 3 days by CD3 cross-linking and the cell-surface expression of CD25 was quantified by flow cytometry. Results (Fig. 3, see inset) indicate that after 3 days of TCR stimulation, cells had undergone between one and five cycles of proliferation, as measured by CFSE dilution.

Concomitantly, we observed an increase in CD25 surface expression in dividing cells; CD25 was up-regulated at the onset of the first cell division and remained constantly high until the fifth cell division. Cells that remain CFSE<sup>hi</sup> and CD25<sup>+</sup> most likely represent resting cells that have not been stimulated. We consistently observed that ~5% of the proliferating cells failed to up-regulate CD25 in the presence of exogenous IL-2 alone. This proportion increased to ~20% when exogenous IL-2 and IL-4 were added simultaneously (Fig. 3, lower panel). Interestingly, for both IL-2- and IL-2 + IL-4-treated cells, the proliferating CD25<sup>neg</sup> cell sub-population were found in cells that underwent only one division, as well as in cells that have achieved up to six rounds of cell proliferation, strongly suggesting that the absence of CD25 at the surface of these cells is not due to a decrease in its expression during
Moreover, the addition of exogenous IL-4 alone during TCR stimulation leads to an increase in the proportion of CFSElow and CD25^+ as compared with cells activated in the absence of any exogenous cytokines (Fig. 3, upper panel). Our results suggest that IL-4 promotes the development of a previously uncharacterized CD8^+ T cell sub-population that proliferates normally upon TCR stimulation, but which fails to exhibit a 'typical' activated phenotype, as defined by the up-regulation of CD25 expression.

The biological functions of IL-4 are achieved through the heterodimerization of the IL-4Rα chain and the gamma common chain (γc), first identified as a component of the IL-2R (7). The γc chain is also shared by other cytokine receptors (IL-7R and IL-15R), whereas the IL-4Rα chain also functions as a component of the IL-13R. We therefore examined if one of these cytokines could also promote the generation of proliferating CD25^+ CD8^+ T cells, as observed in the presence of IL-4. CFSE-labeled naive CD8^+ T cells were TCR stimulated in the presence of exogenous IL-2, IL-4, IL-7, IL-13 or IL-15 was also added at the onset of stimulation. After 3 days, cells were stained with a mAb directed against CD25 and the proportion of CFSElow CD25^- cells within the CD8^+ T cell population was determined by flow cytometry (Fig. 4). In contrast to IL-4, addition of exogenous IL-7, IL-13 or IL-15 does not significantly alter the frequency of CFSElow CD25^- cell population as compared with addition of IL-2 alone, evidencing the unique effect of IL-4 on naive CD8^+ T cell activation.

**Fig. 3.** Effect of IL-4 on naive CD8^+ T cell proliferation and CD25 expression. CFSE-labeled cells were activated for 3 days using anti-CD3 (2 μg ml^-1) and anti-CD28 (1 μg ml^-1) either alone or in presence of IL-2 (100 U ml^-1) and/or IL-4 (500 U ml^-1). Autologous irradiated PBMCs were added at a 1:2 (PBMC:T cell) ratio. After 3 days of activation, cells were labeled with PE-conjugated anti-CD25 antibody. Cells were analyzed using a FACSscan flow cytometer. Results are representative of at least five independent experiments.

**Fig. 4.** The proportion of CFSElow CD25^- cells is not affected by other cytokines. CFSE-labeled cells were activated for 3 days using plate-coated anti-CD3 (2 μg ml^-1) and anti-CD28 (1 μg ml^-1) in presence of exogenous IL-2 (100 U ml^-1), IL-4 (500 U ml^-1), IL-7 (20 ng ml^-1), IL-13 (20 ng ml^-1) or IL-15 (20 ng ml^-1). Irradiated autologous PBMCs were added at 1:2 (PBMC:T cell) ratio. Cells were analyzed for CFSE dilution and CD25 expression using a FACSscan flow cytometer. Results are representative of two independent experiments.
IL-4 prevents Bcl-2 down-regulation induced by CD3 cross-linking

Proteins from the Bcl-2 family, endowed with either anti- or pro-apoptotic functions, are well known for their ability to regulate susceptibility to apoptosis in many cell types (28, 29). IL-4 has been shown to induce the up-regulation of Bcl-2 in murine T cells (18) as well as in human B cells (30). Therefore, we tested the impact of addition of IL-4 on the level of expression of Bcl-2 in anti-CD3-activated naive CD8+ T cells cultured with IL-2 and IL-4, either alone or in combination. After 3 days of stimulation, cells were lysed and the cell lysates were electrophoresed in SDS polyacrylamide gel. Proteins were then transferred onto PVDF membrane and probed with antibodies directed against Bcl-2 (Fig. 5). Non-activated naive CD8+ T cells contain high levels of Bcl-2 (lane 2). As previously described (31), CD3 cross-linking led to a sharp decrease in Bcl-2 expression level, which became barely detectable (lane 3). Addition of exogenous IL-2 at the onset of T cell priming did not prevent Bcl-2 down-regulation (lane 5). In contrast, CD8+ T cells activated in the presence of IL-4 (either alone or in combination with IL-2) continued to express Bcl-2 (lane 4 and 6), albeit at lower levels than non-activated cells. These results demonstrate that IL-4 partially prevents the decay of Bcl-2 expression following TCR-mediated activation of naive CD8+ T cells, which might explain, at least in part, the lower susceptibility to apoptosis of CD8+ T cells activated in the presence of IL-4.

IL-4 inhibits GrB up-regulation following activation of naive CD8+ T cells

Differentiation of naive CD8+ T cells into effector cells is characterized by the acquisition of cytolytic functions (32). As previously mentioned, IL-4 has been reported to exert divergent effects on the acquisition of cytolytic properties in CD8+ T cells (9–15). To investigate the role of IL-4 on the development of these functions following CD8+ T cell activation in our model, we analyzed the level of expression of GrB, a potent apoptosis-inducing protease of CTLs (33), in cells activated in the presence of IL-2 and/or IL-4 (Fig. 6). While GrB was not expressed in non-activated naive CD8+ T cells (lane 1), it was detected in CD8+ T cells having a memory phenotype (i.e. CD45RO+) (lane 2). Anti-CD3 stimulation of naive CD8+ T cells induces an increase in the level of GrB expression (lane 3), and addition of exogenous IL-2 at the
onset of stimulation further enhanced the up-regulation of GrB expression of 1.7-fold (lane 5). In contrast, stimulation of CD8+ T cells in the presence of IL-4 led to a 7-fold decrease in the expression of the lytic effector molecule when compared with cells activated in the absence of exogenous cytokines (lane 4 versus lane 3). This down-regulation of Granzyme expression was even more important (15-fold lower) when compared with cells activated in the presence of exogenous IL-2 (lane 4 versus lane 5). Moreover, IL-4 partially inhibited (~2.5-fold) the up-regulation of GrB induced by the addition of IL-2 (compare lane 5 and lane 6). These results indicate that IL-4 significantly inhibits GrB up-regulation in activated CD8+ T cells, and therefore influences the differentiation of naive CD8+ T cells into cytolytic effectors.

We then analyzed GrB protein expression by intracellular flow cytometry to determine if the CFSElow CD25- cells generated in the presence of IL-4 express lower levels of GrB. Fig. 7(A) shows that 80% of the CFSElow CD25+ are GrB positive when cells proliferate in the presence of IL-2 alone or IL-2 + IL-4, while the proportion of GrB-positive cells in the CFSEhigh (cells that do not proliferate) is negligible. In contrast, within the CFSElow CD25− compartment, only 35% of the cells are GrB positive (Fig. 7A and B). These results show that the inhibition of GrB up-regulation, in response to IL-4, is mainly observed within the CFSElow CD25− sub-population, indicating that CD8+ T cell activation in the presence of IL-4 will lead to the generation of cells that are able to proliferate while expressing low levels of the cytotoxic effector molecule, GrB.

The phenotype of the CFSElow CD25− and the CFSElow CD25+ CD8+ subsets was further characterized; we analyzed the expression levels of several surface markers including CD27, CD62L, OX40 and CD45RA (data not shown). Results (n = 2) showed that both CD25− and CD25+ compartments expressed similar levels of CD27, CD62L or OX40. On the other hand, CD45RA expression was down-regulated in both CFSElow CD25− and the CFSElow CD25+ CD8+ sub-populations when compared with CFSEhigh cells. Of note, CD45RA level of expression always remained 2-fold higher in the CFSElow CD25− CD8+ sub-population as compared with the CFSElow CD25+ sub-population. Taken together, those results show that TCR activation of naive CD8+ T cells in the presence of IL-4 leads to the generation of a sub-population of activated cells that do not express CD25 (but express similar level of other surface markers including OX40, CD27 and CD62L as compared with the activated CD25+ compartment) and contain no or low level of GrB, suggesting an inability to exert cytotoxic function.

Discussion

It is well described that cytokines have the ability to profoundly influence T cell development and differentiation, both in vitro and in vivo (34). In this report, we demonstrate that exposure to IL-4 during primary activation of CD8+ T cells (i) partially protects cells from AICD and (ii) promotes the development of a sub-population of CD8+ T cells that proliferate normally but that does not up-regulate CD25 and GrB expression.

We provide two possible mechanisms by which IL-4 could protect activated CD8+ T cells from apoptosis. First, addition of IL-4 at the time of TCR stimulation partially blocks the up-regulation of the Fas receptor on activated CD8+ T cells. Fas ligation has been shown to trigger apoptosis of activated T cells by engaging the cascade of caspase activation, leading to the cleavage of vital cellular proteins (35). The failure of CD8+ T cells to up-regulate levels of Fas at the cell surface in the presence of exogenous IL-4 in our system could protect activated CD8+ T cells from Fas-triggered apoptosis. Second, CD8+ T cells activated in the presence of IL-4 maintain high levels of the anti-apoptotic molecule Bcl-2. The mechanism by

![Fig. 7. The CFSElow CD25- cells express less GrB than CFSElow CD25+. (A) CFSE-labeled naive CD8+ T cells were activated using coated anti-CD3 (2 μg ml−1) and soluble anti-CD28 (1 μg ml−1) with IL-2 (100 U ml−1) alone or in presence of IL-4 (500 U ml−1). Autologous irradiated PBMCs were added at a 1:2 (PBMC:T cell) ratio. After 3 days of activation, cells were labeled with PE-CD25, washed, fixed with 2% PFA and incubate with Alexa 700–GrB antibodies in saponin buffer. Cells were then analyzed using flow cytometry. Left panels represents GrB expression in the gated sub-populations (light gray: CFSEhigh, black: CFSElow CD25− and dark gray: CFSElow CD25+). Results are representative of two experiments. (B) The level of expression of GrB is expressed as a percentage of GrB-positive cells gated on CFSE−/CD25−, CFSE−/CD25+ and CFSE+/CD25− cells for cells treated with IL-2 and IL-4. The mean ± SD of two experiments is shown.](https://example.com/fig7.png)
which IL-4 promotes the expression of Bcl-2 is unclear but most probably involves the activation, through the IL-4R signaling pathway, of Akt/PKB, which is known to regulate the expression of Bcl-2 family proteins including Bcl-2 (7). Experimental evidence strongly suggests that a pathway involving Bcl-2 proteins is directly involved in protecting T cells from apoptosis. For example, in vivo experiments have shown a decrease in the levels of Bcl-2 as T cells undergo apoptosis (36). Moreover, the over-expression of Bcl-2 increases T cell responses to viral challenge, most likely by preventing the death of activated T cells following pathogen clearance (37). Therefore, our results strongly suggest that IL-4, by inhibiting Fas expression and maintaining Bcl-2 expression following T cell activation, controls CD8+ T cell longevity and that it might be an important factor involved in the generation of long-term memory CD8+ T cells. Supporting this hypothesis, it was demonstrated that naive CD8+ T cells exposed to exogenous IL-4 for a short period during in vitro TCR stimulation have an increased ability to survive and to generate long-term memory T cells following in vivo adoptive transfer (38).

Evidence supporting the inhibitory effect of IL-4 on the acquisition of cytotoxic functions by CD8+ T cells has been provided by studies using various in vivo models. It was shown that antigen-specific CD8+ T cells from IL-4-deficient mice display superior cytolytic activities as compared with T cells from IL-4+/+ mice (14). In contrast, over-expression of IL-4 during the course of an immune response favors the development of antigen-specific CTLs having diminished perforin/granzyme-mediated cytotoxicity (14, 39, 40). The marked IL-4-mediated decrease in GrB expression by a sub-population of CD8+ T cells (Fig. 6) strongly suggests that these cells have a limited cytolytic potential because of the central role of GrB in granule-mediated cytolysis (41). Supporting this possibility, Kienzle and colleagues have demonstrated, using a murine T cell clonal culture system, that TCR stimulation of naive CD8+ T cells in the presence of IL-4 for several days induces a sub-population of CD8+ T cells expressing low levels of perforin and Granzyme mRNA that possess limited cytolytic functions (11, 42). Similar results were previously reported by another group using a different experimental system (43). Interestingly, in these three studies, the decrease in cytolytic functions was associated with a marked down-regulation of CD8 expression at the surface of the T cells, a phenomenon that was not observed in our experiments. This discrepancy could be due to intrinsic differences between mouse and human T cells or to differences in the model/experimental settings used to perform the experiments, most notably the length and duration of the cultures. However, we demonstrate that activated CD8+ T cells that fail to induce GrB expression also fail to up-regulate CD25, a marker normally expressed by T cells following TCR stimulation. Interestingly, the IL-4-induced loss of CD25 expression at the surface of these cells was not due to an abortive stimulation since these cells were induced to proliferate at levels comparable to those of CD25+ CD8+ T cells (at least up to six rounds of cell proliferation), but most probably due to an ability to induce the pathway leading to transcription of the CD25 gene or to the inhibition of cell-surface expression of CD25. Further studies will be required to test whether other activation pathways are modulated in CD8+ T cells activated in the presence of IL-4.

There is mounting evidence that distinct subsets of CD8+ T cells having different functional properties (termed Tc1 and Tc2), analogous to their CD4 counterparts (Th1 and Th2), exist in both rodents and humans. The differentiation of these subsets appears to be mainly influenced by the presence of different cytokines in the T cell microenvironment, with IFN-γ and IL-12 promoting the development of Tc1 cells and IL-4 inducing the differentiation into Tc2 cells (44, 45). Vukmanovic-Stijeci et al. have shown that Tc2 T cell clones (i.e. generated in the presence of IL-4) are more resistant to AICD and express higher levels of CD40L, leading to the hypothesis that these cells could provide help to B cells (46, 47). Therefore, addition of exogenous IL-4 during CD8+ T cell stimulation in our system probably promotes the differentiation of CD8+ T cells into Tc2-like cells possessing distinct functional properties as compared with ‘classical’ type 1 CTLs. By virtue of their diminished cytolytic functions and increased resistance to AICD, these Tc2 cells might play a regulatory role or induce other immune defense mechanisms during the immune response, as opposed to the direct killing of target cells mediated by Tc1 cells, thereby contributing to the diversity of effector functions during an immune response. Further studies on IL-4-mediated CD8+ T cell differentiation should lead to a deeper understanding of the function of this AICD-resistant, low cytolytic CD8+ CD25− T cell sub-population during the immune response.

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Abbreviations

AICD activation-induced cell death
CFSE 5,6-carboxyfluorescein diacetate succinimidyl ester
CIHR Canadian Institutes of Health Research
DOC deoxycholic acid
GrB Granzyme B
[3H]Tdr [3H]-thymidine
PI propidium iodide
PVDF polyvinylidene difluoride

References

of high levels of IL-2 or gamma interferon.
not inhibit the development of RSV-specific memory cytotoxic cells.
of respiratory syncytial virus (RSV) and interleukin-4 (IL-4) does not inhibit the development of RSV-specific memory cytotoxic T lymphocytes, whereas priming is diminished in the presence of high levels of IL-2 or gamma interferon.


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