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Cellular FLIP (Long Isoform) Overexpression in T Cells Drives Th2 Effector Responses and Promotes Immunoregulation in Experimental Autoimmune Encephalomyelitis

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Cellular FLIP (c-FLIP) is an endogenous inhibitor of death receptor-induced apoptosis through the caspase 8 pathway. It is an NF-κB-inducible protein thought to promote the survival of T cells upon activation, and its down-regulation has been implicated in activation-induced cell death. We have generated transgenic mice overexpressing human c-FLIP long form (c-FLIPL) specifically in T cells using the CD2 promoter (TgFLIP L). TgFLIPL mice exhibit increased IgG1 production upon stimulation by a T cell-dependent Ag and a markedly enhanced contact hypersensitivity response to allergen. In addition to showing augmented Th2-type responses, TgFLIP L mice are resistant to the development of myelin oligodendrocyte glycoprotein 35–55 peptide-induced experimental autoimmune encephalomyelitis, a Th1-driven autoimmune disease. In vitro analyses revealed that T cells of TgFLIP L mice proliferate normally, but produce higher levels of IL-2 and show preferential maturation of Th2 cytokine-producing cells in response to antigenic stimulation. After adoptive transfer, these (Th2) cells protected wild-type recipient mice from experimental autoimmune encephalomyelitis induction. Our results show that the constitutive overexpression of c-FLIPL in T cells is sufficient to drive Th2 polarization of effector T cell responses and indicate that it might function as a key regulator of Th cell differentiation. The Journal of Immunology, 2004, 173: 6619–6626.

Strict regulation of peripheral T cells responses is important for control of the adaptive immune response to prevent the development of autoimmunity and prolonged responses to exogenous Ags that might lead to immune pathology. Classical mechanisms of apoptosis counterbalance proliferation and differentiation pathways to shape appropriate and timely effector T cell responses after TCR stimulation, which must regress thereafter, but allow for memory cell formation when inflammatory cues coexist. Death receptors (DR) of the TNF receptor superfamily are known to be important for the regulation of lymphocyte homeostasis and immune function (1, 2). Fas and TNF receptor type I are responsible for mediating TCR-induced apoptosis in activated lymphocytes (3–5) by a process known as activation-induced cell death (AICD) (6). There is also good evidence that DR-signaling pathways play critical roles in T cell activation and proliferation (7, 8). It has been shown that Fas costimulates proliferation and cytokine production in resting T cells upon activation of the TCR (9) and that downstream signaling components such as Fas-associated death domain protein (FADD) (10–13) and caspases 3 and 8 (14–17) are required for this effect. Therefore, efficient molecular switch mechanisms are necessary for regulating the outcome of bifunctional DR signaling pathways during T cell activation.

Cellular FLIP (c-FLIP) is an endogenous inhibitor of the FADD-caspase 8 proapoptotic signaling pathway downstream of DR (18), which is induced upon NF-κB activation as part of an antiapoptotic program (19, 20). It exists in long (c-FLIPL) and short (c-FLIPS) isoforms, both of which form heterodimers with procaspase 8 within the death-inducing signaling complex (DISC) and block its activation (21). c-FLIPL interaction completely blocks caspase 8 processing, whereas c-FLIPS engagement allows partial processing of both caspase 8 and c-FLIPL, which remain bound to the DISC as 43-kDa forms, thereby preventing the recruitment and processing of additional procaspase 8zymogens (21). Recent evidence indicates that c-FLIPL not only functions to block caspase 8 activation, but also positively signals the activation of NF-κB and MAPK ERK pathways (22, 23). c-FLIPL may therefore serve as a modulator between apoptotic and proliferative responses downstream of DR in T cells after TCR stimulation.

The biological role of c-FLIP is poorly understood, mainly because of embryonic lethality associated with the targeted deletion of c-FLIP in mice (24). c-FLIP-deficient cells are specifically sensitive to DR-mediated apoptosis, confirming its importance for cytotoxic protection (24). Studies in which c-FLIP was overexpressed in bone marrow cells of mice using retroviral vectors led to accumulation of activated B cells, autoantibody production, and development or exacerbation of autoimmune disease (25, 26). However, the specific contribution of c-FLIP overexpression in T cells to...
c-FLIP<sub>L</sub> PROMOTES IMMUNE DEVIATION IN EAE

Materials and Methods

Generation of c-FLIP<sub>L</sub> transgenic mice

The SalI-Xhol fragment (1.5 kb) encoding human c-FLIP<sub>L</sub> cDNA and an in-frame 3′ c-Myc tag (provided by Dr. C. Vincenz, University of Michigan Medical School, Ann Arbor, MI, and Dr. V. Dixit, Genentech, South San Francisco, CA) (28) were inserted in a human CD2 promoter cassette at the EcoRI site (provided by Dr. D. Kiossis, National Institute for Medical Research, London, U.K.) (29). The 12.7-kb SalI-Xhol fragment was microinjected into pronuclei of F1 (CBA×C56BL/6) fertilized oocytes as previously described (30). Two transgenic lines were produced (TgFLIP<sub>L</sub>) carrying and expressing approximately five (Tg36) and one (Tg39) copies of the transgene, as estimated by Southern and slot blot hybridization analyses, and were backcrossed for at least five generations into the C57BL/6 background. Similar results were obtained from the two lines in basic functional characterization analyses, and heterozygous mice from the higher expressing Tg36 line and their non-Tg control littermates (NCL) were used for the experiments described in this study unless otherwise stated. Mice were kept under specific pathogen-free conditions in the animal unit of Hellenic Pasteur Institute. All animal procedures were approved by national authorities and conformed to European Union guidelines.

Immunoprecipitation and Western blot

Thymocytes, splenic CD3<sup>+</sup> T cells, and enriched splenic B cells were isolated from naive mice and lysed in 50 mM Tris-HCl (pH 7.4), 250 mM sucrose, 1 mM EDTA, 1 mM EGTA, 10 mM NaF, 1% Triton X, 1 mM benzamidine, 10 μg/ml aprotinin, 1 mM sodium orthovanadate, and 0.2 mM PMSF. Total protein lysates (300 μg) were incubated with 5 μg of anti-c-FLIP<sub>L</sub> Ab (from Covance Research Products, Berkeley, CA) overnight at 4°C, followed by 50 μl of protein A-Sepharose slurry (Sigma-Aldrich) for 3 h. Protein-Ab complexes were analyzed by SDS-PAGE and Western blot using anti-human FLIP mAb (I/1000; NF6; Alexis, San Diego, CA), HRP-conjugated goat anti-mouse IgG(L) (1/500; Jackson Immunoresearch Laboratories, West Grove, CA), and the ECL Plus detection system (Amersham Biosciences, Arlington Heights, IL). Splenic B cells were enriched by C-mediated lysis of T cells using anti-CD3 mAb (clone 145-2C11; BD Pharmingen, San Diego, CA), followed by guinea pig Ab (Serion Immunodiagnostics, Worbzum, Germany). Western blots of tissue protein lysates (100 μg) were analyzed using anti-human usorin Ab (gift from Dr. D. Nicholson, Merck Frosst Centre for Therapeutic Research, Montreal, Canada) and anti-β-tubulin Ab (BD Pharmingen) to control for loading.

Apoptosis assays

Thymocytes were plated at 2 × 10<sup>6</sup> cells/well in 24-well plates and incubated with increasing concentrations of Jo2 anti-Fas mAb (BD Pharmingen) for 6 h. Apoptotic cells were identified with bioxin-annexin V (BD Pharmingen), followed by FITC-streptavidin, and were quantitated with a FACS-Calibur using CellQuest software (BD Biosciences, Mountain View, CA). Specific apoptosis was calculated using the following formula: (% experimental apoptosis – % spontaneous apoptosis)/(100 – % spontaneous apoptosis) × 100. For assessment of AICD, splenic CD3<sup>+</sup> T cells were enriched using affinity columns (R&D Systems, Minneapolis, MN), plated at 2 × 10<sup>6</sup> cells/ml in flat-bottom, 96-well plates, and stimulated with 1 μg/ml plate-bound anti-CD3 mAb (145-2C11; BD Pharmingen) and 20 U/ml murine IL-2 (Roche, Indianapolis, IN) for 24 h, followed by 50 U/ml IL-2 for 24 h. Viable cells were recovered by Histopaque-1077 (Sigma-Aldrich, St. Louis, MO) density centrifugation, restimulated with 4 μg/ml plate-bound anti-CD3 mAb for 24 h, and stained with annexin V/propidium iodide for the identification of apoptotic cells.

T cell priming and proliferation assays

Isolated mononuclear splenocytes were stimulated in triplicate at 2 × 10<sup>6</sup> cells/ml in flat-bottom, 96-well plates coated with anti-CD3 mAb (0.0625–1 μg/ml) for 72 h. Cells were pulsed with 1 μCi/5 × 10<sup>5</sup> cells [H]thymidine (ICN Radiochemicals, Irvine, CA) for the last 16 h of culture, and [H]thymidine incorporation was measured by liquid scintillation counting (Wallac, Turku, Finland). Alternatively, T cells were primed in vivo by immunizing mice s.c. with 50 μg of myelin oligodendrocyte glycoprotein 35–55 peptide (MOG<sub>35–55</sub>) or 100 μg of chicken egg OVA (Sigma-Aldrich) emulsified in CFA (Sigma-Aldrich) supplemented with 800 μg of H37Ra Mycobacterium tuberculosis (Difco, Detroit, MI). Draining lymph nodes (LN) were removed at different times after immunization, and isolated cells were cultured for 72 h in 96-well plates in RPMI 1640 (In vitrogen Life Technologies, Gaithersburg, MD) containing 10% heat-inactivated FCS, 50 μM 2-ME, and increasing concentrations of MOG<sub>35–55</sub> or OVA. Cell proliferation was measured as described above. Results are expressed as the stimulation index (ratio between radioactivity counts of cells cultured in presence of Ag and cells cultured with medium alone).

Measurement of cytokines

IL-4 and IFN-γ ELISA kits (Pierce, Rockford, IL) were used to measure cytokine secretion from LN cell culture supernatants according to the manufacturer’s instructions. The limits of detection for IL-4 and IFN-γ ELISAs were <5 and <10 pg/ml, respectively. In addition, the mouse Th1/Th2 cytokine cytometric bead array kit (BD Biosciences) was used to measure cytokine levels in culture supernatants according to the manufacturer’s instructions.

Contact hypersensitivity response

Mice were sensitized by application of 100 μl of 4% 4-ethoxymethylene-2-phenyl-2-oxazolin-5-one (oxazolone; Sigma-Aldrich) in acetone to the shaved abdomen and air-drying. After 5 days, both sides of one ear of sensitized or naive mice were painted with 50 μl of 1% oxazolone in acetone, and the other ear was treated with 50 μl of vehicle alone. Ear thickness was measured 24 and 48 h postchallenge using an electronic caliper gauge (Dyer, Lancaster, PA). Ear swelling was calculated as the difference in thickness between the oxazolone-painted and the vehicle-painted ears.

Ab responses to T cell-dependent Ag

Mice were immunized i.p. with 50 μg of trinitrophenyl-keyhole limpet hemocyanin (TNP-KLH; Biosearch Technologies, Novato, CA) in CFA and boosted i.p. with 5 μg of TNP-KLH 28 days later. Mice were bled from the tail under a thermal source 7, 20, and 35 days after priming, and sera were collected for measurement of anti-TNP Abs by ELISA as previously described (31).

EAE induction and evaluation

EAE was induced by s.c. tail base injection of 150 μg of MOG<sub>35–55</sub> emulsified in CFA supplemented with 800 μg of H37Ra M. tuberculosis (Difco) on days 0 and 7. Mice also received an i.p. injection of 200 μg of pertussis toxin (Sigma-Aldrich) on days 0 and 2. Mice were assessed daily for clinical signs according to the following scale: 0, normal; 1, limp tail; 2, hind limb weakness; 3, hind limb paralysis; 4, forelimb paralysis; 5, moribund or dead (0.5 gradations represent intermediate scores). Mice were allowed free access to food and water throughout the experiment.

Adaptive transfer

For adoptive transfer of TgFLIP<sub>L</sub> T lymphoblasts, transgenic mice were immunized with 150 μg of MOG<sub>35–55</sub> and 10 days later splenocytes and LN cells were pooled and boosted in culture with 20 μg/ml MOG<sub>35–55</sub> for 72 h. After extensive washes, 7 × 10<sup>6</sup> cells were transferred i.p. 4 h before active EAE induction, and mice were followed for clinical signs of the disease.

Histopathological analysis

Mice were transcardially perfused with ice-cold 4% paraformaldehyde in PBS under deep anesthesia. CNS tissues were postfixed in the same fixative.
for 3 h at 4°C and processed for standard histopathological analysis. Inflammation was visualized by staining with H&E, whereas demyelination was demonstrated by a Luxol Fast Blue/periodic acid-Schiff stain.

**Statistical analysis**

Data were evaluated using Student’s t test, and p < 0.05 was considered significant. Data are reported as the average of all observations ± SEM.

**Results**

**c-FLIPL overexpression in T cells inhibits Fas-mediated cell death, but does not alter AICD after anti-CD3 stimulation**

All the evidence available to date concerning the biological function of c-FLIPL has linked it to the FADD-caspase 8 signaling pathway, specifically as an inhibitor of caspase 8-mediated apoptosis and more recently as a positive mediator of activation signals in T cells. The physiological significance of regulated levels of c-FLIPL levels during T cell activation, however, is not known. To study the role of c-FLIPL in T cell function and in the development of Th1 and Th2 polarized immune responses in vivo, we generated two transgenic lines in which c-FLIPL was expressed specifically and constitutively in T cells of the C57BL/6 mouse strain under the direction of the human CD2 promoter and enhancer (Tg-FLIPL mice). Immunoprecipitation (Fig. 1a) and Western blot (Fig. 1b) analyses of protein extracts from enriched cell populations and whole tissues showed T cell-specific expression of the transgene in the Tg36 line, whereas expression in the single-copy Tg39 transgenic line was undetectable by these methods. Expression of the transgene in both lines was confirmed by functional analyses, which demonstrated that thymocytes from c-FLIPL transgenic mice are resistant to Fas-induced apoptosis compared with NCL (Fig. 1, c and d). Mice from both lines developed normally, with no obvious phenotypic abnormalities. The structure and histology of all organs appeared normal, and lymphocyte populations in the thymus, LN, and spleen were normal, as determined by FACS analysis, although a trend toward a decreased CD3+ population in spleen of Tg36 mice was noted (data not shown). Also, AICD in response to repeated stimulation of the TCR with anti-CD3 mAb was normal in transgenic T cells (data not shown). These results are in full agreement with those previously reported for transgenic T cells constitutively overexpressing a murine c-FLIPL transgene (27) and add support to evidence that c-FLIPL is not important for the regulation of AICD.

**Altered help of c-FLIPL-expressing T cells to Ab production in response to a T cell-specific Ag**

To determine the functional significance of c-FLIPL in T cell responses in vivo, we first studied T cell help to Ab production in response to immunization with the T cell-specific Ag TNP-KLH. TgFLIPL mice showed efficient switching from IgM to IgG production. Generally, higher levels of IgM were observed in the transgenic mice by day 20 postimmunization and were maintained through day 35 (Fig. 2a). No changes were detected in the total amount of secreted IgG Ab, but there was an increase in the production of IgG1 Ab isotype by TgFLIPL mice after both primary (day 0) and secondary (day 28) TNP-KLH immunizations compared with NCL mice (Fig. 2c). An early and transient increase in IgG2a production was also noted (Fig. 2d, day 7). Otherwise, levels of IgG2a, IgG2b, and IgG3 in TgFLIPL mice were normal. The finding that TgFLIPL mice produce higher amounts of IgG1, which is considered the major IgG isotype preferentially induced by IL-4-secreting Th2 cells, after IgM to IgG switching occurs indicates that c-FLIPL overexpression in T lymphocytes might alter effector T cell cytokine production.

**T cells from TgFLIPL mice shape more efficient contact hypersensitivity responses**

To further investigate whether TgFLIPL T cells show a Th2 bias in vivo, we challenged transgenic mice with the chemical hapten oxazolone, which was used to generate a contact hypersensitivity reaction that is mediated predominantly by Th2 cytokines (32). Ear swelling of oxazolone-sensitized mice was significantly increased in transgenic compared with NCL groups at 24 and 48 h after ear challenge (Fig. 3). We also noted, in two independent experiments, enhanced ear swelling of nonsensitized TgFLIPL at the 24 h point, although the reason for this is not known (Fig. 3a). The enhanced contact hypersensitivity response to oxazolone in c-FLIPL transgenic mice provides evidence that c-FLIPL expression enhances the Th2 polarization of activated T lymphocytes and primes a more robust and persistent allergic reaction.

**TgFLIPL mice are resistant to MOG-EAE**

EAE induced in C57BL/6 mice by immunization with the MOG35–55 peptide is a chronic, initially relapse-remitting, and subsequently progressive autoimmune disease targeting the CNS and is a widely accepted animal model for multiple sclerosis (33). Disease is driven by myelin-specific Th1 effector cells, is largely Ab-independent, and is characterized by inflammation and primary demyelination of spinal cord and brain tissues caused mainly by activated macrophages, eventually leading to irreversible axonal damage and neuronal degeneration (33). We were interested to test the sensitivity of TgFLIPL mice to MOG-EAE to determine whether c-FLIPL expression by T cells would also enhance Th1 responses.

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**Figure 1.** T cell-specific expression of the c-FLIPL transgene blocks Fas-mediated apoptosis of thymocytes. a, Immunoprecipitation of cellular extracts from TgFLIPL and NCL mice with anti-Myc Ab, detected using anti-human FLIP Ab (top row). Coomassie Blue-stained cellular extracts are shown (bottom row) that correspond to 1/38th of the cell lysate used to perform the immunoprecipitation. b, Western blot of total protein lysates from lymphoid tissues of TgFLIPL and NCL mice detected using anti-human usurpin and anti-mouse β-tubulin Ab. Functional analyses (c and d) showed resistance to Jo2 anti-Fas mAb-mediated apoptosis of TgFLIPL thymocytes (n = 2) compared with NCL (n = 2), as demonstrated by annexin V immunostaining. The experiment was performed for both Tg36 (c) and Tg39 (d) lines.
immune responses or whether it would continue to drive Th2 polarization even under strongly Th1-polarizing conditions. Immunization of NCL mice with MOG35–55 in CFA resulted in typical MOG-EAE, showing 100% penetrance and severe clinical symptoms reaching maximal scores of 3.5 (Fig. 4a). In the initial stages of disease, clinical remissions were incomplete, and at later stages, disease became nonremitting. In contrast, c-FLIPL mice showed resistance to the development of MOG-EAE (Fig. 4a). The initiation of disease was delayed, and the clinical course was much milder compared with NCL, even though disease penetrance remained high (87.5%). Moreover, remissions were characterized by complete resolution of disease. In a small number of mice, disease became nonremitting in the chronic phase. Overall, TgFLIPL mice did not develop the severe wasting symptoms and neurological defects that are associated with EAE in wild-type mice and were characterized by a much healthier appearance (Fig. 4b).

To determine whether EAE in TgFLIPL mice showed the typical Th1-mediated inflammation or features of Th2-mediated inflammation such as polymorphonuclear cell infiltrates, mice with the maximal clinical score from each group, but not representative of the mean clinical scores, were chosen for histological examination. CNS tissues from NCL showed lesions characteristic of MOG-EAE, with meningeal inflammation and perivascular cuffs consisting mainly of activated macrophages in both brain and spinal cord, and perivenuous and subpial demyelination. These lesions could be seen at both early (day 17) and late (day 78) disease time points (Fig. 5, a–c, g, and h). Interestingly, when present, disease in TgFLIPL mice also showed the typical features of MOG-EAE, with no evidence for a Th2-type inflammatory reaction (Fig. 5, d–f). Tissues taken at late time points (day 78) from transgenic mice in which disease had developed and resolved were normal, with no signs of inflammation or demyelination (Fig. 5i). These data clearly show that c-FLIPL expression in T cells protects mice from the development of EAE, significantly reducing the severity of disease and facilitating complete resolution at later time points.

**TgFLIPL** T cells display normal priming, but abnormally prolonged Ag-specific reactivity in vivo

Previous studies have shown that c-FLIPL can influence TCR-mediated activation of ERK and NF-κB signaling pathways (22), so we examined whether the immune deviation observed in TgFLIPL mice in vivo could be associated with alterations in T cell proliferation in response to TCR activation. Splenocytes isolated from naive TgFLIPL mice proliferated normally after stimulation of the TCR/CD3 receptor complex using plate-bound anti-CD3 mAb (Fig. 6a). In addition, TgFLIPL LN cells, isolated from mice
10–13 days after immunization with MOG$_{35-55}$ or OVA, showed the same degree of proliferation as control mice when restimulated with Ag in vitro (Fig. 6, b and c). Interestingly, the proliferative responses of TgFLIP$_L$ LN cells isolated 50 days after immunization with MOG$_{35-55}$, and thus reflecting the behavior of long term surviving Ag-specific lymphocytes, were significantly enhanced compared with that of NCL cells (Fig. 6d), indicating that c-FLIP$_L$ overexpression might act to extend the survival of effector Ag-specific T cell pools or enhance memory cell formation.

Enhanced early cytokine production and skewing toward Th2 differentiation in T cells from TgFLIP$_L$ mice

To examine whether altered cytokine production by c-FLIP$_L$ T cells could be responsible for the Th2-biased phenotypes observed in vivo and for resistance to EAE, we measured cytokine production by transgenic T cells in response to stimulation. TgFLIP$_L$ LN cells showed enhanced IL-2 production compared with NLC mice and altered balance of Th1/Th2 cytokines. Specifically, autoantigen-stimulated TgFLIP$_L$ LN cells showed a modest increase in the production of the Th2 cytokines IL-4 and IL-5, accompanied by reduced production of the Th1 cytokines TNF-α and IFN-γ at both early and late time points after initial MOG$_{35-55}$-specific T cell maturation (Fig. 7). This finding indicates that the constitutive expression of c-FLIP$_L$ in T cells during their activation induces a shift toward Th2 differentiation. To exclude the possibility that c-FLIP$_L$ T cells have an intrinsic defect in IFN-γ production, we measured cytokine production by T cells purified from naive mice and stimulated for 24 h with 1 μg/ml plate-bound anti-CD3. TgFLIP$_L$ T cells efficiently produced IL-2, IFN-γ, and IL-4 (Fig. 7). Taken together, these findings suggest that the constitutive expression of c-FLIP$_L$ in T cells during activation allows the FADD-caspase 8 DR signaling pathway to mediate increased early cytokine secretion and to promote a Th2 differentiation program.
Adoptive transfer of TgFLIPL MOG35–55-specific lymphoblasts prevents induction of EAE

We examined directly whether TgFLIPL MOG35–55-specific Th2 cells could serve as immunosuppressive cells in vivo. By adoptive transfer, we tested whether TgFLIPL MOG35–55-specific lymphoblasts could protect wild-type recipient mice from EAE development. Splenocytes were isolated from MOG35–55-immunized Tg-FLIPL mice and then boosted in vitro with peptide. Cytokine analysis confirmed a Th2 bias for TgFLIPL lymphocytes, whereas lymphocytes isolated from wild-type mice showed a Th1 bias (data not shown). After adoptive transfer of TgFLIPL MOG35–55-specific lymphoblasts or PBS to produce a baseline disease control, recipient wild-type mice were immunized for EAE induction. All control mice developed severe EAE (Fig. 8). In contrast, recipient mice of TgFLIPL donor lymphocytes developed a delayed and milder form of EAE. TgFLIPL-specific MOG35–55-specific cells significantly inhibited the development of EAE in wild-type recipients, causing a 4-day delay in the onset of symptoms, and reducing the clinical score and penetrance of EAE from 100% to 50% over the study period (Fig. 8). These adoptive transfer studies provide evidence that MOG35–55-specific, c-FLIPL-expressing T cells differentiate into suppressive T cells in vivo that have the potential to ameliorate autoimmune disease.

Discussion

c-FLIPL is expressed at high levels in naive resting T cells and during early stages of activation when these cells are resistant to apoptosis (34). c-FLIPL expression is down-regulated when T cells become susceptible to FasL-mediated apoptosis, and this process is regulated by IL-2 (35). However, there is evidence that apoptosis signaling through the FADD-caspase 8 pathway is not essential for AICD. Mice in which caspase 8 is conditionally deleted in T cells (17), and transgenic mice constitutively overexpressing c-FLIPL (Ref. 27 and this study) display normal T cell AICD. Other evidence indicates that the expression of c-FLIPS, rather than c-FLIPL, is associated with the regulation of Fas-mediated death of activated T cells (36). The physiological role of c-FLIPL during T cell activation, therefore, remains to be determined.

In this study we show that c-FLIPL expressed in T lymphocytes, in addition to blocking DR-mediated cell death, can alter cytokine expression to promote Th2 differentiation and immune regulation of the Th1-mediated autoimmune disease EAE. Mice overexpressing a human c-FLIPL transgene specifically in their T cells display strong hypersensitivity reactions to the contact allergen oxazolone.
The mechanism by which incoming TCR-triggered signals are modulated by DR signaling is not yet clear. One possibility is that DR-mediated signals directly regulate TCR signaling components. A second possibility is that DR-mediated signals cross-talk with costimulatory signals from members of the CD28 family such as CD28 or ICOS, both of which have been implicated in Th2 differentiation (48, 49). Indeed, naïve T cells gain the capacity for Fas-dependent costimulation when they are activated in the presence of CD28 costimulation or T cell differentiation cytokines, such as IL-4 or IL-12 (50). Third, c-FLIP$_L$ engagement at the DISC can divert apoptotic signals to NF-$\kappa$B and ERK signaling pathways by the recruitment of TNF-associated factors 1 and 2 and receptor-interacting protein to the DISC (22). NF-$\kappa$B activity has been shown to be required for development of the Th2 cell phenotype and allergic airway inflammation in mice and to augment the expression of GATA-3 (51, 52). Taken together, the available evidence suggests that c-FLIP$_L$ expression in newly activated T cells may influence T cell maturation toward a Th2 phenotype through two interrelated mechanisms: a direct antiapoptotic effect that extends T cell life and a positive signaling effect that sustains T cell activation.

Our results demonstrate that c-FLIP$_L$ can play an important role in the control of autoimmunity through the regulation of cytokine production and T cell differentiation. It will be interesting to establish whether this mechanism can be regulated by DR ligands expressed at sites of T cell priming, immune privilege, or inflammation. Indeed, TNF can down-regulate autoimmune responses when overexpressed systemically or at target sites, such as pancreatic $\beta$-cells in transgenic mice, and this is associated with the increased secretion of Th2 cytokines (53). The novel function of c-FLIP$_L$ as a modulator of the quality of effector T cell responses alters the significance of DR-mediated apoptosis in the immune system and hints that failure of T cells to undergo caspase 8-mediated apoptosis is not always associated with deleterious events altering T cell homeostasis, but may, instead, prime them for a regulatory phenotype. The physiological requirements for such a regulation and the potential use of this knowledge in clinical applications should be subjects of further research.

Acknowledgments

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