**Cell Death and Differentiation (1998) 5,** 539–546

http://www.stockton-press.co.uk/cdd

**Meeting Report**

# DATELINE Aix-les-Bains

**Thomas Brunner[1,2] and Laurent Genestier[3]**

[1]Institute for Pathology, University of Berne, 3010 Berne, Switzerland; [2]La Jolla Institute for Allergy and Immunology, San Diego, CA 92121, USA;
[3]Corresponding author Thomas Brunner, Department of Immunopathology, Institute for Pathology, University of Berne, Murtenstr. 10, 3010 Berne, Switzerland, e-mail: brunnert@patho.unibe.ch

**INSERM Philippe Laudat Conference on 'Activation-induced cell death and peripheral tolerance: prospects for therapeutic intervention'**
Aix-les-Bains, France, November 2–6, 1997

**Abbreviations:** DR3, death receptor 3; TNF-RI, TNF receptor 1; ASM, acidic sphingomyelinase; tTG, tissue transglutaminase; FLIP, FLICE-inhibitory protein; FLAME, FADD-like anti-apoptotic molecule; AICD, activation-induced cell death

While our body is exposed daily to an enormous number of antigens and potentially harmful pathogens, our immune system is, under normal circumstances, capable of defending the organism and eliminating such intruders efficiently. Thus, disease such as those mediated by persisting bacterial or viral infections are relatively rare or only short lasting. However, it also becomes obvious from this observation that immune responses and in particular those mediated by immunocompetent cells, have to be terminated as quickly and efficiently as they have been initiated, to avoid chronic inflammation resulting in tissue destruction and potentially in autoimmune disease. While immunological research has come up with different potential control mechanisms for any given immune reaction, none of them appears to be universal. Antigen-specific induction of unresponsiveness in B and T lymphocytes has been recognised by researches already decades ago as a mechanism of immune regulation. The idea that immune responses can be regulated through antigen-specific induction of apoptotic cell death has evolved only in the last ten years. While both mechanisms are under intense investigation it is still poorly defined and understood to what extent tolerance and activation-induced cell death co-operate in the regulation of immune homeostasis, whether they represent separate mechanisms or might be even identical under certain conditions. These issues have been recently discussed at the INSERM Philippe Laudat conference held November 2–6 1997 in Aix-les-Bains, France, organised by J.-P. Revillard, M. Goldman, D. Kabelitz and H. Waldmann.

## Basic mechanisms of cell death

While most immunologists are investigating cell death of single cells in a multicellular organism, such as the human body, P. Golstein (Marseilles, France) presented evidence that common mechanisms of apoptotic cell death apply also to unicellular organisms. Upon shortage of food supply Dictyostelium single cell amoebae aggregate to form a sporangium and spores that can restart the Dictyostelium life cycle again. During the process of sporangium formation cells of the stem appear to undergo a form of cell death that resembles in many ways apoptotic cell death observed in multicellular organisms, like chromatin and cytoplasmic condensation whereas the lack of phagocytes to remove apoptotic cells appears to be compensated through autodigestive vacuoles of the dying cells (Cornillon *et al*, 1994). Thus, in the case of Dictyostelium single cells appear to cooperate in the formation of a temporary multicellular structure and undergo cell death in order to maintain the species.

Given the fact that many different cell types undergo similar forms of apoptosis and that common pathways of signal transduction have been defined, the question of the central mechanisms of apoptosis has been posed. G. Kroemer (Villejuif, France) discussed in a 'heptalogue' seven requirements for a central executioner of apoptosis and discussed evidence that mitochrondria might fulfil these requirements (Kroemer *et al*, 1997). Mitochondrial permeability transition is common to apoptotic cell death induced by all triggers and precedes morphological changes of apoptosis. Thus different signals appear to converge at the level of mitochondria which co-ordinate further downstream signals leading to the different manifestations of apoptosis events. The universal requirements of the mitochondria for energy production in eukaryotic cells thus inseparably connects life and apoptotic cell death in one cellular compartment and ensures a common pathway of apoptotic cell death The idea that the release of cytochrome C from the mitochondria represents such a converging event of common apoptotic pathways is further supported by the finding that inhibitors of apoptosis, such as Bcl-2 and $Bclx_L$ are located at the outer mitochondrial membrane where they control the release of cytochrome C and thus further downstream events (Golstein, 1997; Kluck *et al*, 1997; Yang *et al*, 1997).

Still controversial in this unifying picture of the role of mitochondria in apoptotic cell death is the role of Bcl-2 in CD95-induced cell death: while many scientists have found no protective role for Bcl-2 in CD95-induced cell death, indicating the possibility that alternative pathways of apoptotic cell death might bypass the mitochondria (Memon *et al*, 1995; Strasser *et al*, 1995), others have

observed protection (Asoh and Ohta, 1997; Itoh *et al*, 1993; Weller *et al*, 1995). Thus, no rule without exception, and no exception without possible explanation. P. Krammer (Heidelberg, Germany) discussed that CD95, DR (death receptor) 3, TRAIL receptor 2 (DR5) and TNF receptor I (TNF-RI) all rapidly activate FLICE (caspase 8) through oligomerization of their intracellular death domains upon receptor triggering, leading to a proteolytically active FLICE that can directly activate further downstream caspases, like CPP32 (caspase 3), thus bypassing the requirement for the mitochondria. Similar to the death domain containing receptors, cytotoxic granules and in particular granzyme B might also directly cleave FLICE and thus initiate apoptotic cell death through this common pathway (P. Krammer).

In favour of a protective role for Bcl-2 in CD95-induced apoptosis were the results presented by R. Testi (Rome, Italy). He pointed out that CD95 ligation leads to an early activation of PC-PLC and acidic sphingomyelinase (ASM) and production of ceramide in a death-domain and caspase (caspase 8?) dependent way. Cells that lack ASM activity, such as those from patients with Nieman-Pick disease, appear to be less sensitive to CD95 crosslinking and have delayed mitochondrial permeability transition but show normal CD95-induced cell death after reconstitution with ASM. Ceramide is a precursor of GM3 ganglioside which can be converted to GD3 ganglioside by the ST8 sialyltransferase. Both CD95 ligation and ceramide addition lead to transient GD3 synthesis, and GD3 and overexpression of ST8 induce apoptosis and altered mitochondrial permeability transition (De Maria *et al*, 1997). The ASM pathway might thus represent a parallel and/or amplifying pathway to the direct activation of the caspase cascade after CD95 ligation and might connect the CD95 induced apoptotic pathway to the control of the mitochondria. As shown previously, apoptotic cell death induced by direct ceramide addition is blocked by Bcl-2 (Martin *et al*, 1995). To what extent the ASM pathway participates in CD95 induced apoptosis in different cell types might thus explain some of the discrepancies on the role of Bcl-2 in this death-inducing pathway.

Ceramide production as a second messenger of apoptotic pathways appears not to be unique to CD95 and TNF-RI ligation. N. Bonnefoy-Berard (Lyon, France) illustrated that antibodies to HLA class I can induce CD95 independent ceramide production and apoptotic cell death in primary activated lymphocytes. As for CD95 ligation, MHC class I induced ceramide production is blocked by the caspase inhibitor ZVAD but is enhanced by YVAD and DEVD. YVAD- and DEVD-sensitive caspases appear therefore to be not only downstream of ceramide production but also downstream of the mitochondria since both inhibitors do not block mitochondrial permeability transition.

Proteolytic cleavage of diverse cytoplasmic, membrane associated and nuclear substrates by caspases or potentially other caspase-activated proteases certainly represents a key event in apoptotic cell death leading to the distinct morphology. Yet unsolved is the question what prevents the cell from leaking and thus releasing potentially harmful autoantigens (Casciola-Rosen *et al*, 1995; Casiano *et al*, 1996). Under normal conditions apoptotic cells are efficiently phagocytosed and removed without induction of inflammation or autoimmune responses. Nevertheless, many protease substrates, cleaved during apoptotic cell death are known as potential autoimmune antigens (Casciola-Rosen *et al*, 1995; Casiano *et al*, 1996). M. Piacentini (Rome, Italy) discussed the possibility that tissue transglutaminase (tTG) activity might polymerise protease substrates thus preventing the release of antigenic peptides into circulation. The calcium- and GTP-dependent enzyme tTG is induced in the thymus upon induction of apoptosis via glucocorticoids, TCR stimulation or DNA damage. Similarly, $CD4^+$ T cells in HIV infected patients express tTG and expression is further enhanced during the progression of the disease (M. Piacentini; M.-L. Gougeon, Paris, France) (Amendola *et al*, 1996). Intriguingly, the induction of tTG during apoptotic cell death is inhibited by the caspase inhibitor ZVAD. Overexpression of tTG in fibroblasts sensitises the cells to TNF$\alpha$ and also induces spontaneous cell death. Although it is currently difficult to elucidate whether tTG expression represents only an epiphenomenon or is unseparably linked to apoptotic cell death and to proper elimination of dying cells, the role of tTG as a regulatory enzyme in apoptotic cell death has to be considered and should be further explored.

## Activation and inhibition of the TNF receptor family signaling cascade

In recent years a steadily increasing number of death inducing receptors with sequence homology to the TNF receptor family has been described (Wallach *et al*, 1997). Although the precise physiological role of all these receptors in apoptotic cell death in different cell types and differential stages is still unclear, evidence emerges that different receptors of this family might engage common pathways. CD95, TNF-RI, DR3 and TRAIL-R2 (DR5) all lead to a rapid, death-domain dependent activation of FLICE (caspase 8), capable of activating other caspases and further downstream events (P. Krammer, Heidelberg, Germany; M. Thome, Lausanne, Switzerland; D. Wallach, Rehovot, Israel). Caspase 8 exists in two major isoforms (a and b) which both are recruited to the receptor via death effector domain interaction of the N-terminal pro-domain with the homologous domain of the adapter molecule FADD. Why primary activated T cells are insensitive to CD95 induced apoptosis but gradually acquire sensitivity (Klas *et al*, 1993), has been subject to extensive speculations. M. Thome, P. Krammer and D. Wallach provided evidence that virally encoded (Herpes simplex and Pox virus) or cellular molecules, homologous to FLICE, might account for this resistance to CD95-induced apoptosis. Cellular FLIP (FLICE-inhibitory protein) (Irmler *et al*, 1997), also called CASH (caspase homologue) (Goltsev *et al*, 1997) or FLAME (FADD-like anti-apoptotic molecule) (Srinivasula *et al*, 1997) and by many other names, is expressed in resting T cells and persists during the first days after primary activation. Similarly, most melanomas, known to be fairly insensitive to CD95 ligation, express FLIP. Both, viral and cellular FLIP appear to block CD95 induced apoptosis through competitive binding to FADD, thus blocking

the signaling cascade at the level of FLICE (caspase 8) activation. Overexpression of viral and cellular FLIP thus blocks CD95-, TRAIL-R- and DR3-induced recruitment of FLICE to the receptor and subsequent apoptosis.

In a more generalised view, our current understanding of the signaling cascades of the death domain containing receptors of the TNF-R family (CD95, TNF-RI, DR3, DR5) induced apoptosis is coherent and integrates many of our *in vivo* and *in vitro* observations: engagement of the receptors sequesters the adapter molecule FADD via death domain interactions which subsequently recruits caspase 8 via death effector domain interaction, leading to a proteolytically active tetramer capable of engaging further downstream events in the apoptosis cascade. D. Wallach, however, reminded the audience that life (or death) of a cell might not be as simple and that many observations do not fit into this simplified view. Death domains, initially described as death transducing domains in the TNF-R family, are more ubiquitous than we thought and are found in growing numbers of molecules, like p105 NF$\kappa$B and related molecules, which to our current knowledge have little to do with apoptosis. Nonetheless, overexpression of death domains induces apoptotic cell death. Similarly, caspase 8 appears to consist of up to ten different isoforms ($\alpha$1, $\alpha$2, $\alpha$3, $\beta$1, $\beta$2, $\beta$3, $\beta$4 etc.). Although some isoforms lack functional protease regions, they nevertheless may induce cell death in some cells. Even more controversial is the unique role of TNF-R family members associated signaling molecules in transducing apoptotic cell death. Caspase 8, FADD and TRADD gene deficient mice do not show an apoptosis deficient phenotype but rather die all during embryonic development *in utero*. In addition, mice transgenic for dominant-negative FADD under the control of the proximal lck promoter, directing its expression to the thymus, do not show reduced thymic deletion but rather show accelerated negative selection. Thus, signals transduced through the CD95 and TNF-R do not only result in apoptotic cell death but must activate other fundamentally important cellular functions as well. In trying to understand these controversial results, we are thus encouraged to consider apoptotic cell death as mediated through the TNF-R family not any more as an isolated and distinct function but to ingrate these findings into a more global view of cellular activation leading either to cell death or survival (Alderson *et al*, 1993; Lee *et al*, 1997; Yeh *et al*, 1997).

## Transcriptional control of apoptotic T cell death

The transcriptional regulation of CD95-L expression has received increasing attention. TCR induced CD95-L expression is a crucial element in AICD and cytotoxic effector functions of $CD4^+$ T cells (Brunner *et al*, 1996). However, a growing number of publications demonstrate diverse additional functions of CD95-L also in non-hematopoietic cells and tissues, such as the eye, testis, tumor cells, hepatocytes, fibroblasts and probably more to be described (Brunner *et al*, 1996; French and Tschopp, 1996). Thus, it remains to be elucidated what signals regulate CD95-L expression in all these different cell types. There is increasing evidence that stress signals as induced by chemotherapeutic drugs or irradiation can induce CD95-L expression in T cells and also in non-lymphoid cells (Friesen *et al*, 1996; Fulda *et al*, 1997; Kasibhatla *et al*, 1998; Muller *et al*, 1997). P. Krammer reported that bleomycin induces CD95/CD95-L dependent apoptotic cell death in hepatomas. Bleomycin induces both expression of CD95 and CD95-L and expression co-localises on the same cell. In hepatomas drug-induced CD95/CD95-L dependent cell death appears to require the functional expression of p53 since drug induced apoptosis and CD95 expression was only observed in p53 positive cells (Muller *et al*, 1997). p53 seems to control CD95 expression via a p53 responsive element within the CD95 gene but not in the CD95 promoter.

The role of p53 in T cell apoptosis is more controversial. While DNA damage-induced apoptosis in thymocytes appears to require the expression of p53, normal susceptibility towards DNA damage is observed in mature T cells (Strasser *et al*, 1994). Similarly, T. Brunner (Bern, Switzerland) provided evidence that UV and topoisomerase II inhibitor induced apoptosis of p53-deficient T cells is comparable to those in wild type cells. In contrast, DNA damage induced apoptosis appears to be dependent on CD95 and CD95-L expression since a soluble CD95 fusion protein blocks apoptosis. DNA damage induces rapid expression of CD95-L in T cells in a process that requires the activation of the JNK pathway and of the transcription factors AP-1 and NF$\kappa$B. Sequence analysis and binding experiments further revealed that AP-1 and NF$\kappa$B bind to specific sites in the CD95-L promoter (Kasibhatla *et al*, 1998). Thus, it remains to be seen whether CD95/CD95-L dependent cell death in different cell types is differentially regulated by transcription factors such as p53.

Resting T cells are insensitive to AICD but rather acquire sensitivity after primary activation and initial proliferative phase (Klas *et al*, 1993; Russel *et al*, 1991). Thus, entry into cell cycle appears to prime T cells for AICD. The transcription factor c-Myc has been shown to be crucial for cell cycle progression and has also been implicated in cell death. T. Brunner reported that AICD in T cells is dependent on c-Myc expression. Downregulation of c-Myc expression specifically blocks activation-induced CD95-L expression whereas CD95 expression is unaffected. CD95-L seems to be a direct transcriptional target of c-Myc since c-Myc binds to a non-canonical responsive element in the CD95-L promoter. Similarly, overexpression of dominant-negative inhibitors of the transcription factor c-Myc block CD95-L promoter activity whereas overexpression of c-Myc enhances its activity. These findings are in agreement with the recent report by Hueber *et al*, that c-Myc-dependent apoptosis in serum-starved fibroblasts is dependent on CD95 and CD95-L expression (Hueber *et al*, 1997). Thus the transcriptional control of CD95-L via c-Myc might assure that only T cells in cell cycle and thus expressing c-Myc can efficiently transcribe CD95-L and thus undergo AICD.

Another transcription factor involved in T cell apoptosis is GFI-1 (T. Möröy, Essen, Germany) (Zornig *et al*, 1996). The zink finger protein GFI-1 is specifically expressed in T

cells and thymocytes within few hours after cell activation. Forced overexpression of GFI-1 in the thymus results in very low numbers of thymocytes with no cells at the CD4/CD8 double positive stage and the few remaining ones at the double negative stage. In contrast, GFI-1 overexpression in mature T cells results in protection from IL-2 withdrawal and AICD, however, it does not affect DNA damage-, dexamethasone- or CD95-induced apoptosis. Whether GFI-1 is involved in the expression of death-inducing ligands, like CD95-L or TNF, is currently unknown but is an intriguing hypothesis.

## CD95/CD95-L interactions in health and disease

While the importance of CD95/CD95-L interactions for the immune homeostasis of $CD4^+$ T cells is almost undoubted, exciting recent data indicate that the role of CD95/CD95-L interaction in health and disease might be more widespread than we initially thought (Brunner *et al*, 1996). Virus-induced loss of $CD4^+$ T cells is believed to be a major event in HIV-induced immunodeficiency syndrome (AIDS). Several reports indicate that this loss is due to apoptotic cell death of the $CD4^+$ T cells (Ameisen, 1995) and that HIV-induced CD95-L expression might be, at least in part, responsible for this apoptotic cell death (Lynch *et al*, 1996). Thus, infected and sensitised cells may not only die via autonomous CD95/CD95-L interaction but may also kill non-infected sensitised T cells activated during infection of the human host (Kameoka *et al*, 1997; Samuelsson *et al*, 1997; Westerndorp *et al*, 1995) (M. Gougeon). Since most viruses pursue a strategy of avoiding host cell death rather than inducing it, HIV induced CD95-L expression might also serve another purpose. As shown for immune privileged sites (Bellgrau *et al*, 1995; Griffith *et al*, 1995), CD95-L expression might lead to inefficient cytotoxic T cell responses against virally infected host cells, thus ensuring viral replication and spreading of the disease (Johnson, 1997). A. Badley (Ottawa, Canada) suggested even another way by which CD95-L expression might be responsible for $CD4^+$ T cell loss during HIV infection. He reported that monocytic cell lines as well as monocyte-derived macrophages can express CD95-L upon HIV infection (Badley *et al*, 1996). In lymphonodes of infected patients, CD95-L expression co-localises with macrophage markers. Since macrophages can serve as a reservoir of monocytotrophic strains and these strains induce a faster progression of the disease, it is likely that HIV-induced CD95-L expression in macrophages might contribute to the rapid loss of $CD4^+$ T cells during HIV infection (Badley *et al*, 1996).

Similar strategies of immune escape have been proposed for tumor cells (Walker *et al*, 1997). Although increased frequencies of anti-tumor cytotoxic T cells are found in lymph nodes of melanoma patients anti-tumor responses appears to be inefficient and the death of the patient is more than likely. Why melanomas are such aggressive tumors has been subject of extensive investigation and several immune escape mechanisms have been suggested. Recent data presented by P. Romero (Lausanne, Switzerland), now indicate that many melanoma cell lines and primary isolates from patients express high levels of CD95-L and that melanoma-expressed CD95-L can induce apoptosis in activated T cells. Furthermore, CD95-L expressing melanoma lines cause a more rapid death in transfer experiments in mice than CD95-L negative lines (Hahne *et al*, 1996). Reports by other groups indicate that CD95-L expression is not restricted to melanomas but many tumors derived from different tissues are capable of expressing CD95-L and might use this defense mechanism as lethal weapon against cytotoxic T cell responses (Walker *et al*, 1997).

While virus-induced and tumor cell-expressed CD95-L might account as a disease-promoting factor, can the same strategy be used to avoid disease? Starting with the initial observations by Griffith and colleagues (Griffith *et al*, 1995) and Belgrau and collaborators (Bellgrau *et al*, 1995) scientists have pursued the idea of inducing transplantation tolerance by local expression of CD95-L with enthusiasm, however with divergent results. This topic has also been the ground for intense discussions during this meeting. While some groups have found inhibited rejection of pancreatic islets by co-transplantation of CD95-L transfected myeloblasts (Lau *et al*, 1996); others found no protection from autoimmune-destruction of $\beta$-islet cells by overexpression of CD95-L but observed rather acceleration of the onset of diabetes (Allison *et al*, 1997; Chervonsky *et al*, 1997; Kang *et al*, 1997) (R. Mueller, T. Brunner, D. Green and N. Sarvetnik, unpublished results). Mixed leukocyte reactions appear to be efficiently inhibited by CD95-L overexpressing COS cells through selective apoptosis of cycling T cell blasts (R. Schwinzer, Hannover, Germany). Similarly, CD95-L positive H-$2K^b$ transgenic testis allografts are strongly infiltrated shortly after transplantation into $K^b$ T cell receptor transgenic mice but cellular infiltrates are quickly cleared by induction of apoptosis and grafts survive indefinitely (M. Hoffman, Hannover, Germany). In contrast, transplantation of CD95-L expression tumor cells lead to accelerated neutrophil-mediated rejection of the allograft and survival of the mice, whereas mice transplanted with wild type tumor died quickly (Seino *et al*, 1997). Thus, while it has been tempting to assume that localised expression of CD95-L could be therapeutically used in transplantation immunology, we first have to understand which co-factors enhance CD95-L mediated immune privilege and what factors might even promote accelerated rejection of CD95-L expression allografts.

While we have based the *in vivo* importance of CD95/CD95-L interactions in the immune homeostasis on the analysis of the CD95 (*lpr*) and CD95-L (*gld*) mutant mice, little information has been obtained so far on the pathology of genetic defects of the CD95/CD95-L system in humans. Recent data now indicate that genetic CD95/CD95-L defects in humans are quite common and may represent one of the underlying mechanisms of autoimmunity observed in these individuals. A. Fischer (Paris, France) reported of 43 cases with genetic defects in the CD95 or CD95-L gene. Heterozygous defects in the CD95 gene were found to be most common (40 cases) whereas homozygous defects in CD95 (two cases) and

heterozygous defects in CD95-L (1 case) are very rare. Approximately 75% of all mutations in CD95 are found within the death domain, leading to a defect of CD95 mediated apoptosis. Most patients develop lymphoproliferative disease within the first 5 years of life, with dramatic enlargement of the spleen and excess of $CD4^-CD8^-$ T cells in the periphery. Many patients also develop autoimmune diseases and in some cases even a higher incidence of tumor formation, such as lymphomas, adenomas and hepatomas, is observed. Thus, genetic CD95/CD95-L defects in mice and humans appear to lead to similar pathologies and further support the importance of CD95/CD95-L interactions in immune homeostasis.

## Modulation of apoptosis by cytokines and costimulatory signals

As pointed out earlier, resting T cells are insensitive to AICD but acquire sensitivity after primary activation (Klas *et al*, 1993; Russel *et al*, 1991). Thus, T cells have to enter cell cycle to reach an AICD-susceptible stage (R. McDonald, Lausanne, Switzerland). IL-2 not only promotes T cell proliferation but also enhances susceptibility to AICD (Lenardo, 1991). Thus, signals through the IL-2 receptor can control apoptotic cell death in T lymphocytes and may represent an autocrine regulatory loop (M. Lenardo, Bethesda, USA). The role of IL-2 in regulation of CD95-dependent and -independent apoptosis have been subject of various presentations at this meeting. J. Russel (St. Louis, USA) reported that some muteins of IL-2 show a dissociation of AICD and proliferation promoting activity. These mutants are still capable of interacting with the receptor and inducing proliferation but are unable to sensitise T cells for AICD. Thus, induction of cell cycle progression and susceptibility to apoptotic cell death appear to be two independent functions of IL-2, although it is currently unclear how this can be distinguished on the level of the IL-2 receptor. This idea was further supported by L. Genestier (San Diego, USA) and J.-P. Revillard (Lyon, France) who found that anti-CD95- or anti-HLA class I-induced apoptosis in human T cell blasts is blocked by cyclosporin A, FK506, anti-IL-2 or anti-IL-2 receptor (CD25). In contrast, block of G1/S transition of the cell cycle by aphidicolin does not alter susceptibility to CD95- or HLA class I-mediated apoptosis (Fournel *et al*, 1996; Genestier *et al*, 1997). The *in vivo* relevance of IL-2 induced promotion of apoptotic cell death was further underlined by A. Schimpl (Würzburg, Germany) (Kneitz *et al*, 1995). $CD4^+$ T cells, but not $CD8^+$, from IL-2-deficient mice show normal TCR-induced proliferation *in vitro* but have impaired peripheral deletion in response to superantigen *in vivo*. In contrast to *lpr* and *gld* mice, IL-$2^{-/-}$ mice do not accumulate $CD4^-CD8^-B220^+$ T cells and show normal expression of CD95 and CD95-L. However, the sensitivity to CD95 ligation and ceramide is strongly reduced which can be partially compensated by addition of exogenous IL-2. This reduced sensitivity to AICD and CD95 ligation appears to result in strong autoimmune disease and many animals often die after 10 weeks of age. Since survival of these mice is enhanced by crossing IL-$2^{-/-}$ mice with TCR tg mice, i.e. mice with a reduced TCR repertoire, this model further supports the importance of antigen-induced peripheral deletion in the control of immune homeostasis *in vivo*.

Diverse cytokines not only promote differentiation and proliferation but may also act as survival factors and thus antagonise apoptotic cell death. Intradermal immunisation of volunteers with antigen leads to infiltration of $CD45RO^+CD4^+$ and $CD45RO^+CD8^+$ T cells which peaks at day 7 after immunisation and decreased thereafter. This time-dependent of the infiltrate correlates with the expression of Bcl-2 (A. Akbar, London, United Kingdom). T cell activation further induces expression of IL-15 and IL-2 which parallels Bcl-2 expression. T cells isolated from infiltrates are very susceptible to cytokine deprivation and restimulation and die quickly after *in vitro* culture. The addition of IL-2 or IL-15 induces $Bclx_L$ expression, rescues cells from apoptotic death and allows restimulation via TCR. Thus, cytokines like IL-2 may not only promote AICD but may also maintain T cell survival during the course of an immune response.

Similar to T cells, germinal center B cells can undergo AICD upon crosslinking of the B cell receptor (BCR). Whether or not AICD in B cells is CD95/CD95-L dependent is still controversial (Daniel *et al*, 1997; Hahne *et al*, 1996; Samuelsson *et al*, 1997; Scott *et al*, 1996; Truman *et al*, 1997). Nevertheless, germinal center and memory B cells express high CD95 levels and are thus potential targets for activated CD95-L expressing T cells. T Defrance (Lyon, France) (Defrance *et al*, 1997) discussed the complex crosstalk of signals through CD40, BCR and IL-4 receptor on AICD and CD95 sensitivity. Crosslinking of the BCR induces AICD in germinal center B cells which can be rescued by the T cell-derived IL-4 or CD40 ligation. Comparable to thymocytes, the absence of any signal will lead to death by neglect whereas BCR ligation alone will induce AICD. The death of the different B cell subpopulations during their maturation seems to be further regulated by the expression of death effector gene such as c-myc, p53 and Bax. Thus, B cell maturation is tightly coupled to T cell activation which ensures that autoreactive B cells will not survive this antigen-specific T cell dependent selection process.

The role of co-stimulatory molecules like CD4 and CD2 in apoptotic cell death in T cells remains a controversial issue. Both, CD4 and CD2 ligation have been reported to lead to apoptotic cell death by a CD95/CD95-L dependent mechanism (J.-P. Revillard) (Wang *et al*, 1994), whereas others have found that CD2-induced apoptosis proceeds CD95-independent (Mollereau *et al*, 1996). In contrast, D. Kabelitz (Langen, Germany) reported that CD4 crosslinking by soluble antibodies or HIV gp160 can block AICD in T cells by inhibition of activation-induced CD95-L expression. Similarly, co-stimulation via CD2 may block AICD in T cell hybridomas by inhibition of activation-induced CD95 expression (E. Ayroldi, Perugia, Italy) (Ayroldi *et al*, 1997). Thus, stimulation via CD4 or CD2 may promote or antagonise apoptosis depending on the absence or presence of TCR signals and, as suggested by D. Lynch and P. Krammer, depending on the epitopes that the antibodies recognise.

## Tolerance, apoptosis and immune regulation

Although its importance for the regulation of immune responses is generally accepted, the mechanistic events leading to tolerance induction are still quite obscure. While T cells can be rendered tolerant towards a second encounter with antigen several possible mechanisms might be responsible for such non-responsiveness. Neonatal treatment with antigen will lead to central deletion (negative selection) of antigen-specific thymocytes and tolerance will be life-long. To what extent antigen-induced T cell deletion also applies to tolerance induction in adults with mature circulating antigen-specific T cells is unclear and certainly alternative explanations need to be considered. As discussed above, high antigen concentrations or persistent delivery may also induce peripheral deletion of mature antigen-specific T cells by AICD. Induction of tolerance by chronic activation of peripheral T cells and induction of peripheral deletion could explain to a certain degree why induction of peripheral tolerance is often dependent on multiple antigen applications and might be lost after removal of the antigen. After a single injection of high dose of SEB extensive deletion of responsive V$\beta$s is observed after initial expansion and non-responsiveness to SEB is longlasting (Webb *et al*, 1990). Some experimental systems have indeed revealed that apoptotic cell death of antigen-specific T cells might (at least) participate in peripheral tolerance induction (R. Liblau, Paris, France) (Liblau *et al*, 1996). Others even suggested that peripheral tolerance may not only be mediated through deletion of antigen-responsive T cells but may also be mediated through antigen-specific apoptosis or T cell-mediated killing of antigen presenting cells and thus the disapperance of presentable and recognisable antigen (O. Leo, Rhode-St-Genese, Belgium; C. Servet, Lyon, France) (Muraille *et al*, 1997). Antigen-specific deletion of mature T cells is certainly one possible explanation for peripheral tolerance but may not explain everything. In particular, we would have problems to explain how tolerance can be 'infectious' or transferred (H. Waldman, Oxford, United Kingdom) by cells that have undergone or will undergo AICD. In addition, SEB injection never leads to a complete disappearance of all V$\beta$8$^+$ T cells, nevertheless the remaining ones are unresponsive to re-encounter with the superantigen. Similarly, very low concentrations of SEB do not lead to peripheral deletion but still can induce tolerance towards restimulation with SEB in an apparently V$\beta$-independent way (O. Leo) (Muraille *et al*, 1997).

Thus, alternative mechanisms of tolerance induction have to exist which may act independently or in concert with AICD in T cells. J. Bluestone (Chicago, USA) discussed that engagement of CTLA-4 on T cells might represent such a non-apoptosis-mediated pathway of tolerance induction or suppression (Bluestone, 1997). Engagement of CTLA-4 efficiently blocks T cell proliferation and IL-2 production, and might thus antagonise co-stimulatory signals mediated via B7/CD28 interactions. Similarly, inhibition of CTLA-4 by antibodies enhances SEB-induced expansion of V$\beta$8$^+$ T cells *in vivo*, however does not block SEB-induced T cell depletion. Whereas CTLA-4 activation does not engage apoptotic cell death in T cells, CD28 co-stimulation can rescue cells from anti-CD3-induced apoptosis. CTLA-4 rather appears to suppress T cell expansion by inhibition of the Ras pathway associated with the TCR signaling (Calvo *et al*, 1997).

Additional support that peripheral tolerance does not necessarily require apoptotic cell death of antigen-specific T cells was also provided by L. Chatenoud (Paris, France), S. Cobbold (Oxford, United Kingdom), M. Goldman (Bruxelles, Belgium) and H. Waldman (Oxford, United Kingdom). Treatment of mice with non-depleting anti-CD3, -CD4 or -CD8 antibodies results in long-lasting tolerance of T cells and non-responsiveness to skin grafts or autoantigens in NOD pancreas (Chatenoud *et al*, 1997; Marshall *et al*, 1996). Non-responsiveness or altered responses to antigen might also be achieved through immune deviation. L. Adorini (Milan, Italy) discussed that autoimmune NOD mice during the course of diabetes exhibit a strong Th1 response. Continuous treatment with soluble antigen not only induces immune deviation by a shift towards a Th2 response but also induces peripheral tolerance (Adorini and Trembleau, 1997). Whether or not this immune deviation is due to antigen-induced apoptosis of Th1 cells and selective survival of cells committed to the Th2 lineage, as proposed in other studies (Ramsdell *et al*, 1994; Zhang *et al*, 1997) (D. Kabelitz, Langen, Germany), is currently unknown but a possibility to be considered. Similarly, it is unclear whether immune deviation-mediated tolerance is due to unresponsiveness or altered responses of the resulting Th2 cells, e.g. reduced cytotoxic potential. Although additional research has to be done to bring light into many remaining questions, the intriguing observation remains that cytokines are important regulators of immune deviation, peripheral deletion and tolerance.

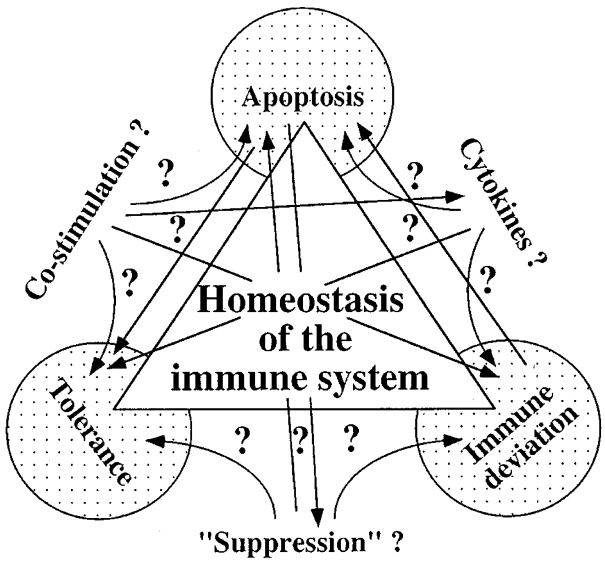


**Figure 1** Regulation of the homeostasis of the immune system and some of the many remaining question-marks

Antigen-induced apoptosis, tolerance and immune deviation are interacting in a complex way for one single goal: the homeostasis of the immune system. All mechanisms of immune regulation certainly represent interesting targets for immunotherapeutical approaches. However, before therapies can be developed for the cure of allergic diseases, cancer, autoimmunity or to prevent transplant rejection the principle mechanisms and interaction between these possible immunomodulatory events have to be understood (Figure 1). And we most likely will have to integrate a new, or better long forgotten, term in our unifying concepts of immunotherapy, the 'suppressor' cells (H. Waldmann, Oxford, United Kingdom). We might thus say the 'S' word soon in public again (Green and Webb, 1993).

## Acknowledgements

The Philippe Laudat conference on activation-induced cell death and peripheral tolerance was supported by INSERM. The authors would like to thank C. Mueller, Institute for Pathology, Berne, Switzerland, for critical reading of the manuscript and helpful discussions, and J. Laissue for support.

## References

Adorini L and Trembleau S (1997) Immune deviation towards Th2 inhibits Th1-mediated autoimmune diabetes. Biochem. Soc. Trans. 25: 625 – 629

Alderson MR, Armitage RJ, Maraskovsky E, Tough TW, Roux E, Schooley K, Ramsdell F and Lynch DH (1993) Fas transduces activation signals in normal human T lymphocytes. J. Exp. Med. 178: 2231 – 2235

Allison J, Georgiou HM, Strasser A and Vaux DL (1997) Transgenic expression of CD95 ligand on islet beta cells induces a granulocytic infiltration but does not confer immune privilege upon islet allografts. Proc. Natl. Acad. Sci. USA 94: 3943 – 3947

Ameisen JC (1995) From cell activation to cell depletion. The programmed cell death hypothesis of AIDS pathogenesis. Adv. Exp. Med. Biol. 374: 139 – 163

Amendola A, Gougeon ML, Poccia F, Bondurand A, Fesus L and Piacentini M (1996) Induction of "tissue" transglutaminase in HIV pathogenesis: evidence for high rate of apoptosis of CD4+ T lymphocytes and accessory cells in lymphoid tissues. Proc. Natl. Acad. Sci. USA 93: 11057 – 11062

Asoh S and Ohta S (1997) Bcl-2 completely blocks Fas-mediated apoptosis in mtDNA-depleted HeLa cells. Biochem. Biophys. Res. Commun. 237: 659 – 662

Ayroldi E, Migliorati G, Cannarile L, Moraca R, Delfino DV and Riccardi C (1997) CD2 rescues T cells from T-cell receptor/CD3 apoptosis: a role for the Fas/Fas-L system. Blood 89: 3717 – 3726

Badley AD, McElhinny JA, Leibson PJ, Lynch DH, Alderson MR and Paya CV (1996) Upregulation of fas ligand expression by human immunodeficieny virus in human macrophages mediates apoptosis of uninfected T lymphocytes. J. Virol 70: 199 – 206

Bellgrau D, Gold D, Selawry H, Moore J, Franzusoff A and Duke R (1995) A role for CD95 ligand in preventing graft rejection. Nature 377: 630 – 632

Bluestone JA (1997) Is CTLA-4 a master switch for peripheral T cell tolerance? J. Immunol. 158: 1989 – 1993

Brunner T, Yoo NJ, Griffith TS, Ferguson TA and Green DR (1996) Regulation of CD95 ligand expression: a key element in immune regulation. Behring Inst. Mitt. 97: 161 – 174

Calvo CR, Amsen D and Kruisbeck AM (1997) Cytotoxic T lymphocyte antigen-4 (CTLA-4) interferes with extracellular signal-related kinase (ERK) and Jun NH2-terminal kinase (JNK) activation, but does not affect phosphorylation of T cell receptor zeta and ZAP70. J. Exp. Med. 186: 1645 – 1653

Casciola-Rosen LA, Anhalt GJ and Rosen A (1995) DNA-dependent protein kinase is one of a subset of autoantigens specifically cleaved early during apoptosis. J. Exp. Med. 182: 1625 – 1634

Casiano CA, Martin SJ, Green DR and Tan EM (1996) Selective cleavage of nuclear autoantigens during CD95 (Fas/APO-1)-mediated T cell apoptosis. J. Exp. Med. 184: 765 – 770

Chatenoud L, Primo J and Bach JF (1997) CD3 antibody-induced dominant tolerance in overtly diabetic NOD mice. J. Immunol. 158: 2947 – 2954.

Chervonsky AV, Wang Y, Wong FS, Visintin I, Flavell RA, Janeway CA and Matis LA (1997) The role of Fas in autoimmune diabetes. Cell 89: 17 – 24

Cornillon S, Foa C, Davoust J, Buonavista N, Gross JD and Golstein P (1994) Programmed cell death in dicytostelium. J. Cell. Sci. 107: 2691 – 2704

Daniel PT, Oettinger U, Mapara MY, Bommert K, Bargou R and Dorken B (1997) Activation and activation-induced death of human tonsillar B cells and Burkitt lymphoma cells: lack of CD95 (Fas/APO-1) ligand expression and function. Eur. J. Immunol. 27: 1029 – 1034

De Maria R, Lenti L, Malisan F, d'Agostino F, Tomassini B, Zeuner A, Rippo MR and Testi R (1997) Requirement for GD3 ganglioside in CD95- and ceramide-induced apoptosis. Science 277: 1652 – 1655

Defrance T, Billian G, Krammer PH and Lagresle C (1997) Fas-dependent and Fas-independent mechanisms for selection of the mature human B cell repertoire. Ann. N.Y. Acad. Sci. 815: 67 – 74

Fournel S, Genestier L, Robinet E, Flacher M and Revillard JP (1996) Human T cells require IL-2 but not G1/S transition to acquire suspectibility to Fas-mediated apoptosis. J. Immunol. 157: 4309 – 4315

French LE and Tschopp J (1996) Constitutive Fas ligand expression in several non-lymphoid mouse tissues: Implication for immune-protection and cell turnover. Behring Inst. Mitt. 97: 156 – 160

Friesen C, Herr I, Krammer PH and Debatin KM (1996) Involvement of the CD95 (APO-1/Fas) receptor/ligand system in drug-induced apoptosis in leukemic cells. Nat. Med. 2: 574 – 577

Fulda S, Sieverts H, Friesen C, Herr I and Debatin KM (1997) The CD95 (APO-1/Fas) system mediates drug-induced apoptosis in neuroblastoma cells. Cancer Res. 57: 3823 – 3829

Genestier L, Paillot R, Bonnefoy-Berard N, Waldmann H and Revillard JP (1997) T cell sensitivity to HLA class I-mediated apoptosis is dependent on interleukin-2 and interleukin-4. Eur. J. Immunol. 27: 495 – 499

Golstein P (1997) Controlling cell death. Science 275: 1081 – 1082.

Goltsev YV, Kovalenko AV, Arnold E, Varfolomeev EE, Brodianskii VM and Wallach D (1997) CASH, a novel caspase homologue with a death effector domain. J. Biol. Chem. 272: 19641 – 19644

Green DR and Webb DR (1993) Saying the "S" word in public. Immunol. Today 14: 523 – 525

Griffith TS, Brunner T, Fletcher SM, Green DR and Ferguson TA (1995) Fas (CD95)/Fas-ligand-induced apoptosis as a mechanism of immune privilege. Science 270: 1189 – 1192

Hahne M, Renno T, Schroeter M, Irmler M, French L, Bornard T, MacDonald HR and Tschopp J (1996) Activated B cells express functional Fas ligand. Eur. J. Immunol. 26: 721 – 724

Hahne M, Rimoldi D, Schroter M, Romero P, Schreier M, French LE, Schneider P, Bornard T, Fontana A, Lienhard D, Cerottini J and Tschopp J (1996) Melanoma cell expression of Fas (Apo-1/CD95) ligand: implication for tumor immune escape. Science 274: 1363 – 1366

Hueber AO, Zornig M, Lyon D, Suda T, Nagata S and Evan GI (1997) Requirement for the CD95 receptor-ligand pathway in c-Myc-induced apoptosis. Science 278: 1305 – 1309

Irmler M, Thome M, Hahne M, Schneider P, Hofmann K, Steiner V, Bodmer JL, Schroter M, Burns K, Mattmann C, Rimoldi D, French LE and Tschopp J (1997) Inhibition of death receptor signals by cellular FLIP. Nature 388: 190 – 195

Itoh N, Tsujimoto Y and Nagata S (1993) Effect of bcl-2 on Fas antigen-mediated cell death. J. Immunol. 151: 621 – 627

Johnson PR (1997) Upregulation of Fas ligand by simian immunodeficiency virus – anefarious mechanism of immune evasion? J. Exp. Med. 186: 1 – 5

Kameoka M, Suzuki S, Kimura T, Fujinaga K, Auwanit W, Luftig RB and Ikuta K (1997) Exposure of resting peripheral blood T cells to HIV-1 particles generates CD25+ killer cells in a small subset, leading to induction of apoptosis in bystander cells. Int. Immunol. 9: 1453 – 1462

Kang SM, Schneider DB, Lin Z, Hanahan D, Dichek DA, Stock PG and Baekkeskow S (1997) Fas ligand expression in islets of Langerhans does not confer immune privilege and instead targets them for rapid destruction. Nat. Med. 3: 738 – 743

Kasibhatla S, Brunner T, Genestier L, Faris M, Echeverri F, Mahboubi F, Nel A and Green DR (1998) DNA damaging agents induce expression of Fas-ligand and subsequent apoptosis in T lymphocytes via the activation of NF-kappaB and AP-1. Mol. Cell, In press.

Klas C, Debatin K-M, Jonker RR and Krammer PH (1993) Activation interferes with the APO-1 pathway in mature human T cells. Intern. Immunol. 5: 625 – 630

Kluck R, Bossy-Wetzel E, Green DR and Newmeyer DD (1997) The release of cytochrome c from mitochondria: a primary site for Bcl-2 regulation of apoptosis. Science 275: 1132 – 1136

Kneitz B, Hermann T, Yonehara S and Schimpl A (1995) Normal clonal expansion but impaired Fas-mediated cell death and energy induction in interleukin-2-deficient mice. Eur. J. Immunol. 25: 2572 – 2577

Kroemer G, Zamzami N and Susin SA (1997) Mitochondrial control of apoptosis. Immunol. Today 18: 44 – 51

Lau HT, Yu M, Fontana A and Stoeckert CJ (1996) Prevention of islet allograft rejection with engineered myoblasts expressing FasL in mice. Science 273: 109 – 112

Lee SY, Reichlin A, Santana A, Sokol KA, Nussenweig MC and Choi Y (1997) TRAF2 is essential for JNK but not NF-kappaB activation and regulates lymphocyte proliferation and survival. Immunity 7: 703 – 713

Lenardo MJ (1991) Interleukin-2 programs mouse alpha beta T lymphocytes for apoptosis. Nature 353: 858 – 861

Liblau RS, Tisch R, Shokat K, Yang X, Dumont N, Goodnow CC and McDevitt HO (1996) Intravenous injection of soluble antigen induces thymic and peripheral T-cell apoptosis. Proc. Natl. Acad. Sci. USA 93: 3031 – 3036

Lynch DH, Campbell KA, Miller RE, Badley AD and Paya CV (1996) FasL/Fas and TNF/TNFR interactions in the regulation of immune responses and disease. Boehring Inst. Mitt. 97: 175 – 184

Marshall SE, Cobbold SP, Davies JD, Martin GM, Phillips JM and Waldmann H (1996) Tolerance and suppression in a primed immune system. Transplantation 62: 1614 – 1621

Martin SJ, Takayama S, McGahon AJ, Corbeil J, Kolesnik RN, Reed JC and Green DR (1995) Inhibition of ceramide-induced apoptosis by Bcl-2. Cell Death Differ. 2: 253 – 257

Memon SA, Moreno MB, Petrak D and Zacharchuk CM (1995) Bcl-2 blocks glucocorticoid – but not Fas- or activation-induced apoptosis in a T cell hybridoma. J. Immunol. 155: 4644 – 4652

Mollereau B, Deckert M, Deas O, Rieux-Laucat F, Hirsch F, Bernard A, Fischer A, Lynch DH, Charpentier B, Le Deist F and Senik A (1996) CD2-induced apoptosis in activated human peripheral T cells: a Fas-independent pathway that requires early protein tyrosine phosphorylation. J. Immunol. 156: 3184 – 3190

Muller M, Strand S, Hug H, Heinemann EM, Walczak H, Hoffmann WJ, Stremmel W, Krammer PH and Galle PR (1997) Drug-induced apoptosis in hepatoma cells is mediated by the CD95 (APO-1/Fas) receptor/ligand system and involves activation of wild-type p53. J. Clin. Invest. 99: 403 – 413

Muraille E, De Smedt T, Andris F, Pajak B, Armant M, Urbain J, Moser M and Leo O (1997) Staphyloccocal enterotoxin B induces an early and transient state of immunosupression characterized by V beta-unrestricted T cell unresponsiveness and defective antigen-presenting cell functions. J. Immunol. 158: 2638 – 2647

Ramsdell F, Seaman MS, Miller RE, Picha KS, Kennedy MK and Lynch DH (1994) Differential ability of Th1 and Th2 T cells to express Fas ligand and to undergo activation-induced cell death. Int. Immunol. 6: 1454 – 1553

Russel JH, White CL, Loh DY and Meleedey-Rey P (1991) Receptor-stimulated death pathway is opened by antigen in mature T cells. Proc. Natl. Acad. Sci. USA 88: 2151 – 2155

Samuelsson A, Sonnerborg A, Heuts N, Coster J and Chiodi F (1997) Progressive B cell apoptosis and expression of Fas ligand during human immunodeficiency virus type 1 infection. AIDS Res. Hum. Retroviruses 13: 1031 – 1038

Scott DW, Grdina T and Shi Y (1996) T cells commit suicide, but B cells are murdered! J. Immunol. 156: 2352 – 2356

Seino K, Kayagaki N, Okumura K and Yagita H (1997) Antitumor effect of locally produced CD95 ligand. Nat. Med. 3: 165 – 170

Srinivasula SM, Ahmad M, Ottilie S, Bullrich F, Banks S, Wang Y, Fernandes-Alnemri T, Croce CM, Litwack G, Tomaselli KL, Armstrong RC and Alnemri ES (1997) FLAME-1. a novel FADD-like anti-apoptotic molecule that regulates Fas/TNFR1-induced apoptosis. J. Biol. Chem. 272: 18542 – 18545

Strasser A, Harris AW, Huang DC, Krammer PH and Cory S (1995) Bcl-2 and Fas/APO-1 regulate distinct pathways to lymphocyte apoptosis. EMBO J. 14: 6136 – 6147

Strasser A, Harris AW, Jacks T and Cory S (1994) DNA damage can induce apoptosis in proliferating lymphoid cells via p53-independent mechanisms inhibitable by Bcl-2. Cell 79: 329 – 339

Truman JP, Choqueux C, Tschopp J, Vedrenne J, Le deist F, Charron D and Mooney N (1997) HLA class II-mediated death is induced via Fas/Fas ligand interactions in human splenic B lymphocytes. Blood 89: 1996 – 2007

Walker PR, Saas P and Dietrich P-Y (1997) Role of Fas ligand (CD95L) in immune escape. The tumor cell strikes back. J. Immunol. 158: 4521 – 4524

Wallach D, Boldin M, Varfolomeew E, Beyaert R, Vandenbeele P and Fiers W (1997) Cell death induction by receptors of the TNF family: towards a molecular understanding. FEBS Lett. 410: 96 – 106

Wang ZQ, Dudhane A, Orlikowsky T, Clarke K, Li X, Darzynkiewicz Z and Hoffmann MK (1994) CD4 engagement induces Fas antigen-dependent apoptosis of T cells in vivo. Europ. J. Immunol. 24: 1549 – 1552

Webb S, Morris C and Sprent J (1990) Extrathymic tolerance of mature T cells: clonal elimination as a consequence of immunity. Cell 63: 1249 – 1256

Weller M, Malipiero U, Aguzzi A, Reed JC and Fontana A (1995) Prorooncogene bcl-2 gene transfer abrogates Fas/APO-1 antibody-mediated apoptosis of human malignant glioma cells and confers resistance to chemotherapeutic drugs and therapeutic irradiation. J. Clin. Invest. 95: 2633 – 2643

Westerndorp MO, Frank R, Ochsenbauer C, Stricker K, Dhein J, Walczak H and Debatin K-M, PK (1995) Sensitization of T cells to CD95-mediated apoptosis by HIV-1 Tat and gp120. Nature 375: 497

Yang J, Liu X, Bhalla K, Kim CN, Ibrado AM, Cai J, Peng T-I, Jones DP and Wang X (1997) Prevention of apoptosis by Bcl-2: release of cytochrome c from mitochondria blocked. Science 275: 1129 – 1132

Yeh WC, Shahinian A, Speiser D, Kraunus J, Billia F, Wakeham A, de la Pompa JL, Ferrick D, Hum B, Iscove N, Ohashi P, Rothe M, Goeddel DV and Mak TW (1997) Early lethality, functional NK-kappaB activation, and increased sensitivity to TNF-induced cell death in TRAF2-deficient mice. Immunity 7: 715 – 725

Zhang X, Brunner T, Carter L, Dutton R, Roger P, Bradley L, Sato T, Reed JC, Green DR and Swain SL (1997) Unequal Death in T helper cell (Th)1 and Th2 effectors: Th1, but not Th2, effectors undergo rapid Fas/FasL-mediated apoptosis. J. Exp. Med. 185: 1837 – 1849

Zornig M, Schmidt T, Karsunky H, Grzeschiczek A and Moroy T (1996) Zink finger protein GFI-1 cooperates with myc and pim-1 in T-cell lymphomagenesis by reducing the requirements for IL-2. Oncogene 12: 1789 – 1801