Autoimmune Lymphoproliferative Syndrome – Impaired Regulation of the Immune Response by Impaired Induction of Apoptosis

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Key Words

ALPS · Apoptosis · Autoimmune lymphoproliferative syndrome · Caspase · CD95

Summary

Autoimmune lymphoproliferative syndrome (ALPS) is an inherited disease hindering apoptosis of lymphocytes. It includes mutations in genes coding for various components of the apoptotic pathway: CD95 (ALPS 0 and ALPS Ia), CD178 (ALPS Ib) and caspase-10 (ALPS II). Moreover, patients with genetically inherited caspase-8 or other components of apoptosis-inducing pathway deficiencies (ALPS III) have been described recently. In addition, ALPS-like clinical patterns, including Dianzani's autoimmune lymphoproliferative disease (DALD), were characterized. In this review, we summarize currently known types of ALPS and characterize their immunological and molecular background.

Schlüsselwörter

ALPS · Apoptose · Autoimmunes lymphoproliferatives Syndrom · Caspase · CD95

Zusammenfassung

Das autoimmune lymphoproliferative Syndrom (ALPS) ist eine vererbte Störung des Apoptoseprozesses in Lymphozyten. Es umfasst Mutationen in Genen, die der Expression verschiedener Komponenten des Apoptoseprozesses zugrunde liegen: CD95 (ALPS 0 und ALPS Ia), CD178 (ALPS Ib) und Caspase 10 (ALPS II). Kürzlich wurden zudem Patienten mit ALPS III beschrieben. Unter dem Begriff ALPS III werden derzeit genetisch bedingte Defekte von Caspase 8 oder anderen Komponenten des Apoptose induzierenden Signaltransduktionsweges zusammengefasst. Darüber hinaus sind ALPS-ähnliche klinische Bilder bekannt, wie die Dianzani-Erkrankung (Dianzani's autoimmune lymphoproliferative disease; DALD). Die vorliegende Übersichtsarbeit fasst die derzeit bekannten ALPS zusammen und beschreibt ihren immunologischen und molekularen Hintergrund.

Introduction

In 1967, Virginia C. Canale and Carl H. Smith described 5 cases of children between 3 and 14 years of age that were, during a period of 13 years, admitted at The New York Hospital due to lymphoma-like symptoms [1]. The newly defined disease, called the Canale-Smith syndrome or autoimmune lymphoproliferative syndrome (ALPS) [2–4], was characterized by onset of symptoms between 1 month and 2 years of age, including lymphadenopathy, varying degrees of autoimmune hemolytic anemia, neutropenia and thrombocytopenia [1, 5], as

well as an increase of peripheral T cell receptor $\alpha\beta$ (TCR $\alpha\beta$) CD4⁺ CD8[−] double negative (DN) T cells [6–8]. Further research was enabled by the discovery of 2 mice MRL strand mutants, *lpr* and *gld*, displaying an autoimmune lymphoproliferative phenotype [9]. However, despite extensive studies, the genetic background of the syndrome described by Canale and Smith remained unidentified until the early 1990s. Finally, the molecular characteristic of ALPS was established after the discovery and cloning of CD95 (also known as Fas/APO-1/TNFRSF6) [10–12] and its ligand CD178 (FasL/APO-1L) [13–14].

Death Receptors and Induction of Apoptosis

The CD178 (CD95L/FasL/APO-1L) molecule is a physiological ligand for CD95. It belongs to the ever expanding tumor necrosis factor (TNF) family which also includes TNF, lymphotoxin (LT)- α/β , TRAIL/APO-2L, TRANCE/RANKL, LIGHT, TWEAK/APO-3L, APRIL, BAFF/TALL-1, CD70/CD27L, CD153/CD30L, CD154/CD40L, AITRL/GITRL, CD134L/OX40L and ectodysplasin A (EDA) [15–17].

Similarly to other members of the TNF family, CD178 is synthesized as a type 2 transmembrane protein that could be cleaved by matrix metalloproteinase (MMP)-7 to form a less active soluble molecule [18]. The tightly controlled expression of CD178 is restricted to activated T cells and natural killer (NK) cells, thus indicating the key role it plays in the mechanisms of cytotoxicity [19, 20]. Recently, it has been postulated that, in addition to the precise transcriptional regulation and MMP-7-dependent posttranslational modification, the activity of CD178 could also be controlled by an alternative pre-mRNA splicing [21, 22]. On the other hand, the constitutive expression of CD178 was found in the testis and the eye, where it is supposed to maintain the immunoprivilege site status [23, 24].

The CD95 molecule, also known as Fas, APO-1 or, most recently, TNFRSF6, is a plasma membrane-anchored type-1 glycoprotein which is a typical representative of the TNF receptor (TNFR) superfamily [16, 25–27]. The members of this family are characterized by extracellular fragments containing up to 6 cysteine-rich domains (CRDs). The TNFR superfamily is divided into 2 groups. The first group comprises glycoproteins with an intracellular functional domain known as ‘death domain’ (DD). DD is responsible for apoptosis-inducing signal transmission, and thus determines the biological activity of the receptor molecule [25, 26]. The remaining molecules are lacking of DD and, therefore, they activate other signaling pathways [26, 28].

CD95 consists of 3 extracellular CRDs, a transmembrane anchoring segment and an intracellular DD formed of 80 amino acid residues [29]. The expression of CD95 is ubiquitous but particularly abundant in T lymphocytes and activated B lymphocytes, thus underlying the significance of this molecule in the immune homeostasis [20, 30]. The binding of CD178 to the CD95 receptor molecule is supposed to induce homotrimer formation and trigger the apoptosis signaling pathway [27]. Recently, some authors postulated the occurrence of previous receptor trimerization through the pre-ligand association domain (PLAD) [31, 32]. According to that hypothesis, CD178 binding would result in the formation of multiple CD95 trimeric clusters with the engagement of 6 or more receptor molecules that enabling the multiplication of the apoptosis-inducing signal.

CD95 DD cluster formation induces conformational changes leading to the attachment of the Fas-associated DD (FADD) adapter protein [33]. The FADD molecule is composed of its

own DD and another domain, known as the death effector domain (DED). Binding of the adapter molecule occurs through homophilic DD interaction between the DD of the CD95 receptor and that of the FADD, resulting in the formation of the death-inducing signaling complex (DISC) [27, 30]. The DED component of the FADD adapter protein is responsible for further binding of the pro-enzyme pro-caspase-8, (formerly known as FADD-like IL-1-converting enzyme; FLICE) and/or pro-caspase-10 (FLICE2) [34–37]. Both belong to the family of caspases (cysteine-aspartate-specific proteases) which are synthesized as inactive pro-enzymes. Both are composed of a caspase-recruiting domain (CARD) and large p20 and small p10 protease subunits. The activated caspases can further activate downstream effector pro-caspases, such as caspase-3, -6 and -7, which are responsible for the cleavage of cellular substrates and finally lead to cell death [30, 38]. In addition, active caspase-8 may also cleave the cytosolic Bid protein which induces mitochondrial outer membrane permeabilization (MOMP) and release of serine proteinase HtrA2/Omi and cytochrome C from the mitochondria [39–41]. The cytochrome C attaches to apoptotic protease-activating factor-1 (APAF-1) and activates pro-caspase-9 [42, 43].

Recent studies have shown that, similarly to other death receptors, the CD95 signaling pathway is much more complex than originally assumed [30]. The number of proteins that interact with and modulate DISC activity is still increasing and includes: death-associated protein (DAXX) [44], receptor-interacting protein kinase (RIP)-1 [45], RIP-associated protein with DD (RAIDD) [28], FLICE-inhibitory protein (FLIP) [46], FADD-interacting serine/threonine kinase (FIST/HIPK3) [47], Fas-associated factor (FAF)-1 [48], Fas-associated protein phosphatase (FAP)-1 [49], FLICE-associated huge protein (FLASH) [50], the Src kinase family members Lck and Fyn [51], the ubiquitin-like protein sentrin and its ligase ubiquitin-conjugating enzyme Fas-associated protein (UBC-FAP) [52, 53] and many others. Therefore, in addition to the caspase cascade, CD95 triggering involves several caspase-independent signals, such as the activation of sphingomyelinases and generation of ceramides, activation of p38 mitogen-activated protein kinase (MAPK), extracellular signal-regulated kinase (ERK), as well as c-Jun N-terminal kinase (JNK) and nuclear factor (NF)- κ B pathways (fig. 1) [15, 30, 54]. Unexpectedly, a broad activation of the cellular metabolism by the CD95 death receptor might result, in certain circumstances, not only in apoptosis inhibition and cell survival but in cell proliferation [30, 43]. On the other hand, however, such a pleiotropic mechanism enables the effective substitution of defective or lacking single component or even entire signaling pathway. This could explain, at least in part, the usually weak biological consequences of impaired apoptosis induction caused by a single mutation [27, 30, 43].

It is noteworthy that, in addition to the previously mentioned inhibitor FLIP, the activity of caspases is also controlled by 2 big families of apoptosis modulators – the Bcl/Bax superfamily

Fig. 1. The CD178-CD95 signaling pathway. Grey areas correspond to constituents of the pathway, presumably affected in the respective ALPS types.

ly and inhibitors of apoptosis (IAPs) [30, 43]. It has been well documented that some of them could play a key role in the regulation of apoptosis during immune response [30, 43, 55]. Pronounced upregulation of Bcl-2 and FLIP has been proven to occur in the initial phase of lymphocytes activation [55]. This could explain the marked resistance of activated lymphocytes to apoptosis induction via CD95, although the latter is upregulated, too. Within a few days, at high levels of IL-2, both apoptosis inhibitors are downregulated, resulting in re-sensitization of the lymphocytes to CD178, followed by extensive elimination of activated cells [56–58].

Immunological Aspects of Autoimmune Lymphoproliferative Syndrome

The interaction between the CD178 and CD95 molecules results in extensive apoptosis of activated lymphocytes (AICD, activation-induced cell death) and is currently considered an important regulatory mechanism of lymphocyte action [20, 59, 60]. According to this concept, the apoptosis-mediated silenc-

ing of immune response is crucial for controlling the peripheral lymphocyte pool size. Otherwise, it would expand as a result of further responses during life. Therefore, due to disturbances in the CD178-CD95 pathway, impaired apoptosis induction in lymphocytes will result in their accumulation and the enlargement of peripheral lymphatic organs (lymph nodes and spleen), thus leading to the development of typical symptoms of ALPS – lymphadenopathy and splenomegaly [4, 7, 8]. As previously suggested, enlargement of lymphatic organs is due to both increased proliferation and impaired AICD. Immunostaining of ALPS patient-derived lymph nodes showed normal T cell phenotypes (predominantly CD4+) and an apoptosis frequency comparable to healthy controls in the germinal centers, but an increase of DN T cells in the paracortical T-zone. They displayed positive staining for the proliferation marker Ki-67, numerous mitotic figures and significantly decreased apoptosis, detected by TUNEL analysis (in situ detection of fragmented DNA) [61].

The most significant immunological abnormality found in ALPS patients is the pronounced increase in circulating TCR $\alpha\beta$ CD3+ CD4– CD8– DN T lymphocytes [62]. In healthy individuals, this population constitutes less than 1–2% of lymphocytes in the peripheral blood [62, 63], thymus and skin [61]. The role and origin of this population remain unclear. Interestingly, it has been shown that DN T cells express T cell intracellular antigen(TIA)-1 and perforin, markers of CD8+ cytotoxic T lymphocytes [61]. Moreover, DN T cells found in ALPS patients express HLA-DR which is considered a previous activation marker [62]. However, Sneller et al. [6] and Fuss et al. [64] have shown that DN T cells derived from ALPS patients poorly respond to mitogen or antigen and do not express cytokines after in vitro stimulation, thus displaying anergic behavior. On the other hand, some authors have proposed that DN T lymphocytes could play a regulatory role for syngeneic CD4+ CD8+ T cells in immune responses against alloantigens [65] or even kill allogeneic tumor target cells [66]. Taken together, these findings support the thesis that DN T cells originate from chronically activated CD8+ populations [61]. However, this subject requires further studies. Recently, a human model for studying DN T cells has been described, where the CD8 mutation resulting in a significantly increased DN T cell count and mild immunodeficiency might facilitate better comprehension of the biology of this population [67]. Also, it is noteworthy that, in addition to TCR $\alpha\beta$ DN T cells, an expansion of TCR $\gamma\delta$ DN T cells and CD8+ CD57+ T cells, as well as a decrease in CD4+ CD25+ regulatory T cells have been observed in ALPS patients [68, 69]. Nevertheless, the immunological and clinical significance of these populations remains yet to be clarified.

The silencing of immune reactions presumably decreases the risk of autoimmunity. Indeed, it has been proven that an uncontrolled immune response that continues after elimination of the invading pathogen would result in cross-reactive recognition of self-antigens by activated lymphocytes [70, 71].

Therefore, similarly to T lymphocytes, also function and homeostasis of B lymphocytes is under the control of the CD178-CD95 system [72]. In addition, impaired regulation of immune responses in ALPS is associated with predominance of the Th2 subpopulation. Consequently, ALPS patients display a Th2-characteristic cytokine profile, including decreased secretion of IL-2 and IFN- γ , whereas IL-4, IL-5 and IL-10 levels are significantly elevated compared to healthy subjects [64]. These conditions promote polyclonal B cell activation and expansion with a significant increase in the CD5+ B cell subpopulation, and hypergammaglobulinemia with autoreactive antibodies, predisposing to development of autoimmune manifestations [69]. In fact, as reported by several groups, such antibodies, especially IgG and IgA directed against erythrocytes, platelets, anti-cardiolipin and anti-nuclear antibodies, anti-dsDNA and anti-U1-RNP were found in ALPS patients [7, 73]. Thus, the most frequent ALPS-associated autoimmune symptoms include autoimmune hemolytic anemia, thrombocytopenia and neutropenia, glomerulonephritis, polyneuropathy, urticaria and vasculitis [63]. Interestingly, ALPS symptoms usually occur at the age of 1 month to 2 years. However, a few cases of patients with onset of symptoms at more advanced age, with the oldest reported patient being age 54, have also been reported [73, 74].

In addition, since the CD178-CD95 system plays an important role in anti-cancer defense, its impaired function could bias towards hematological or solid cancer development. Indeed, in some ALPS patients, Hodgkin's or non-Hodgkin's lymphomas, liver carcinoma (with hepatitis C infection), multiple thyroid or breast adenomas and basal cell carcinomas were observed [61, 75, 76]. This predisposition is particularly visible in individuals with combined defects, e.g. impaired CD95-dependent pathway accompanied by mutation of genes encoding granzymes or perforin [76, 77].

Murine Models of Autoimmune Lymphoproliferative Syndrome

The mouse model of ALPS was first described in the MRL strain by Andrews et al. [78] and was further characterized as an autosomal recessive *lpr* (lymphoproliferation) mutation that was mapped to the mouse chromosome 19 [79]. Subsequently, Roths et al. [80] identified another mutant very similar to *lpr* which was designated as *gld* (generalized lymphoproliferative disorder) and, as an autosomal recessive mutation, was localized on the mouse chromosome 1 [81]. Furthermore, the allelic *lpr* mutation in the CBA/KIJms mouse strain coexisting with heterozygous *gld* mutation and, therefore, designated as *lpr^{cg}* (*lpr* complementing *gld*), has been described [82].

The MRL/*lpr* as well as *gld* mutants displayed lymphadenopathy and splenomegaly, with accumulation of the unusual subsets of T cells and autoantibody-producing B cells, with large

Fig. 2. The proposed algorithm of the ALPS classification.

amounts of IgG and IgM autoantibodies, including anti-DNA and rheumatoid factor antibodies. The development of autoimmune diseases, predominantly arthritis and nephritis, causes the mice to die at approximately 5 months of age. The phenotype of the lymphocytes that accumulate in the lymph nodes and spleen is similar to that observed in ALPS patients, including CD4⁺ CD8[−] DN T cells [9, 83].

The further genetic studies on *lpr* and *gld* mice identified the genes affected by these mutations, thus confirming the close molecular similarity to human ALPS. In *lpr* mice, a splicing defect and thus premature termination and extremely reduced expression of the CD95 receptor is caused by the insertion of a retroviral early transposable element (ETn) into intron 2 of the *FAS* gene [84, 85]. The ETn carries poly(A) adenylation signals (AATAAA) on the long terminal repeat (LTR) sequences, and thus it is believed that the *FAS* transcript that starts from exon 1 terminates prematurely in intron 2 and is sliced [9, 86].

In contrast to the *lpr* mutants, *lpr^{cg}* mice express the full length *FAS* mRNA and CD95 receptor molecule. However, in *lpr^{cg}*

mice, due to a point mutation (T to A) in the intracellular DD resulting in an amino acid change (from isoleucine to asparagine), the apoptotic signal through CD95 is not transmitted [9, 12].

The *gld* mutation consisting of a point mutation (T to C), affects the C-end of the CD178 molecule. This results in the replacement of phenylalanine with leucine, thus disabling the CD178-CD95 interaction [14, 85].

Types and Characteristics of Human Autoimmune Lymphoproliferative Syndrome

As mentioned before, ALPS patients display a characteristic phenotype that includes lymphadenopathy and splenomegaly, increase in TCR $\alpha\beta$ CD4⁺ CD8⁺ DN T cells and autoimmune symptoms. Thus, they assemble the basic clinical criteria, which, associated with mutation analysis, are required to identify ALPS. In spite of a few exceptions, ALPS patients have at least 3 of the listed symptoms. Moreover, in humans, diagnosis of ALPS can be confirmed by functional analysis of patient lymphocyte sensitivity to anti-CD95 antibody-induced apoptosis in vitro [27]. The proposed algorithm of the ALPS classification is shown in figure 2.

According to particular genotypic characteristics, ALPS patients are classified as follows: ALPS 0 (homozygous *FAS*/CD95 mutation) [8, 87], ALPS Ia (heterozygous *FAS*/CD95 mutation) [27], ALPS Ib (*FASLG*/CD178 mutation) [88], ALPS II (caspase-10 mutation) [89] and ALPS III (target unknown) [27] (fig. 1).

ALPS 0

The first case of ALPS 0 was characterized by Rieux-Laucat et al. [8] in a patient born to consanguineous parents. The infant displayed massive lymphoproliferation suggesting a prenatal onset. A further 2 cases were very similar, with prenatal onset of extreme lymphoproliferation and lung infiltration, leading to the early death of 1 patient [87, 90]. Lymphoproliferation involved both T and B lymphocytes, with peripheral blood lymphocytes comprising more than 70% of DN T cells. A great number of mitotic figures in the spleen and lymph node sections suggested active lymphocyte proliferation rather than a simple accumulation. The patient's lymphocytes were not responding to anti-CD95 antibody-induced apoptosis in vitro. On the other hand, some apoptotic lymphocytes were observed on the blood smears, thus suggesting activation of alternative death pathways [27].

Molecular analysis has shown that ALPS 0 is caused by complete CD95 molecule deficiency as a result of a homozygous null mutation. In 2 cases, the mutation affected exon 9 which encodes the DD of the CD95 receptor. These mutants, although non-functional, should be present on the cell surface.

However, presumably, the defective protein might be unstable and prematurely eliminated, or, as a novel-peptide, is accumulated in the endoplasmic reticulum [91]. Another stop-coding mutation that affects the extracellular domain of the receptor, results in premature termination and generation of short CD95 forms which do not anchor in the plasma membrane [63].

ALPS Ia

In contrast to homozygous ALPS 0 patients, only around 70% of heterozygous carriers of CD95 mutations exhibit clinical symptoms. The amplitude of the defect differs between each ALPS Ia patient. Symptoms usually commence between 0 and 5 years of age, with most cases involving a range of 6–12 months [7, 63]. Lymphoproliferation is the most prominent symptom leading to hepato-splenomegaly and lymphadenopathy. Lymphoproliferation and subsequent accumulation of T and B lymphocytes in the paracortical areas of lymphoid organs results in their enlargement without altering the overall structure. In all cases, a DN T cell subpopulation was found. However, its percentage varies between 2 and 61% [63].

Autoimmune symptoms are the second most frequent clinical manifestation of ALPS. As previously described, they include autoimmune hemolytic anemia, thrombocytopenia, neutropenia, urticaria, vasculitis as well as glomerulonephritis and Guillain-Barré syndrome [63]. Furthermore, recently, Bona et al. [92] proved that mutations affecting CD95 might lead to other autoimmune diseases. They found that the impaired function of CD95 might be associated with an increased frequency of autoimmune thyroid diseases, including Grave's disease (GD) and Hashimoto's thyroiditis (HT) in pediatric patients.

ALPS Ia is characterized by heterozygous mutations of the *FAS*/CD95 gene. The majority of so far reported *FAS* mutations (approximately two thirds) affect exon 9 which encodes the DD. At least 1 patient has been described with an exon 8-located mutation [93]. The deletion of exon 8 which contains 25 nucleotides leads to a frameshift and premature termination after 3 missense codons. Recently, Goldman et al. [5] described 2 patients with novel mutations. The first patient displayed a base pair substitution in the 5' splice sequence of intron 8, which resulted in deletion of exon 8. The second patient exhibited a new complex deletion resulting in a frameshift and missense substitution within exon 9. Clinical symptoms appear in approximately 75 and 90% of patients with truncated and missense-mutated intracellular portion of CD95, respectively. DNA changes were also found by Bona et al. [92] in exons 2, 3 and 7. All of these mutations corresponded to silent DNA changes or common polymorphisms.

Approximately 30% of *FAS*/CD95 mutations deal with the extracellular domain. Usually, they are accompanied by a less significant impairment of CD95-mediated apoptosis induction

and clinical penetrance of approximately 30%. These mutations reveal a defective CD95 molecule which is either incapable of anchoring in the plasma membrane, or, more likely, unable to interact with CD178 [27].

ALPS Ib

ALPS Ib was ascribed to a patient with systemic lupus erythematosus accompanied with lymphoproliferation. Interestingly, the clinical manifestations did not include DN T cells and splenomegaly. Therefore, due to the absence of basic symptoms of 'normal' ALPS, the case was classified as ALPS Ib. The genetic studies revealed a dominant *FASLG/CD178* mutation, similar to that of *gld* mice [88].

ALPS II

A number of patients have been described with clinical symptoms of ALPS, including impaired CD95-mediated lymphocytes apoptosis, but without mutation of the *FAS/CD95* gene [7, 89]. Intriguingly, triggering of other death receptors, including TNF-RI/TNFRSF1A/CD120a, DR3/TNFRSF12/APO-3, DR4/TNFRSF10A/TRAIL-R1/APO-2 and DR5/TNFRSF10B/TRAIL-R2/KILLER, in these patients, also revealed impaired induction of apoptosis [63]. This could suggest a defective shared element in the apoptotic pathways induced by each of the receptors mentioned above. Indeed, it has been proven that these patients displayed mutations affecting the initiator caspase-10 [36, 37, 89].

Amazingly, in addition to the accumulation of T and B lymphocytes, patients with mutated caspase-10 demonstrated the accumulation of dendritic cells, a unique feature not observed in any other ALPS type. Moreover, they exhibited exceedingly acute autoimmune hemolytic anemia, thrombocytopenia, optic neuritis and meningitis [63].

Recently, Chun et al. [94] described 2 siblings, a 12-year-old female and an 11-year-old male, with defective lymphocyte homeostasis and apoptosis and impaired activation of T and B lymphocytes and NK cells. Molecular analysis revealed caspase-8 mutations. Interestingly, further studies showed that mutated caspase-10 and caspase-8 may cross-inhibit, although DISC recruitment is not impaired [36]. Also, 1 case of a 5-year-old female with an ALPS-like phenotype, impaired CD95-mediated induction of apoptosis and decreased expression of caspase-8 has been reported by our group. However, due to the absence of DN T cells, the described patient presumably should not be classified as a typical ALPS patient [95].

Due to the fact that caspase-10 and -8 mutations alter 2 different elements on the same level of the apoptotic pathway, it is plausible that 2 distinct subtypes of ALPS II should be considered, rendering e.g. ALPS IIa for caspase-10 and ALPS IIb for caspase-8 (fig. 2).

It is noteworthy that one of the difficulties with ALPS II is the lack of a respective mouse model, since caspase-10 which takes part in the apoptotic pathway in humans, does not exist in mice. Thus, homozygous caspase-8 deficiency in mice leads to embryonic lethality clearly demonstrating its importance in development [96].

ALPS III

Over 30 patients with a mild form of typical ALPS symptoms have been investigated by the group of Rieux-Laucat [63]. The patients displayed hypergammaglobulinemia and an increase in DN T cells in the peripheral blood. Surprisingly, they did not reveal any defects of the CD178-CD95 pathway, with no molecular abnormality established so far [63]. It has been proposed that another apoptotic pathway could be afflicted. This thesis might be supported by animal studies with knockout mice lacking other death receptors, such as DR3 or DR6. DR3-deficient mice displayed impaired apoptosis during negative selection in the thymus, resulting in the generation of autoreactive T cells, whereas DR6-lacking animals revealed an increased proliferation of CD4⁺ T cells with significant Th2 polarization [97, 98]. Nevertheless, the significance of both receptors in CD95-independent homeostasis of the immune system and development of ALPS symptoms requires further investigation. Moreover, the involvement of other components of death receptor-activated pathways, including JNK, MAPK or some of the apoptosis regulators, cannot be excluded [63].

Dianzani's Autoimmune Lymphoproliferative Disease

Aside from the 5 ALPS types, Dianzani et al. [74] have reported several patients with symptoms similar to that of ALPS. These unrelated patients exhibited decreased function of CD95, without any detectable *FAS/CD95* or *FASLG/CD178* mutations. Most of the patients' parents were CD95-resistant, which would imply an inherited trait. The T cells of the majority of the patients were also resistant to apoptosis induced by ceramides, thus suggesting the possibility of mutations affecting the CD95 pathway downstream from the death receptor. In contrast to ALPS patients, these individuals lack DN T cells.

Since this disease is similar to ALPS but does not exhibit the complete pattern of ALPS, it has been named autoimmune lymphoproliferative disease (ALD). To avoid confusion with another ALD acronym (for adrenoleukodystrophy), the name Dianzani's ALD (DALD) has been proposed in the OMIM NCBI site (www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM) to describe this disease which lacks any recognized CD178-CD95 mutations and DN T cell expansion [74].

DALD seems to be genetically heterogeneous, with the final outcome of the disease differing among families, thus demon-

strating different genetic backgrounds. As a result, several gene alterations may be needed to cause the disease. Given the observations of Dianzani et al. [99], it was suggested by the authors that different subjects carry different mutations, and the accumulation of these mutations may lead to the disease. This was deduced from the fact that in most cases both parents displayed defective CD95 function but did not manifest the disease. In many cases, the patients displayed resistance to both anti-CD95 antibody-induced and ceramide-induced cell death, whereas many of their parents presented resistance to only one of them. Moreover, the DALD families also revealed an increased frequency of cancers with no particular type dominating.

Conclusions

The impaired induction of apoptosis observed in ALPS patients, results from mutations altering the CD178-CD95 system. Recently, in addition to CD178 and CD95, the role of other molecules, including caspase-10 and -8 as well as other death receptors, has been investigated in the pathogenesis of ALPS. Thus, future studies should focus on further clarification of molecules involved in ALPS II and III, and in particular on the connection between apoptosis and other signaling pathways. It is noteworthy that ALPS could also serve as a very attractive model for the better understanding of several aspects of immune system function, including immune response regulation, induction of autoimmune reactions and development of cancer.

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