at Duke Comprehensive Cancer Center, Durham, NC, USA.

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Autoimmune lymphoproliferative syndrome: a multifactorial disorder

Frédéric Rieux-Laucat[1-3] and Aude Magerus-Chatinet[1-3]

[1]INSERM, Unité 768, Hôpital Necker; [2]Université Paris Descartes; [3]Hôpital Necker, APHP, Paris, France.
E-mail: frederic.rieux-laucat@inserm.fr

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Homeostasis of peripheral lymphocytes is maintained by numerous mechanisms including anergy, suppression and apoptosis. The last can be triggered by specialized receptors belonging to a subgroup of the tumor necrosis factor receptor superfamily called death receptors.[1] Fas, also termed tumor necrosis factor receptor superfamily 6 (TNFRSF 6), CD95 or Apo-1, is the prototypic member of the death receptor family. Its intracellular domain of 60 to 80 amino acid residues called the death domain allows homotypic interactions with a cytoplasmic death domain-containing protein named the Fas-associated death domain (FADD). FADD contains another domain called the death-effector domain (DED) mediating the recruitment of DED-containing cystein proteases such as pro-caspase-8 and pro-caspase-10 (also called Flice and Flice-2, respectively) in humans. These pro-enzymes are processed in their active form into a death-inducing signaling complex which, in turn, triggers a biochemical cascade composed of other pro-caspases and culminating in apoptosis. Initiation of this process can be inhibited by recruitment of an inactive caspase analog, the Flice inhibitory protein (FLIP). Finely tuned stoichiometry of FLIP, caspase-8 and caspase-10 regulates the stability and pro-apoptotic activity of the death-inducing signaling complex. This Fas-induced cell death can be triggered by cell-cell contact between a Fas-ligand-positive effector cell and a Fas-positive target, or following chronic stimulation through the antigen T-cell receptor (TCR). This activation-induced cell death is mainly controlled by Fas and Fas-ligand.[2]

The role of Fas in human lymphocyte homeostasis was illuminated by the discovery of Fas mutations in patients with autoimmune lymphoproliferative syndrome (ALPS).[3] ALPS was first described in 1967 by Canale and Smith and is now recognized as a chronic or recurrent, non-malignant lymphoproliferative condition frequently accompanied by autoimmune manifestations (mostly autoimmune cytopenias). Manifestations usually appear in the first 5 years of life (median onset at 3.5 years). The most frequent presentation of ALPS is a benign lymphoproliferation limited to lymphoid organs. Enlargement of spleen

and lymph nodes is the most prominent feature of the lymphoproliferation in nearly all ALPS patients. Autoimmunity is the second most salient feature of ALPS. Autoimmune cytopenias account for more than 80% of the autoimmune manifestations and are, essentially, autoimmune hemolytic anemia, thrombocytopenia and, sometimes, neutropenia. ALPS is, therefore, an exclusion diagnosis for Evans' syndrome which is defined by a combination of autoimmune hemolytic anemia and thrombocytopenia without a known etiological cause. A recent study showed that a large proportion of patients with Evans' syndrome did, in fact, have ALPS (with *FAS* mutations).[4] Organ-specific autoimmunity, such as uveitis, dermatitis, glomerulonephritis, hepatitis, thyroiditis, Guillain-Barré syndrome and type-I diabetes are also occasionally found in ALPS patients.

A hallmark of ALPS is the finding of a high proportion of $CD4^-CD8^-$ $TCR\alpha\beta^+$ T cells, called double-negative T cells. $TCR\alpha\beta^+$ double-negative T cells normally account for less than 2% of $TCR\alpha\beta^+$ T cells (or less than 1.5% of total lymphocytes). Double-negative T cells express activation markers such as HLA-DR and CD69, but also lack CD25 expression while paradoxically they express CD45RA, a marker of naïve T cells. They also express granzyme A, a marker of cytotoxic T cells,[5,6] whereas expression of perforin is controversial. The repertoire of double-negative T cells is polyclonal but more restricted than that of CD4 and CD8 T cells. A detailed repertoire analysis and CDR3 sequencing showed that double-negative T cells essentially emerge from the peripheral $CD8^+$ T-cell population,[7] likely following active proliferation *in vivo*. It is believed that the proportion of double-negative T cells varies as a function of ongoing, *in vivo* cell proliferation. In contrast, these cells are unable to proliferate *in vitro*. Double-negative T cells have features of "terminal" cells such as expression of CD57 and shortened telomeres (Rieux-Laucat *et al., unpublished results*). These cells may undergo apoptosis as a consequence of activation of Fas-independent pathways of apoptosis[5] (see below). Double-negative T cells produce low amounts of interleukin-2 and interferon-γ, but very high amounts of interleukin-10 and soluble Fas-ligand. For all these reasons double-negative T cells are considered as a marker of the disease rather than as a real disease-causing population of cells. Plasma concentrations of soluble Fas-ligand and interleukin-10 can also be used as biomarkers of ALPS.[8,9] The number of B cells in the blood of ALPS patients is usually normal, although gammaglobulinemia G and A is a common feature of ALPS. This cell lineage has been poorly studied in ALPS.

ALPS is a consequence of defective lymphocyte apoptosis mostly associated with germline or somatic dominant mutations of the *FAS* gene (ALPS-*FAS*).[10] In rare instances, mutations of the Fas-ligand (ALPS-*FASL*),[11] or signaling molecules such as caspase-10 (ALPS-*CASP10*)[12] have been described. Mutations of Fas abrogate the formation of the death-inducing signaling complex or the Fas/Fas-ligand interaction (either by modifying the structure of Fas or by precluding its membrane expression). The consequences of these defects can be quantified *in vitro*, on activated T cells either upon oligomerization of *FAS* by an agonistic antibody, or after restimulation through the TCR and endogenous expression of *FASL*. However there is no correlation between the magnitude of the apoptosis defect and severity of the disease.[3] Moreover, some Fas mutations may not be sufficient to trigger the disease, as asymptomatic carriers of germline *FAS* mutations have been described (partial clinical penetrance). T cells of these asymptomatic carriers exhibit a functional defect that is of the same magnitude as that of symptomatic patients. This observation implies that there are modifiers of the onset of ALPS.

Genetically undetermined ALPS are named ALPS-U whereas a phenotypic variant of ALPS was termed Dianzani's autoimmune lymphoproliferative disease (DALD).[10] DALD patients present with the same clinical symptoms as ALPS, but do not have an increase of double-negative T cells.[13] Although DALD T cells are resistant to Fas-induced apoptosis, no molecular cause for the disease has so far been identified. The comparative study of ALPS and DALD is, therefore, of great interest to better understand the molecular and cellular mechanisms involved in these similar lymphoproliferative diseases, as well as to decipher their underlying genetic bases. It could well be that the genetic defect causing DALD might also be involved as a modifier of ALPS-*FAS*. Alternatively, these diseases could be the consequence of different genetic defects impairing a common biochemical or functional pathway. Dianzani's group has been studying DALD and ALPS for years. They initially observed increased osteopontin concentrations in the sera of DALD patients.[14]

Osteopontin is a phosphorylated glycoprotein secreted by macrophages and activated T cells. It has both cytokine and chemoattractive functions, which may favor lymphoproliferation and autoimmunity by inhibiting activation-induced cell death. Nevertheless the underlying mechanisms of this last observation remained unexplained. In this issue of the journal, Boggio *et al.*[15] provide some elucidation of the potential mechanisms involved in DALD and possibly in ALPS. As for the observations regarding osteopontin, Boggio *et al.* used a cDNA array analysis to detect another over-expressed transcript, which turned out to be that of tissue inhibitor of metalloproteinase 1 (TIMP-1). It is particular interesting that the functions influenced by TIMP-1 include inhibition of activation-induced cell death in Hodgkin's lymphoma, a frequent malignant complication in ALPS. In addition, Boggio *et al.* showed that TIMP-1 inhibited both Fas-induced cell death and activation-induced cell death *in vitro*. Correlatively they found elevated TIMP-1 levels in the serum of both ALPS and DALD patients. This is an interesting finding since the TIMP-1-mediated inhibition could potentially worsen the apoptosis defect observed in ALPS and DALD patients. In order to substantiate this observation it would be very interesting to assess TIMP-1 levels in sera of ALPS-*FAS* patients and of healthy relatives carrying the same *FAS* mutation. An increase in the former but not in the latter would support a role of TIMP-1 in the onset of ALPS. It, therefore, remains to be understood whether the overexpresion of TIMP-1 is a cause or a consequence of the disease. The scenario might be different in DALD. Indeed, Dianzani and his colleagues previously demonstrated that elevated osteopontin levels were associated with particular combinations of poly-

morphisms and that these genotypes were associated with an increased risk of the development of DALD.[14] As demonstrated in the work by Boggio *et al.*, elevated osteopontin levels could mediate TIMP-1 overexpression, and both molecules could thus impair Fas-induced cell death and activation-induced cell death. It is not yet clear whether this is the causal event accounting for the apoptosis defect observed in DALD or whether it worsens a defect of apoptosis as a consequence of another genetic defect that remains to be identified. Other unanswered questions are what is the signaling pathway of this inhibiting mechanism and what is the trigger of the TIMP-1 over-expression in ALPS . It could be either a consequence of the proliferation itself, or a consequence of an additional genetic defect. In any case this might be considered as a "modifier" which could potentiate the partial Fas-induced cell death defect observed for some Fas mutations with low clinical penetrance.

Finally, if the pathological role of TIMP-1 is confirmed *in vivo*, these findings could point to a central role of apoptosis in key check-points of self-tolerance. Additionally they could constitute a step forward in the understanding of the pathophysiological mechanisms involved in ALPS and DALD, and could pave the way to the understanding of other autoimmune diseases.

Frédéric Rieux-Laucat is an Investigator at INSERM U768. Aude Magerus-Chatinet is a Post Doctorate fellow at INSERM U768.

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Antenatal treatment of fetal alloimmune thrombocytopenia: a current perspective

Cheryl A. Vinograd and James B. Bussel

Division of Hematology, Department of Pediatrics, Weill Medical College of Cornell University, New York, NY, USA

E-mail: jbussel@med.cornell.edu *doi:10.3324/haematol.2010.030148*

(Related Original Article on page 1921)

Fetal alloimmune thrombocytopenia is caused by maternal sensitization to paternally-derived antigens on fetal platelets, most commonly HPA-1a.[1] It occurs in approximately 1 in 1000 live births and is the commonest cause of severe fetal and neonatal thrombocytopenia, and of intracranial hemorrhage in neonates born at term.[2] Since there is currently no routine screening, first-time cases of fetal alloimmune thrombocytopenia are generally identified following the birth of a markedly thrombocytopenic neonate. Antenatal management is thus only possible in subsequent pregnancies.

Intracranial hemorrhage is the most devastating complication of fetal alloimmune thrombocytopenia and often occurs antenatally. Assessment of projected clinical severity is thus based on the development of intracranial hemorrhage in a previous sibling. If there is such a history of intracranial hemorrhage, the chance of this complication occurring again in the next pregnancy is extremely high in