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From single-cell signature to prognostic factors: the case of Sézary syndrome

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“CD60 expression associated to the concurrent CD49d and CD26 deficiency constitutes a peculiar signature of Sézary syndrome...”

Sézary syndrome (SzS), the most aggressive form of cutaneous T-cell lymphoma (CTCL), is an extra-nodal non-Hodgkin's lymphoma characterized by the presence of an epidermotropic circulating clonal CD4⁺ T-cell population with hyper-convoluted or cerebriform nuclei, also known as Sezary cells (SC) [1]. It has been proposed that the neoplastic T-cell clone in SzS belongs to the Th2 type, thus giving reason for the eosinophilia and the elevated IgE levels frequently recorded in SzS [1].

SC phenotype

SC phenotype has been largely investigated from both a morphological [2] and molecular point of view.

Flow cytometry analysis has been widely used to typify SC and a great number of surface antigens has been proposed as a specific marker, mainly focusing on the assessment of the lack or over expression of molecules usually expressed on CD4⁺ T lymphocytes.

Down regulation of CD7 expression was originally suggested as a typical attribute of SS circulating CD4⁺ T cells [3], but the physiological appearance of CD7⁺ T cells in aged individuals prompted to the identification of more reliable markers [4]. The lack of Dipeptidyl peptidase IV (CD26/DPPIV), a 110 kDa glycoprotein involved in T-cell proliferation and activation, has been described in SC [2]. CD26/DPPIV plays an important regulatory role during the migration of lymphocytes and is able to cleave several important cytokines and chemokines [5], such as SDF-1, generating an inactive chemokine. SDF-1 is abundantly

detected at the skin level in SS patients and the loss of CD26 from SC might act as a reinforcing mechanism of SDF-1-directed epidermotropism [6].

Both the over expression of 9-*O*-acetylated form of the GD3 ganglioside (CD60) [2] and lack of $\alpha 4$ integrin (CD49d) expression have also been suggested as further markers of SC [2].

The ganglioside GD3 (Neu5Ac α 8Neu5Ac α 3Gal β 4GlcCer) is a proapoptotic intracellular second lipid messenger, acting through TNF- α , FAS/FASL or β -amyloid pathways [7]. The 'acetylated form' of GD3 (9-*O*-acetyl GD3), that is, CD60, rarely expressed in circulating human T cells [8], may totally abolish apoptosis [7] and has already been observed in melanoma, breast cancer and in a number of tumor cell lines [9].

$\alpha 4 \beta 1$ (CD49d) integrin is able to bind to VCAM-1, a surface molecule expressed on the endothelium only after activation, thus mediating the passage of lymphocytes right through the vessel wall. The peripheral T-cell clone observed in SzS is actually lacking CD49d, therefore it can be hypothesized that SC, even though skin committed (expression of CLA, CCR4, CXCR4), are not only incapable of transferring through the endothelial vessel wall, but also of adhering to the extracellular matrix.

CD60 expression associated to the concurrent CD49d and CD26 deficiency constitutes a peculiar signature of SC, since such phenotype is found in <5% of normal CD4⁺ T cells (CD7⁺, CD26⁺, CD49d⁺ and CD60⁺). Furthermore, the

chemokine receptor expression profile observed in SC (CCR4⁺, CCR7⁺ and CXCR4⁺) [6] suggests that the malignant clone on SzS belongs to a Th2 central memory (CM) subset [10,11].

TCR V β repertoire

The human TCR V β region locus located on chromosome 7 at band 7q34 comprises at least 65 V β segments, that can be clustered into 25 subfamilies, most (V β 2, V β 4, V β 6 and V β 13) or less frequently (V β 10 and V β 18) represented in normal peripheral blood [12].

The evaluation of the TCR V β family by FACS analysis, is considered to be the best way to quantify the tumor burden in CTCL [13], taking into account that the panel of commercial MoAbs covers about 70% of all TCR-V β domains, and therefore might fail in several cases [14]. A skewed usage of TCR-V β 5.1 or TCR-V β 2, followed by V β 12 and V β 13.1 families is observed in patients affected by SzS, while several V β families are constantly under-represented (V β -3, -4, -9, -14, -16, -18 and -21.3) [15,16].

The frequent usage of several families may simply reflect their higher frequency of expression in normal controls [12], but could also be caused by chronic environmental stimuli, as already described in atopic dermatitis [17].

Staphylococcus aureus strains are often present in the cutaneous T-cell lymphomas skin, producing several enterotoxins (SE) with super-antigenic (sAg) properties and consequently inducing the appearance of skewed T-cell clones [18]. For example, TSST-1 may stimulate selectively TCR-V β 2 bearing cells, SE-C may bind both TCR-V β 5.1 and TCR-V β 13.1, SE-B and SE-C TCR-V β 17 and SE-E TCR-V β 5.1 [18]. It is widely known that T-cell oligoclonality may be caused by recurring sAg stimulation and the expression of CD60 on SC, providing antiapoptotic properties, could play a further pathogenetic role [19].

FACS analysis & survival rate

Several prognostic indicators of unfavorable outcome in SzS have been reported to date, such as circulating SC count, high CD4/CD8 ratio, advanced patient age, high lactate dehydrogenase serum levels and elevated white blood cell count [20]. CD60 over expression and loss of CD49d on SC, as measured at the time

of the first diagnosis, is also strictly associated with a significantly lower survival rate [16]. A poorer outcome was also detected in SzS patients able to synthesize specific IgE to environmental or food allergens; whereas total IgE levels do not seem to allow the prediction of survival [21]. The expression of CD60 in circulating SC of IgE⁺ subjects is a further, stronger negative prognostic factor in SS [21]. Conversely, the cumulative absence of risk factors such as over expression of CD60, lack of CD49d and specific IgE reactivity identify a small group of patients with a very good prognosis [16,21]. A strong relationship between atopic conditions and hematopoietic cancers has been suggested [22], thus explaining the possible relationship between pre-existent allergic reactivity and the worst outcome in SzS. Several studies demonstrated a skewed Th2 cytokine profile in SzS. IgE production is the consequence of a Th2 skewed immune-response, and the presence of CD60 on T cells is also associated with functional Th2 profile, given that repeated stimulation of CD4⁺CD60⁺ memory T cells leads to the synthesis of Th2 cytokines such as IL-5, IL-4 and IL-10 [8].

In summary, there is evidence that a skewed usage of several TCR-V β families, particularly V β 2 and V β 5.1, overexpression of CD60, lack of CD26 and CD49d, are strictly associated to a significantly lower survival rate and a Th2 central memory phenotype represent the hallmarks of the malignant T cell clone in SzS.

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