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GENERAL INTRODUCTION
INTRODUCTION

In recent years the molecule programmed death-1 (PD-1) has attracted much attention for several reasons. First, it is a critical element in the negative regulation of cellular immune responses. Second, blockage of the interaction between PD-1 expressed on T cells and its ligand PD-L1 expressed on tumor cells has emerged as a promising approach in the treatment of several cancers. Third, PD-1 is constitutively expressed by a subset of T cells, namely follicular helper T cells which play a key role in the differentiation of germinal center B cells into memory B cells or plasma cells. Fourth, expression of PD-1 has been used as important biomarker in the differentiation between different types of T-cell non-Hodgkin lymphoma. The main focus of the studies in this thesis was to determine whether expression of PD-1 might also be a useful marker in the diagnosis of different types of cutaneous lymphoma.

In this introductory chapter we will first describe general knowledge about the PD-1 molecule and the physiological role of PD-1 in cellular and humoral immunity. Next, expression of PD-1 in different types of nodal non-Hodgkin lymphoma and its diagnostic significance will be presented. Finally, primary cutaneous T-cell lymphoma and outstanding issues will be introduced and PD-1 related important contributions will be explained.

DISCOVERY AND DESCRIPTION OF THE PD-1 MOLECULE

PD-1 (CD279) was originally identified in 1992 by Ishida et al. who were studying dependency of programmed cell death on de novo RNA and protein synthesis. In mouse cell lines that were induced to undergo apoptosis they discovered that the PD-1 gene was activated and involved in this type of cell death.1 Human full-length PD-1 was identified two years later as a type I trans-membrane glycoprotein of 288 amino acids (aa) and the PD-1 gene (named PDCD1) was mapped to chromosome 2q37.3. Ref 2 The extracellular regions of human and mouse PD-1 are relatively highly conserved (approximately 65% homology).3 PD-1 is considered to belong to the immunoglobulin (Ig) superfamily, because the structure of the extracellular domain resembles an Ig variable region (IgV). PD-1 is a monomer molecule and structurally related to family members CD28 and CTLA-4, which are functional as homodimer molecules.4 The extracellular domain of PD-1 is connected to a transmembrane region and an intracellular tail (Figure 1), containing two phosphorylation sites called ITSM (immunoreceptor tyrosine–based switch motif) and ITIM (immunoreceptor tyrosine–based inhibitory motif). Splice variants of the human PD-1 gene have been reported.5 One splice variant that lacks the transmembrane region represents the soluble form of PD-1, which can functionally antagonize the interaction of cell membrane PD-1 with its ligand.6
GENERAL INTRODUCTION

DISCOVERY AND DESCRIPTION OF THE PD-1 LIGANDS

PD-1 has two ligands named PD-L1 (CD274; B7-H1) and PD-L2 (CD273; B7-DC). Human PD-L1, which was discovered in 2000, is 290 aa in size and is encoded by the CD274 gene on chromosome 9.\(^7\) One year later PD-L2 was identified as a glycoprotein consisting of 273 aa and being encoded by the PDCD1LG2 gene (Table 1).\(^8\) The CD274 and PDCD1LG2 genes are located next to each other and separated by only 42 kb of intervening genomic DNA. PD-L1 and PD-L2 share 40% aa identity. Both PD-1 ligands are type I transmembrane molecules and member of the Ig superfamily. Like PD-1, the extracellular part of PD-L1 and PD-L2 contains one IgV–like domain, but unlike PD-1, the extracellular part of PD-L1 and PD-L2 also contains one additional Ig constant region (IgC)–like domain (Figure 1). The intracellular domains of PD-L1 and PD-L2 are relatively short, only about 30 aa, without clear signaling motifs, but it is suggested that these intracellular tails may have a functional roles.\(^9\) PD-L2 has a two- to six-fold higher affinity for PD-1 than does PD-L1. \(^{10}\)

Ligands PD-L1 and PD-L2 also have splice variants and soluble forms with PD-1 binding capacity (Figure 1; Table 1),\(^{11-13}\) but their role is unclear. Soluble PD-L1 is released by activated dendritic cells, but not by macrophages, monocytes or T cells.\(^{14}\) In addition, soluble PD-L1 is also produced by various tumor cells.\(^{14}\)
Although it is typically thought that PD-L1 and PD-L2 interact with PD-1, it is important to note that there are other binding partners for these two ligands (Table 1). PD-L1 has been shown to interact specifically with CD80 (B7-1). Keeping in mind that CD80 can also bind to CD28 and CTLA-4, it is difficult to comprehend how this complex receptor–ligand interactions is exactly balanced. Very recently, it is reported that PD-L2 can also bind to repulsive guidance molecule b (RGMb, also known as DRAGON), which was originally identified in the nervous system as a co-receptor for bone morphogenetic proteins and is expressed by macrophages and other cells of the immune system.

### Table 1. General facts on human PD-1 and ligands PD-L1 and PD-L2

<table>
<thead>
<tr>
<th></th>
<th>Discovery</th>
<th>Aliases</th>
<th>Gene name (chromosome location)</th>
<th>Size (amino acids)</th>
<th>Splice variants</th>
<th>Ligands</th>
</tr>
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<tbody>
<tr>
<td>PD-1</td>
<td>1994</td>
<td>CD279</td>
<td>PDCD1 (2q37.3)</td>
<td>288</td>
<td>4</td>
<td>PD-L1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>PD-L2</td>
</tr>
<tr>
<td>PD-L1</td>
<td>2000</td>
<td>CD274</td>
<td>CD274 (9p24.1)</td>
<td>290</td>
<td>1</td>
<td>PD-1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B7-H1</td>
<td></td>
<td></td>
<td></td>
<td>CD80</td>
</tr>
<tr>
<td>PD-L2</td>
<td>2001</td>
<td>CD273</td>
<td>PDCD1LG2 (9p24.2)</td>
<td>273</td>
<td>2</td>
<td>PD-1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B7-DC</td>
<td></td>
<td></td>
<td></td>
<td>RGMb</td>
</tr>
</tbody>
</table>

In humans, PD-1 can be expressed on T cells, B cells, natural killer T cells, monocytes and myeloid CD11c⁺ dendritic cells (DC) after activation. PD-1 is not present on naïve T cells, but is induced on CD4⁺ and CD8⁺ T cells after T-cell receptor-mediated activation and remains high in case of persistent stimulation with antigen (Table 2). In addition, PD-1 can also be induced on T cells by the so-called common gamma-chain cytokines interleukin 2 (IL-2), IL-7, IL-15 and IL-21, which are important for survival and expansion of T cells. PD-1 is highly expressed on non-functional so-called “exhausted” T cells, which are the result of persistent antigen exposure, caused by chronic viral infections or progressive tumors. Remarkably, follicular helper T (TFH) cells, which are key regulators of germinal center B cell differentiation into plasma cells and memory B cells, have a constitutive high expression of PD-1. Regulatory T (Treg) cells, which suppress immune system activation and promote immunologic tolerance, also have a constitutive high PD-1 expression. Triggering of the B-cell receptor stimulate PD-1 expression on B cells. Freshly isolated NK cells from healthy donors do not express PD-1, but IL-2 can induce PD-1 expression on NK cells. Freshly isolated immature Langerhans cells from healthy donors express PD-1, but lose this expression after maturation.
PD-L1 expression is found on a broad range of immune cells (Table 2), including T cells and antigen-presenting cells, and is upregulated after activation. In particular, type I and type II interferons can upregulate PD-L1 expression. PD-L1 can also be induced on a variety of non-immune cells, such as epithelial cells including keratinocytes and endothelial cells. Of note, tumor cells have been reported to express PD-L1 as well. Expression of PD-L2 has initially been reported to be restricted to activated professional antigen-presenting cells (Table 2), but recently it was shown that PD-L2 is expressed on activated human CD4+ and CD8+ T cells as well.

**SUPPRESSION OF T-CELL FUNCTION BY THE PD-1/PD-L AXIS**

T cells become activated when their T-cell receptor (TCR) recognizes an antigenic peptide–MHC complex that is presented by antigen-presenting cells. TCR are associated with CD3, which actually mediate the activation signal via activation of a series of kinases, such as ZAP70, PI3K, PKC-θ and AKT (Figure 2). T cells concomitantly receive multiple additional antigen-independent signals via receptor/ligand interactions, collectively called costimulation, which can deliver positive or negative signals. Positive costimulation is essential for naïve T cells to become fully activated and if there is no or not enough positive signaling, naïve T cells fail to develop into effector or memory T cells and become nonresponsive to subsequent antigen stimulation, a situation which is often indicated with the term...
1. GENERAL INTRODUCTION

Predominance of negative co-stimulation will induce the development of regulatory T cells, T-cell tolerance or apoptosis. Receptors of the CD28/CTLA-4 family, including PD-1, have a key role in the outcome of T-cell responses, as these receptors provide potent costimulatory or inhibitory signals upon interaction with their corresponding ligands.

Costimulation is highly complex, because some of these receptor and ligands are constitutively expressed while others appear on the cell surface after activation, and in addition, some of the receptors have dual specificity or have similar specificity with different affinity (Figure 2). To provide a detailed description of the costimulatory and inhibitory signals is beyond the scope of this thesis, though an extensive overview can be obtained from references. Only the PD-1/PD-L signaling will be described here, as PD-1 is the primary molecule of interest in this thesis.

Figure 2. Inhibition of T-cell activation by PD-1. Antigen recognition by the T-cell receptor (TCR) provides an activation signal via CD3. Concomitant binding of CD28 to its ligand CD80 or CD86 strongly promotes T-cell activation, while ligation of PD-L1 or PD-L2 to PD-1 causes recruitment of phosphatase SHP-2 that disrupts the TCR and CD28 signaling pathway. A more detailed description is provided in the text.
Upon binding to its ligand, PD-1 becomes associated with TCR and CD28 microclusters that are assembled in the so-called immunological synapse. In addition, phosphorylation of the two intracellular tyrosine-based domains ITSM and ITIM takes place, which leads to the recruitment of phosphatases SHP-2 and possibly SHP-1 as well. Mutagenesis studies have shown that recruitment of SHP-2 by ITSM plays the primary functional role of PD-1 inhibition. The binding of SHP-2 by ITSM results in dephosphorylation of several molecules of the TCR signaling pathway (Figure 2). Altogether, triggering of PD-1 on the T cell leads to inhibition of proliferation and cytokine production, such as IL-2 (which protects against apoptosis), IFN-γ and TNF-α, and promotes T-cell apoptosis through inhibition of survival factor Bcl-xL.

**PD-1+ T FOLLICULAR HELPER CELLS AND B-CELL IMMUNITY**

The differentiation of naïve B cells into memory B cells or antibody-secreting plasma cells in the germinal centers within the B-cell follicles is critically dependent on so-called T follicular helper (TFH) cells. In both humans and mice, TFH cells typically are CD4+ T cells with a high expression of chemokine receptor CXCR5 (chemokine C-X-C motif receptor 5) and CXCL13 (chemokine C-X-C motif ligand 13) and further express ICOS (inducible T cell costimulator), BCL6 (transcription factor B-cell lymphoma 6) and the cytokine IL-21. Of note, TFH cells have a constitutively high expression of PD-1, which is assumed to be one of the key instructive signals to guide the differentiation of germinal center B cells into memory or plasma cells. These markers together are valuable to define TFH cells, but no single marker is sufficient to detect TFH cells as each of them can be found in other T cell subsets. Although TFH are defined by follicular location, T cells fulfilling the phenotypical criteria of TFH can also be detected outside the lymphoid organs, for example in the blood circulation. PD-1 is an important negative regulator of B-cell responses in the periphery. Binding of PD-1 to its ligand causes phosphorylation of the ITSM and ITIM domains of the B-cell receptor, causing recruitment of phosphatase SHP-2, leading to dephosphorylation of key molecules immediately downstream of the B-cell receptor, thereby suppressing the activation of the B cell. Blockade of PD-1 on B cells enhances antigen-specific antibody responses.

**TUMORS AND THE PD-1/PD-L PATHWAY**

In mouse models it has been demonstrated that PD-L1 expressing tumor cells are resistant to killing by CD8+ T cells via the engagement of PD-1 on the T cells by PD-L1 on the tumor cells (Figure 3). This PD-1/PD-L interaction resulted in direct
inhibition of the T-cell mediated cytotoxicity, while blockade of this interaction with antibodies restored the anti-tumor response.\textsuperscript{38-40} These data highlight that tumors can use the PD-1/PD-L pathway as evasion mechanism to escape from the immune system. Indeed, many different tumor types have upregulated PD-L1 expression\textsuperscript{41} and the majority of the tumor infiltrating lymphocytes show PD-1 expression.\textsuperscript{42,43} Therefore, the PD-1/PD-L pathway is generally considered to have a central role in the strategy of tumors to avoid eradication by the host defense system.

The exact mechanism why PD-L1 is expressed by tumor cells is currently unclear. Constitutive oncogene-driven expression of PD-L1 has been suggested, but alternatively, the PD-L1 expression might reflect the adaptation of the tumor to the anti-tumor immune response, a process termed adaptive resistance.\textsuperscript{44} Several studies have investigated possible correlation of the expression of PD-1, PD-L1 and/or PD-L2 with patient prognosis, as reviewed by Sznol \textit{et al.}\textsuperscript{45} Because of the large variation and sometimes even opposite outcomes, these correlation studies should be interpreted with caution. Accumulating data from in vitro cell culture systems and animal models —indicating that PD-1/PD-L interactions form a major suppression mechanism within the tumor microenvironment— created the rationale to develop antibodies against PD-1 and PD-L1 for cancer immunotherapy.
Initial clinical studies using these antibodies demonstrated remarkable anti-tumor activity,\textsuperscript{45,46} validating the PD-1/PD-L pathway as target for cancer immunotherapy.

**PD-1 EXPRESSION IN NODAL T-CELL NON-HODGKIN LYMPHOMA**

Gene expression profiling analysis aimed to compare angioimmunoblastic T-cell lymphoma (AITL) with peripheral T-cell lymphoma, not otherwise specified (PTCL–NOS) clearly demonstrated that tumor cells in AITL show overexpression of several genes characteristic of normal TFH cells, such as $PDCD1$ (encoding PD-1), $BCL6$ and $CXCL13$. These findings strongly support that the TFH cell represent the cell of origin of AITL.\textsuperscript{47} Multiple immunohistochemical studies on AITL specimens confirmed the (co)expression of one or more TFH markers.\textsuperscript{48-56} The pattern of PD-1 reactivity ranged from focal areas of increased numbers of extrafollicular T cells (usually less than 5% of interfollicular cells) to paracortical and diffuse reactivity throughout the lesion. PD-1$^+$ cells were particularly enriched in areas unassociated with lymphoid follicles. The knowledge that AITL arise from these TFH cells provides a rational model to explain several of the peculiar pathological and biological features inherent to this disease, i.e. the expansion of B cells, the intimate association with germinal centers in early disease stages and the striking proliferation of follicular dendritic cells.

Apart from the cases which display peculiar pathological and biological features of AITL, some PTCL–NOS cases showed the expression of TFH markers and may be related to or derived from TFH cells. However, whether nodal follicular PTCL represents an early form of AITL or a distinct entity is unclear.\textsuperscript{57-59} PD-1 is not or rarely expressed on other types of T-cell non-Hodgkin lymphoma (NHL), such as anaplastic large cell lymphoma (ALCL) (Table 3).\textsuperscript{53-55,60}

**PD-1 EXPRESSION IN NODAL B-CELL NON-HODGKIN LYMPHOMA**

Several studies investigated PD-1 expression in nodal B-cell NHL, demonstrating that chronic lymphocytic leukemia (B-CLL) was the only subtype in which the malignant B cells exhibited PD-1 positivity in almost all cases.\textsuperscript{53,61} The strongest PD-1 staining was found in large para-immunoblasts and prolymphocytes located within proliferation centers, whereas small B cells in the surrounding areas showed less positivity. PD-1 is rarely expressed by the neoplastic B cells in follicular lymphoma and diffuse large B-cell lymphoma.\textsuperscript{51,53,61} Other subtypes of B-cell NHL exhibit no PD-1 positivity among the malignant cell population.
B-cell NHL also comprise a variable proportion of PD-1⁺ reactive T cells, showing significantly higher numbers of PD-1⁺ lymphocytes in follicular lymphoma than in diffuse large B-cell lymphoma. Several studies reported that increased numbers of PD-1⁺ T cells in nodal follicular B-cell lymphoma are associated with significantly improved survival, while in contrast, one other study showed that PD-1 positivity is associated with a poorer outcome.

**PRIMARY CUTANEOUS LYMPHOMA**

**Definition**

The term “primary cutaneous lymphoma” refers to a heterogeneous group of different T-cell and B-cell NHL that present in the skin without evidence of extra cutaneous involvement at the time of the diagnosis. It is important to differentiate these cutaneous T-cell lymphoma (CTCL) and cutaneous B-cell lymphoma (CBCL) from their nodal counterparts since they often have a completely different clinical behavior and prognosis and most often require different treatment modalities.

According to the World Health Organization – European Organization for Research and Treatment of Cancer (WHO-EORTC) classification, primary cutaneous lymphoma are subdivided into the two main categories primary CTCL (75–80%) and primary CBCL (20–25%). Within the CTCL category the most common subgroups are: (1) the group of classical CTCL, including mycosis fungoides (MF), variants of mycosis fungoides and Sézary syndrome (SS); (2) the group of primary cutaneous CD30⁺ lymphoproliferative disorders (CD30⁺ LPD); (3) the group of rare and often aggressive cutaneous T/NK-cell lymphoma (Table 4). Within the CBCL category three main types of CBCL are described: primary cutaneous marginal zone lymphoma (PCMZL), primary cutaneous follicle center lymphoma (PCFCL) and primary cutaneous diffuse large B-cell lymphoma, leg type (PCLBCL–LT) (Table 4).
PD-1 expression in primary cutaneous T-cell lymphoma

Recent studies reported PD-1 expression in MF, SS and primary cutaneous small/medium sized pleomorphic T-cell lymphoma. In the following paragraphs these entities will be discussed and the potential significance of investigating PD-1 expression in these conditions will be presented.

- **Mycosis fungoides**

MF is the most common type of the CTCL, which mainly affects adults and accounts for almost 50% of all primary cutaneous lymphoma. MF is clinically characterized by the slow progression from patches to plaques, and even to tumors and in some cases may develop extracutaneous disease.66

Histologically, the early stages of classic MF show superficial band-like or lichenoid infiltrates with atypical small- to medium-sized T cells with highly convoluted (cerebriform) and hyperchromatic nuclei. These atypical T cells might infiltrate into the epidermis (epidermotropism). With progression to tumor stage, the dermal infiltrates become more diffuse, with an increase in the proportion of tumor cells, as well as an increase in the number of blast cells, and the epidermotropism might disappear. The neoplastic T cells in MF commonly show a CD4+CD8− phenotype, but may exhibit a CD4−CD8+ phenotype in some cases. Demonstration of an aberrant phenotype (for example, loss of pan–T-cell antigens, such as CD2, CD3, and CD5) is an important adjunct in the diagnosis of MF. Although MF commonly runs an indolent course, patients developing skin tumors, extracutaneous involvement or erythroderma may have a more unfavorable prognosis.

Wada et al. reported a percentage of more than 50% PD-1+ tumor cells in 6 of 15 cases of patch/plaque stage MF (40%), 9 of 15 cases of tumor stage MF (60%), and in 8 of 11 biopsy specimens from patients with SS (63%).67 In the study of Kantekure et al. 16 of 26 MF biopsy specimens (62%) contained more than 25% PD-1+ tumor cells.68 In this latter study, the authors showed that PD-1 was more frequently expressed at the early patch and plaque stages of CTCL, while PD-1 was expressed to a lesser extent at the tumor stage MF. Of note, this loss of PD-1 expression preferentially affected blast cells. Roncador et al. observed PD-1 expression in 5 of 9 MF cases (56%).51

- **Sézary syndrome**

SS is a leukemic form of CTCL defined by erythroderma, lymphadenopathy and clonal CD4+ T cells in skin and blood (Sézary cells), and generally having a poor prognosis with 25% 5-year disease specific survival. In addition, alopecia,
palmoplantar hyperkeratosis and onychodystrophy might be seen. The diagnosis of SS can be challenging. Especially in the early stages of the disease, it can be difficult to differentiate SS from erythrodermic inflammatory dermatoses (EID). Since the clinical presentation is generally not discriminative and histology may show reactive changes in up to one third of the cases, the diagnosis relies heavily on demonstration of neoplastic cells in the peripheral blood. In the WHO-EORTC classification for cutaneous lymphoma published in 2005, and incorporated in the WHO classification of lymphoid neoplasms in 2008, the diagnosis of SS is based on clinical presentation (erythroderma and lymphadenopathy) and demonstration of a T-cell clone in the peripheral blood (preferably the same clone in skin), in combination with one or more of the following criteria: an absolute Sézary cell count > 1000 cells per mm$^3$; loss of T-cell markers CD2, CD3, CD4 and/or CD5 and/or an expanding population of CD4$^+$ T cells leading to a CD4/CD8 ratio of more than 10. Refs 58, 66

Table 4. WHO-EORTC classification of cutaneous lymphoma with primary cutaneous manifestations*

<table>
<thead>
<tr>
<th>Cutaneous T-cell and NK-cell lymphoma</th>
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<tbody>
<tr>
<td>Mycosis fungoides</td>
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<tr>
<td>MF variants and subtypes</td>
</tr>
<tr>
<td>• Folliculotropc MF</td>
</tr>
<tr>
<td>• Pagetoid reticulosis</td>
</tr>
<tr>
<td>• Granulomatous slack skin</td>
</tr>
<tr>
<td>Sézary syndrome</td>
</tr>
<tr>
<td>Adult T-cell leukemia/lymphoma</td>
</tr>
<tr>
<td>Primary cutaneous CD30$^+$ lymphoproliferative disorders</td>
</tr>
<tr>
<td>• Primary cutaneous anaplastic large cell lymphoma</td>
</tr>
<tr>
<td>• Lymphomatoid papulosis</td>
</tr>
<tr>
<td>Subcutaneous panniculitis-like T-cell lymphoma</td>
</tr>
<tr>
<td>Extranodal NK/T-cell lymphoma, nasal type</td>
</tr>
<tr>
<td>Primary cutaneous peripheral T-cell lymphoma, unspecified</td>
</tr>
<tr>
<td>• Primary cutaneous aggressive epidermotropic CD8$^+$ T-cell lymphoma (provisional)</td>
</tr>
<tr>
<td>• Cutaneous γ/δ T-cell lymphoma (provisional)</td>
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<tr>
<td>• Primary cutaneous CD4$^+$ small/medium-sized pleomorphic T-cell lymphoma (provisional)</td>
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<table>
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<th>Cutaneous B-cell lymphoma</th>
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<tr>
<td>Primary cutaneous marginal zone lymphoma</td>
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<tr>
<td>Primary cutaneous follicle center lymphoma</td>
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<tr>
<td>Primary cutaneous diffuse large B-cell lymphoma, leg type</td>
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* adapted from Willemze et al. 66
Samimi et al. found increased PD-1 expression in the peripheral blood neoplastic T cells in patients with SS, but not in the circulating T cells from patients with MF. They observed a strong decrease in PD-1 expression along with improvement of disease. Moreover, an increase in interferon gamma production was found in cultures of PBMC from patients with SS if the PD-1/PD-L1 interaction was blocked by anti–PD-1 antibodies. They suggested that the high PD-1 expression may contribute to immunosuppression in SS, but mechanisms involved are as yet unknown.

Primary cutaneous CD4+ small/medium-sized pleomorphic T-cell lymphoma

Primary cutaneous CD4+ small/medium-sized pleomorphic T-cell lymphoma (PCSM-TCL) is a CTCL that is included as a provisional entity and rare subtype of primary cutaneous peripheral T-cell lymphoma, not otherwise specified (PTCL-NOS) in recent cutaneous lymphoma classifications (WHO-EORTC 2005; WHO 2008). These PCSM-TCL characteristically present with a solitary plaque or tumor that is generally localized on the face or the upper trunk, and rarely with multiple papules, plaques or tumors. In particular patients presenting with a solitary skin lesion have an excellent prognosis. Histologically, these lymphoma show nodular to diffuse infiltrates with a predominance of CD3+CD4+CD8–CD30– small/medium-sized pleomorphic T cells and a small proportion (<30%) of large CD4+ T cells. In most cases there is a considerable admixture with small reactive CD8+ T cells, CD20+ B cells, including some blast cells, histiocytes, and in some cases plasma cells and eosinophils. The clinical presentation, the architecture and cellular composition of these PCSM-TCL are strikingly similar to those described previously in so-called pseudo-T-cell lymphoma. The relationship between PCSM-TCL presenting with a solitary lesion and pseudo-T cell lymphoma and the most appropriate term for such lesions is a matter of debate.

These PCSM-TCL should be distinguished clinically and histologically from other types of CTCL, in particular PTCL-NOS and tumor-stage MF, as they have a completely different prognosis and require different types of treatment. Recently, Rodriguez-Pinilla et al. reported that medium-sized to large atypical CD4+ T cells in PCSM-TCL showed expression of PD-1, BCL6, CXCL13 and partially CD10, which suggested that these cells have a TFH cell phenotype.

PD-1 expression in primary cutaneous B-cell lymphoma

In the classification of WHO-EORTC (Table 4) three main types of CBCL are described: primary cutaneous marginal zone lymphoma (PCMZL), primary cutaneous follicle center lymphoma (PCFCL) and primary cutaneous diffuse large
B-cell lymphoma, leg type (PCLBCL–LT). PCMZL and PCFCL are indolent types of CBCL having excellent prognosis. PCLBCL–LT is an aggressive type of tumor with a 5-year survival of only circa 50%. Studies about PD-1 expression in CBCL is scarce. Rodriguez-Pinilla et al. reported negative staining for PD-1 in some cases of PCMZL and PCFCL. However, studies on the number and the distribution of reactive T-cells as performed in nodal B-NHL are lacking.

AIMS AND OUTLINE OF THE THESIS

Studies in this thesis were aimed to investigate whether the PD-1 molecule a useful marker in the diagnosis of different types of cutaneous lymphoma. In our first study presented in Chapter 2 we investigated the expression of PD-1 and other TFH cell markers in a large group of patients with PCSM-TCL. The goal of this study was to determine whether PD-1 expression by atypical T cells could serve as a useful diagnostic marker to differentiate between PCSM-TCL and other types of CTCL. In this study we included skin biopsies from patients with MF, cutaneous ALCL and cutaneous PTCL–NOS.

The study described in chapter 2 revealed that PD-1 expression was detected on neoplastic cells in only 2 of 21 MF biopsy samples. This result is in contrast to published studies of other research groups, which reported that PD-1 expression is commonly seen in MF. Chapter 3 describes results of staining for PD-1 and other TFH markers (BCL-6,CXCL-13,CD10) in a large cohort of patients with MF, including patients with erythrodermic MF and SS.

It is well-known that histologic differentiation between SS and erythrodermic inflammatory dermatosis (EID) may be extremely difficult. In Chapter 4 we investigated the expression of PD-1 in skin biopsies of SS patients and patients with various types of EID. The main goal was to investigate if the number and distribution of PD-1+ T cells is useful as an adjunct in the differentiation between SS and EID.

In the continuous search for SS-specific diagnostic biomarkers, we explored in the study described in Chapter 5 the expression of two other potential diagnostic markers, using the same group of patients with SS or EID as presented in chapter 4. These two markers are thymocyte selection-associated high mobility group box protein (TOX) and C-MYC, which is a transcription factor that regulates the global chromatin structure. Increased TOX expression by malignant CD4+ T cells
has been found in MF and SS, but information on TOX expression in EID is lacking. In addition, C-MYC positivity has been demonstrated in a considerable number of infiltrating lymphoid cells in MF and SS, while there is no information on C-MYC expression in EID. We explored in this study if expression of TOX and C-MYC can also be used as diagnostic marker for the differentiation between SS and EID.

In Chapter 6, we investigated the expression of PD-1 in skin sections from primary CBCLs. The aim of this study was to find out if PD-1 is expressed by the neoplastic B cells of these lymphoma. In addition, the number and distribution of PD-1+ T cells was investigated and correlated with clinical behavior.

Chapter 7 summarizes and discusses the findings described in the preceding chapters.
REFERENCES


42. Sfanos KS, Bruno TC, Meeker AK, De Marzo AM, Isaacs WB, Drake CG. Human prostate-infiltrating CD8+ T lymphocytes are oligoclonal and PD-1+. Prostate 2009;69:1694-1703.


64. Richendollar BG, Pohlan B, Elson P, Hsi ED. Follicular programmed death 1-positive lymphocytes in the tumor microenvironment are an independent prognostic factor in follicular lymphoma. Hum Pathol 2011;42:552-557.


