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SYMPOSIUM PAPER

Contribution of the PD-L1/PD-1 pathway to T-cell exhaustion: an update on implications for chronic infections and tumor evasion

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Abstract Recent clinical data support ideas of Programmed death receptor-ligand 1 (PD-L1; also called B7-H1, CD274) playing an important role in immune evasion of tumor cells. Expression of PD-L1 on tumors strongly correlates with the survival of cancer patients. PD-L1 on tumors interacts with the co-inhibitory molecule Programmed death receptor-1 (PD-1, CD279) on T cells mediating decreased TCR-mediated proliferation and cytokine production. In animal tumor models, blockade of PD-L1/PD-1 interactions resulted in an improved tumor control. In addition, exhausted T cells during chronic viral infections could be revived by PD-L1 blockade. Thus, targeting PD-L1/PD-1 interactions might improve the efficacy of adoptive cell therapies (ACT) of chronic infections as well as cancers. Obstacles for a general blockade of PD-L1 might be its role in mediating peripheral tolerance. This review discusses the currently available data concerning the role of PD-L1 in tumor immune evasion and envisions possibilities for implementation into ACT for cancer patients.

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Introduction

Cancer immunotherapies, either based on vaccination with tumor associated antigens (TAA) or adoptive cell therapies (ACT) using TAA-specific T cells, have attracted immunologists as a possible alternative or as an addition to conventional therapies (surgery, chemotherapy and radiation). Despite sufficient homing to tumor sites [1], tumor associated antigen (TAA)-specific T cells so far seldom control tumor growth in a clinical setting [2–4].

The reason for this is the generally accepted notion that the tumor microenvironment can protect tumor cells from immune destruction. Soluble factors as well as membrane-bound molecules, including transforming growth factor β (TGF-β), interleukin (IL)-10, prostaglandin E_2, FAS, CTLA-4 ligands and tumor necrosis factor related inducing ligand (TRAIL) have been found to be expressed by tumors and have been postulated to mediate immune evasion [5–7].

Recently, various molecules have been identified, that can modulate TCR signals [8, 9]. These include besides the well known CD28 and CTLA-4, the recently described molecules PD-1, ICOS and BTLA. Except the latter one, all interact with B7 family ligands. Besides B7-1 and -2, PD-L1 (B7-H1), PD-L2 (B7-DC), ICOS-L (B7-H2, B7 h), B7-H3 and B7-H4 (B7x, B7-S1) have been identified so far.

Within this group of molecules, PD-L1 and B7-H4 are thought to predominately mediate inhibitory signals towards T cells. The strong expression of PD-L1 on various tumors led us and other groups to the hypothesis of this ligand playing an important role in immune evasion of cancer cells [7, 10–12]. Support for our ideas evolves from recent clinical data on long-term

follow-up of renal cell carcinoma (RCC) patients [13], and data from esophageal, gastric and breast cancer patients indicate a correlation of the prognosis with an increased expression of PD-L1 on the cancer cells [14–16]. Thus, a PD-L1/PD-1 blockade might revert the immuno-compromised state of TAA-specific T cells allowing an increase in the efficacy of ACT.

PD-1 and PD-L1/PD-L2

PD-1 is a transmembrane receptor of the Ig superfamily that lacks the relevant motif for binding to B7-1 and B7-2 [17]. It is found to be expressed on thymocytes [18, 19], mature T and B cells following activation [8, 17, 20] and on myeloid cells [18]. Compared to the restricted expression of CTLA-4, this wide expression of PD-1 suggests a broader role in immune regulation [21, 22].

Two ligands for PD-1 (CD279), both belonging to the B7 family, have been identified: PD-L1 (B7-H1, CD274) and PD-L2 (B7-DC, CD273) [23, 24]. Interaction of PD-1 with PD-L1 or PD-L2 has been described to negative regulate cytokine production and proliferation of T cells [23, 25–27]. This inhibitory effect has been shown for CD4+ as well as for CD8+ T cells [23, 26]. Whereas Carter et al. found that murine CD8+ T cells are more susceptible to PD-L1 inhibition, we found human CD4+ T cells transfected with TAA-specific TCR more susceptible compared to the CD8+ counterparts [28]. In addition, some other reports identified co-stimulatory functions of these ligands, possibly mediated via an unidentified receptor different from PD-1 [29–33]. The discordant signaling effects might result from differences in the surface expression of PD-1, a different yet unidentified receptor, or from interfering signals from PD-L2 that has been also shown to mediate inhibitory or co-stimulatory effects [24, 30, 32, 34]. In addition, PD-1 is known to induce tolerance by resting dendritic cells [35]. Whereas many of the tumor models examined the direct interaction of the T-cell with the tumor, recent data indicate that the blockade of PD-L1 also led to improved control of the tumor growth in the presence of antigen presentation by DCs [12].

The cytoplasmatic tail of PD-1 contains an immunoreceptor tyrosine-based inhibitory motif (ITIM) and an immunoreceptor tyrosine switch motif (ITSM). Whereas the former is thought to mediate inhibitory signals, mutational studies indicate that the latter motif is responsible for signaling after PD-1 ligation [36]. This result opens a second explanation for the contrary results after PD-L1/PD-1 interaction. As the ITSM-motif has been shown to be capable of mediating co-stimulatory as well as co-inhibitory signals, depending on the presence or absence of another adaptor molecule (SH2D1A), PD-1 signals might depend on the activation status of the T cell. Indeed, we found WT CD8+ T cells to be superior to PD-1 gene-deficient CD8+ T cells concerning cytokine production in a naïve but not activated status (Blank et al. unpublished data), which, however, does not exclude the theory so far of a second receptor signaling early in activation.

At least in vivo, the status of the antigen-presenting cell (APC) will also alter the outcome of the TCR ligation, as it seems that the inhibitory effects of PD-L1 are mediated predominantly from resting or virus-treated DC, but not from activated DC [35, 37], which might reconcile at least some of the contrary findings.

In vivo models examining the graft survival and diabetes onset using either PD1−/− NOD mice, PD-L1Ig in islet transplantation or PD-L1tg over-expression in pancreatic islets resulted in discordant conclusions [33, 38, 39]. Besides the discussed different activation settings of the T cells in those models, different strain background of the animal models could also play a role for the discordant findings [40].

The variable expression of PD-1 on the T-cell surface, the restricted availability of agonistic PD-1 monoclonal antibodies and the low affinity of the ligand fusion protein hinders so far definitive experiments to delineate the co-stimulatory and/or co-inhibitory effects.

PD-L1 and peripheral tolerance/autoimmunity

The co-inhibitory effects of PD-1 were initially suggested because PD-1-/- mice developed, depending on the strain background, spontaneous autoimmune diseases, such as lupus-like glomerulonephritis, arthritis, gastritis or dilatative cardiomyopathy [41–44]. The effect of PD-L1/PD-1 interaction on immunological tolerance has been examined in 2C and HY TCR transgenic mice. It was found that over-expression of PD-1 inhibits, or in the absence of PD-1 increases, the positive selection [45, 46]. In contrast, we found that on an RAG−/− background, the absence of PD-1 altered the percentage of death by neglect allowing increased numbers of DN T cells to occur in the periphery, but also increased the negative selection, which was not observed in the H–Y system [19]. Using PD-1−/− mice crossed to PD-L1−/− or PD-L2−/−, clearly demonstrated the role of PD-L1/PD-1 interaction during thymic maturation. The perception that the total

numbers of 2C T cells is not changed on an autoreactive background by PD-1 deficiency leads to the conclusion of a normal central tolerance in PD-1 sufficient mice [37]. Under the light of the above-mentioned data showing alterations of the positive selection, the negative selection and death by neglect by PD-1, the previous conclusion needs to be examined further.

Independent of these considerations, the main function of the PD-L1/PD-1 pathway seems to be the regulation of peripheral tolerance [18]. This notion is supported by the various autoimmune diseases found in PD-1 gene-deficient mice. In addition, the observed expression of PD-L1 on human and murine endothelia [47–49], at sites of inflammation in experimental autoimmune encephalomyelitis [50] and in muscle biopsies from patients with inflammatory myopathies [51], supports its postulated role in regulation of peripheral tolerance. The absence of PD-L1 indeed increased the induction of experimental autoimmune hepatitis [52], which could be mediated by the absence of PD-L1, not only on Kupfer cells and monocyte-derived cells within the liver [11, 53], but also by PD-L1 on the liver parechym itself [54]. A definitive proof of the role of peripheral tolerance is the role of PD-L1 in the feto-maternal tolerance. It has been earlier shown that PD-L1 is strongly expressed at this immunological important barrier [55], but just recently data proved the functional relevance in preventing abortion in a mouse model [56].

PD-L1 and T-cell exhaustion during chronic viral infections

During chronic viral infections, functional virus-specific T cells are induced; however, they gradually lose their function during the course of the infection. IFN-γ is a key player in host defences against viral infections, but also has been shown to up-regulate PD-L1 expression predominantly on non-lymphoid tissues, due to the PD-L1 promotor region containing several IFN-γ-responsive elements [11, 49].

Thus, it has been self-evident to examine the role of the PDL-1/PD-1 pathway during chronic viral infections. Recently, R. Ahmed et al. elegantly showed that antiviral immune functions could be restored in vivo by a blockade of PD-L1 in the LCMV infection model. By doing so, they also proved that PD-L1/PD-1 interaction plays a pivotal role during CD8+ T-cell exhaustion from chronic antigenic stimuli [57].

This mechanism might be extended to other chronic viral infections in humans, such as human immunodeficiency (HIV). Indeed, PD-L1 has been shown to be up-regulated during HIV-infection [58] and several recently published studies suggest a role for the PD-1-PD-L1 pathway in exhaustion of virus-specific CD8+ T cells during HIV infection [59–61]. In addition, these groups could show that the PD-1 expression correlates with disease progression and blockade of PD-L1/PD-1 pathway could restore immune functions of the T cells.

It seems that there is a distinct hierarchy of exhaustion, as that with the duration of the infection, the level of antigen exposure increases, the CD4 help decreases and PD-1 expression on antigen-specific T cells increases [62]. Whether these ideas also could be transferred on chronic antigen exposure during ACT in cancer patients needs to be further evaluated.

PD-L1 and immune evasion of tumor cells

Whereas the initial broad detection of PD-L1 was based on mRNA levels, its surface expression was detected only on T- and B-cells, dendritic cells, macrophages, endothelia, epithelia, cardiac myocard, pancreatic islet cells, glial cells, inflamed muscle, keratinocytes and the feto-maternal barrier [20]. Interestingly, PD-L1 surface expression can also be found on almost all tumor entities. It has been detected by immunohistochemistry, for example, on the surface of human cancers of larynx, lung, stomach, colon, breast, cervix, ovary, renal cell, bladder, liver, glioma and melanoma [11, 25, 63]. In vitro experiments indicate that many tumor cell lines also express PD-L1 and/or up-regulate PD-L1 surface expression upon exposure to IFN-γ [11, 20, 63]. This expression is in strong contrast to the expression to B7-1 and B7-2, which are seldom found on tumors. The attributed inhibitory effect of PD-L1/PD-1 interaction earlier led to the hypothesis that tumors escape from the host immune system by negative attenuation of tumor-specific T-cell responses via the PD-L1/PD-1 pathway [7, 64].

In vitro experiments using PD-L1 over-expressing murine tumor cell lines and blocking antibodies against PD-L1 and PD-1 clearly demonstrated that PD-L1 on tumor cells suppresses the cytolytic activity of CD8+ T cells [7, 12]. In addition, others and we could show that endogenous PD-L1 expression on tumor cells are also capable of suppressing T-cell functions, e.g., proliferation and cytokine production [11, 20].

Transfers to in vivo experiments using blocking antibodies of PD-1, PD-L1 or TCR-transgenic mice crossed to PD-1−/− mice revealed accelerated tumor eradication in the absence of PD-L1/PD-1 interaction [7, 12, 20, 65]. In addition, PD-L1 blockade has been

shown to suppress tumor metastasis using melanoma or colon cancer cell lines [66].

Similar to murine studies, others and we recently found PD-L1 to be expressed on human tumor tissues and human tumor lines and to be up-regulated upon IFN-γ exposure. Over-expression of human PD-L1 or interference using anti-PD-L1 blocking antibodies support the notion of tumor-expressed PD-L1 being capable of suppressing T-cell immune functions [11, 28, 63, 67].

Whereas some studies did not indicate a significant relevance of PD-L1 expression for the patients' prognosis in non-small lung cancer [68] or even did not detect PD-L1 at all on acute myeloid leukemia [69], we found in contrast to the latter, frequent expression of PD-L1 on leukemic blasts (Blank et al. unpublished data). Furthermore, a recent paper revealed prognostic value of PD-L1 expression for breast cancer [16], esopgageal cancer [14] and gastric carcinomas [15]. The strongest correlation between cancer patients' survival and tumor-associated PD-L1 expression has been shown within a group of over 300 patients that underwent nephrectomy for clear cell renal carcinoma [13, 70]. Survival rates at 1, 5 and 10 years post-nephrectomy in patients expressing PD-L1 within the primary tumor (compared to PD-L1 negative tumor, in brackets) were 77.3% (94.6%), 41.9% (82.9%) and 36.7% (77.4%). Univariate analysis of these data indicate that patients with a PD-L1-positive tumor nearly four times more likely die from RCC compared to patients with a PD-L1-negative tumor [13].

Based on these in vitro data and the correlation of the clinical prognosis of patients with PD-L1 expression in the tumor microenvironment, it is very likely that the PD-1/PD-L1 pathway plays a pivotal role in tumor-immune evasion from the endogenous immune system, as well as from adoptively transferred tumor-specific or vaccination-induced T cells.

Considerations concerning PD-L1 blockade and ACT

Tumor-specific T cells have been shown to be dysfunctional or anergised [71] and just recently Blankenstein et al. have elegantly shown that immunogenic tumors indeed induced T-cell anergy allowing immune evasion [72]. Continuous exposure to antigen in tumor patients might therefore exhaust T cells, similar to chronic viral infections [57]. Despite differences between chronic antigen stimulation during viral infections and malignancies (tumors seldom express very immunogenic antigens, IFN-γ induction is more likely during viral infections, connective tissues barriers in tumors, etc.) it is tempting to speculate that immunogenic tumors might induce anergy of tumor-specific T cells by expressing PD-L1 on their surface [37]. In the light of many immunotherapy protocols aiming at induction of a type-I immune response [73, 74], even IFN-γ-induced PD-L1 up-regulation on tumor cells might be accountable for the so far often disappointing outcome of tumor immunotherapies. The blockade of the PD-L1/PD-1 pathway might therefore either revert an immuno-compromised state of the cancer patients or prevent inhibition of adoptively transferred T cells during ACT and allow the immune system to eradicate the tumors.

Interfering into the PD-L1/PD-1 pathway by, e.g., an anti-PD-L1 monoclonal antibody has been even in our considerations [10]. However, PD-L1's broad expression on endothelia and its important role in regulation of peripheral tolerance and thus prevention of autoimmunity [18, 37, 75] will require a more specific blockade of the PD-L1/PD-1 pathway or at least extensive testing of binding to normal tissues before in vivo applications.

In addition, the difference between PD-L1 and PD-L2, which share many features, but with contrary outcomes resulting from their blockade, is still poorly understood. Thus, it could be problematic blocking selectively PD-L1 and in doing so allowing an unrestricted PD-L2-mediated, possibly co-stimulatory pathway to act on a broad range of resting peripheral T cells.

Therefore, we would attribute a preference for the blockade of PD-1 on selected tumor- or virus-specific T cells. However, in this case, in advance of such approaches, the bidirectional roles of PD-1 toward co-stimulatory versus co-inhibitory effects have to be dissected clearly.

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