Immune checkpoints in chronic obstructive pulmonary disease

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Immune checkpoints are vital controls of cell-mediated immunity and may play a key role in the pathology of COPD http://ow.ly/vLu430bZVhS

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ABSTRACT Cell-mediated immune responses are vital to the body's defence against infection and play a key role in tumour immunity. T-cell activation and cytotoxic function is tightly regulated by a series of immune-regulatory receptor–ligand interactions or immune checkpoints. These controls limit immune-mediated damage, particularly in the context of chronic infection. However, prolonged signalling through these axes can lead to progressive loss of T-cell function, termed exhaustion.

Understanding of the biology of checkpoints and that exhaustion is reversible has been key to the development of new therapies directed at reversing the dysfunctional status of T-cells, which are dramatically improving outcomes of cancer treatment.

Emerging data suggest that immune checkpoint axes are dysregulated in chronic obstructive pulmonary disease (COPD). T-cells from diseased lungs express the key receptor programmed death (PD)1 and demonstrate loss of cytotoxic function. However, the picture is complex with evidence of downregulation of the associated ligand PDL1 on alveolar macrophages. The resulting impact may be excessive T-cell inflammation as a consequence of acute infection, which may contribute to the pattern of exacerbation and lung damage characteristic of COPD. More work is needed to understand these immune controls in COPD before the therapeutic advances seen in lung cancer can be explored.

Introduction

Cell-mediated immune responses are fundamental to defence against infectious pathogens, particularly viruses [1], and central to the response to tumours [2]. The biology of T-cell responses to antigenic stimuli has been well described, in that acute responses of naïve T-cells to relevant antigens leads to the development of memory cells, which can then rapidly respond to rechallenge by pathogens with resulting clonal expansion, activation and effector function, including cytokine secretion and cytotoxicity, contributing significantly to protective immunity [3–5]. Classically, immune responses to acute infection result in rapid clearance of the pathogen and hence reduction in antigenic load [6]. However, when chronic infection develops [7] or antigen presentation persists due to tumour development [8] a fine balance between T-cell activation and control is required to limit the impact of T-cell-mediated damage while permitting effector function [9]. Recent advances in our understanding of these chronic immune responses have included the identification of numerous inhibitory pathways that are integral to

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coordinating an effective immune response while limiting collateral damage to organs and tissues [10–12]. These immune checkpoints are integral to self-tolerance and consist of an array of ligand–receptor interactions that can significantly modulate T-cell responses [13].

These checkpoints can influence T-cell function at the point of initial antigenic stimulation in the lymph node through receptor–ligand interactions, chiefly with dendritic cells [14]. Here, the role of cytotoxic T-lymphocyte-associated antigen (CTLA)-4 has been established as an important control of T-cell function early in the activation cycle [15]. In addition, these immune checkpoint receptors, which are part of the CD28 family of cell surface receptors [16], play a significant role in controlling the cell-mediated response in tissues where both antigen-presenting cells (APCs) and structural cells may express checkpoint ligands, and both antigenic stimulation and checkpoint ligand expression contribute to a fine equipoise of immune activation and control [17, 18]. Here the programmed death (PD)1 receptor has an established role, in limiting T-cell effector function, especially cytotoxic activity [19]. PD1 and its interaction with the ligands PDL1 and PDL2 leads to modulation of kinases in T-cells [20], affects the duration of T-cell–APC interactions [21] and has a significant effect on regulatory T-cell (Treg) biology [22]. The biology of PDL expression and interaction is complex and incompletely understood; both ligands signal through the same PD1 receptor on T-cells, but have been shown to interact *via* CD80 and potentially other T-cell receptors [23].

T-cell exhaustion

Chronic engagement of checkpoint receptors on T-cells can lead to the phenomenon of T-cell exhaustion [24]. This loss of effector function was first described in the context of chronic viral infections in mouse models, particularly those using genetically modified strains of the lymphocytic choriomeningitis virus [25]. In these models, demonstration that tissue-resident T-cells receiving chronic antigenic and associated PD1 stimulation gradually lose effector function, and consequently the ability to control infections [26]. The exact mechanisms by which receptor engagement leads to the development of an exhausted cellular phenotype are complex. PD1 signalling through the immunoreceptor tyrosine-based switch motif suppresses Akt phosphorylation through PI3 kinase inhibition. These pathways are distinct from those activated through the CTLA-4 receptor [20]. The concept of T-cell exhaustion is that it is not a discrete phenomenon, but a spectrum of dysfunction which begins with loss of proliferative capabilities and proceeds in a hierarchical manner through loss of cytokine production and eventually reduced cytotoxic and interferon (IFN)-γ effector function [27]. An array of receptors has been identified relevant to this process, with clear evidence from early animal studies that this phenomenon may be reversible, with recovery of T-cell effector function seen with blockade of pathways including PD1 [28]. In studies of chronic infection, virus-specific $CD8^+$ T-cells in animal models express other key checkpoint receptors, including lymphocyte-activation gene (LAG)-3, CD244, CD160 and TIM-3 [29]. Better reconstitution of T-cell function is seen when multiple receptors are blocked, suggesting synergistic signalling effects [30]. Similar patterns of cellular expression and T-cell exhaustion have been demonstrated in an array of chronic infections in humans, most notably in tuberculosis [31], HIV [32] and chronic viral hepatitis [16]. Interestingly, T-cell exhaustion has been described in conditions associated with autoimmunity, such as rheumatoid arthritis, suggesting a protective role of checkpoint pathways in this context [33].

Recognition that T-cell exhaustion may limit antitumour responses and that manipulation of immune checkpoint pathways may lead to a gain of effector function quickly led to the exploration of new therapeutic approaches in immune-oncology. In the context of lung disease, therapeutic blockade of the PD1 pathway in nonsmall cell lung cancer has been shown to limit disease progression and improve survival [34, 35]. Indeed, clinical case stratification by PD1 or PDL1 expression is becoming an established strategy in guiding the treatment of this condition [36]. However, in a minority of treated cases checkpoint blockade results in organotypic inflammation, commonly pneumonitis [37]. Here it is postulated that removal of checkpoint controls leads to excessive T-cell activation, tissue inflammation and damage [38]. It is not known what the nature of this unbridled T-cell response is in terms of antigenic target, but therapeutic strategies have evolved to limit effects by reducing dose and duration of therapeutic antibody regimens [39].

Immune checkpoints and chronic obstructive pulmonary disease

The biology of immune checkpoints in the context of chronic lung inflammatory disease is less well understood than in cancer. Chronic obstructive pulmonary disease (COPD) is a condition characterised by an excess of $CD8^+$ T-lymphocytes in the diseased lung and the development of bronchially associated lymphoid tissue rich in effector cells is characteristic of advancing disease [40]. Despite the presence of these potentially protective cells, COPD patients are highly susceptible to the effects of viral and bacterial infections, and both microbial dysbiosis and recurrent exacerbations lead to poor disease outcomes [41]. This paradox could be explained by a loss of immune function due to chronic inflammation and antigenic stimulation in a manner analogous to the tumour microenvironment.

Work in this area is still limited, but an early observation, that increases in PD1 expression were seen on T-cells from COPD patients compared to matched controls and that checkpoint blockade leads to the recovery of IFN-γ release, suggested that these pathways warrant further study in this condition [42]. The majority of the biological effects of cell-mediated immunity is specific to the local effects of tissue-associated lymphocytes which are directly affected by the antigen-rich milieu and inflammatory environment of the organ in question [43]. Therefore, we sought to explore the comparative immune biology of the lung itself and its resident T-cell populations in COPD. This work has focused on the difficult field of lung-specific T-cell function and has used clinically relevant infection models to elicit functional responses [44]. This approach is hampered by access to lung tissue, harvesting of adequate cell numbers and the experimental limitations of using live respiratory pathogens. However, the benefits of this approach are clear in their immediate applicability to human disease. Work in our group by McKendry *et al.* [45] has established that PD1 expression on lung-derived $CD8^+$ T-cells was greater in COPD than controls (16.2% *versus* 4.4%). However, other markers of T-cell exhaustion, such as Tim-3 were not co-expressed on lung-derived T-cells from either COPD donors or controls, suggesting that a fully exhausted phenotype was not present. Furthermore, experimental influenza infection upregulated this expression in both groups, but only control and not COPD-derived $CD8^+$ cells upregulated the cytotoxic degranulation marker CD107a. This suggests some loss of T-cell cytotoxic function in the context of the COPD lung.

Looking at the dynamics of PDL1 expression, we found that while viral infection increased this ligand's expression on alveolar macrophages overall, this increase was less marked in COPD patients relative to healthy subjects, indicating that a loss of checkpoint modulation *via* PDL1 may be present in disease. The relevance of this finding in the model system was confirmed as there was a significant increase in IFN-γ release in the system in COPD compared to controls. Interpretation of these experimental findings in the context of this heterogeneous disease requires caution. However, this may suggest that T-cell responses may be significantly aberrant in the COPD lung, driven by an altered balance between T-cell receptor and APC ligand expression, and that as a consequence acute antigenic stimulation such as that seen during viral infection leads to excessive inflammation, due in part to deranged checkpoint modulation. However, even in a controlled *ex vivo* model the data demonstrated great heterogeneity both in the responses seen to *ex vivo* challenge in COPD patients and smoking controls. Clearly, mechanisms to stratify patients using accessible markers of T-cell status will also be necessary in developing an adequate understanding of the biology suitable to frame therapeutic trials.

The mechanisms by which aberrant PDL1 expression occurs have been further studied by our group. Staples *et al.* [46] established a monocyte-derived macrophage model of T-cell stimulation. Using live influenza infection, we were able to demonstrate that PDL1 was dynamically induced by influenza and that IFN-β plays a key role in the modulation of PDL1 expression. Both augmentation of the culture with IFN-β and knockdown of IFN-β using short interfering RNA produced measurable effects on PDL1 expression in the model and consequent IFN-γ production. This work suggests that innate IFN defects described in airway diseases [47, 48] may play a key role in the control of adaptive, cell-mediated immunity. However, it is still uncertain how the manipulation of checkpoint biology such as that seen in cancer therapeutics can play a role in diseases such as COPD, or whether augmentation of type 1 IFN responses could play a role.

One of the major complicating factors in COPD is the important role that airway infection plays in both acute exacerbations [49] and chronic airway inflammation and hence disease progression [50]. Here the equipoise between immune activation and immune suppression must be even more finely held. Current therapies that alter this balance, for example inhaled corticosteroids, have been shown to increase the risk of severe infectious consequences such as pneumonia [51], presumably due to immunosuppressive effects [52]. The exact role of T-cells in the COPD lung remains incompletely understood; a number of potential antigen-specific targets, both infective [53] and autoimmune, may be present [54] in the inflamed lung. It is currently not certain whether checkpoint blockade would lead to beneficial disease-modifying activity, the eradication of key pathogens or inflammation and lung damage.

Work in peripheral blood has established that proliferation and cytokine production of COPD patient-derived effector T-cells was enhanced in the presence of anti-CTLA-4 or anti-PD1 blocking antibody in response to stimulation with the potentially key antigen P6 from nontypeable *Haemophilus influenzae* [42]. The authors report that anti-PD1 blockade resulted in a moderate enhancement in IFN-γ production, and that treating Treg suppression *via* CTLA-4 blockade may enhance antibacterial immunity in patients with COPD. Therefore, it is not only the effects of checkpoint therapeutic blockade on cytotoxic T-cells that requires further study, but also the effects on Tregs. Furthermore, there is a heterogeneous pattern of chronic infection in the COPD airway which can lead to significant and adverse clinical consequences [41]. Clearly, characterisation of infection risk and even microbial colonisation may be important steps in stratifying COPD patients for immune-modulating therapy.

Conclusion

Further work is required to improve our understanding of immune checkpoint biology in the context of lung infection and inflammation seen in COPD. Key aspects of future work will include description of the nature of the pathological $CD8^+$ T-cell population seen in the lungs of COPD patients. Understanding the repertoire of antigens recognised by these key effector cells, and particularly the question of whether these are pathogen or self (or both) will be crucial. Checkpoint blockade and uncoupling of controls of cytotoxic activity may lead to lung inflammation, especially if the main targets of the resident memory cells in the lung and bronchial associated lymphoid tissue are involved in autoimmune processes. In addition, a more complete description of T-cell activation and regulation status in the lung will be required before the advances in immune modulation seen in cancer can be explored therapeutically in COPD.

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