**Thesis Abstract: Characterisation of Blood Dendritic Cells in Patients with Cancer**

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Dendritic cells (DC) are the key antigen-presenting cells (APC). They play a critical role in initiating specific cellular and humoral immune responses and have been implicated in the defective function of the immune system during cancer progression. Despite the well-demonstrated role of DC in modulating antitumour immunity, only a few studies have investigated the systemic effect of cancer on in vivo circulating DC populations. In the present study, the effects of cancer on the immune system were assessed focusing on how it affects the function of different populations encompassed within the blood DC compartment. The study of the function of cells within the blood DC compartment (as opposed to in vitro generated DC) was chosen despite the fact that it implies significant technical constraints, because they represent the bona fide DC populations. Moreover, blood DC are in their natural state of differentiation and free from the influence of exogenous cytokines thus, reflecting more directly the natural biology of systemic immune responses occurring in vivo.

Given that tumours have been demonstrated to produce a plethora of immunosuppressive factors that exert systemic effects on immune cell function affecting DC, the first stage of this research aimed at reviewing current evidence on the effects of tumours on DC biology. Reports describing the role of tumour-derived factors in the induction of DC dysfunction are examined and discussed in view of the current knowledge to suggest that tumour-induced alteration of DC differentiation, maturation and longevity is one of the crucial mechanisms for tumour-induced immune suppression in cancer.

Therefore, the next stage of the research program investigates the effects of tumours on the frequency and phenotype of circulating DC. To evaluate this, blood DC counts, phenotype and subset distribution were monitored in a cohort of patients with early (Stage I/II, n=95) and advanced (Stage III/IV, n=21) breast cancer showing evidence that the blood DC compartment can be compromised by disease progression. Blood DC numbers were consistently reduced in patients with advanced disease suggesting a diminished availability of DC precursors in patients with more systemic disease. Moreover, a prolonged period of reduced DC counts extending over 48 weeks after tumor resection was documented in patients with early disease. Finally, the blood DC compartment in patients with advanced disease revealed an alteration in (i) the distribution of myeloid (CD11c^+DC) and plasmacytoid (CD123^-DC) subtypes as well as (ii) reduced expression of molecules essential for optimal co-stimulation and antigen presentation to T-cells.

The next stage of research further explores the nature of these alterations. It is demonstrated that the reduction in myeloid and plasmacytoid DC counts is associated with the accumulation of a previously undefined population of HLA-DR^+CD11c^-CD123^- cells lacking markers for most mature hematopoietic lineages (HLA-DR^+ immature cells, DR^+IC) in a cohort of patients with breast (n=120) and prostate (n=10) cancer as well as malignant glioma (n=6). In order to study their functional phenotype, DR^+IC from cancer patients were purified and side-by-side comparisons were performed with their DC counterparts.

Light and electron microscopy revealed that DR^+IC are small cells with poorly developed organelles and condensed chromatin in the nucleus, suggesting immaturity. Phenotypic characterisation showed heterogeneity with variable expression of antigens ascribed to the DC, early B-cell and progenitor lineages. Moreover, in contrast to DC, DR^+IC exhibit limited capac-
ity to capture antigen eliciting reduced proliferation and IFN-γ secretion by allo-reactive T-cells. Finally, increased numbers of these cells correlate with disease status and tumour progression. This is exemplified by the fact that patients with advanced breast cancer demonstrate a significantly larger number of DR^+IC in the circulation than patients with early disease, and also, the observation that in patients with fully-resected malignant glioma, the proportion of DR^+IC in blood is increased when clinical evaluation indicates tumour progression.

The fourth phase of this study evaluates whether DR^+IC could have an impact on the nature of the immune response. For this purpose, DR^+IC and DC were co-purified and their function thoroughly assessed including capacity to capture and present antigens as well as the nature of the T-cell responses generated. In contrast to DC, DR^+IC exhibited a limited response to inflammatory cytokines (TNF-α, IL-1β, IL-6 and PGE₂) or ligands for toll-like receptor (TLR) 4 (Lypopolysaccharide, LPS), TLR3 (viral double-stranded RNA, poly I:C) and TLR9 (bacterial DNA, CpG oligodeoxynucleotide; CpG ODN) in terms of phenotypic maturation (CD40, CD80, CD83, CD86 and HLA-DR) or cytokine secretion (TNF-α IL-10 and IL-12). In addition, in all the systems tested (antigen uptake, allogeneic T-cell proliferation, CTL-elicitation, MHC-II-restricted antigen presentation and cross-presentation), DR^+IC were significantly less efficient than DC. DR^+IC induced poor Th1 (IFN-γ, TNF-α and IL-2) and preferentially induced Th2 bias (IL-4) in activated T-cells. Interestingly, DR^+IC exhibited marked resistance to the pro-apoptotic effect of tumour-derived supernatants and exhibited substantial migratory capacity to inflammatory cytokines in vitro. Finally, ways to differentiate and optimize the function of DR^+IC as antigen presenting cells were investigated. It was found that despite the poor responsiveness to inflammatory or pathogen-derived factors, CD40 stimulation induced phenotypic maturation and secretion of bio-active IL-12, in turn, generating more efficient T-cell activation.

Finally, the implications of the aforementioned findings in relation to tumour-induced immune suppression, DC-based immune monitoring as well as DC-based immunotherapeutic strategies for cancer are discussed. The relevant data are presented to support the notion that disease progression in cancer patients can have significant effects on the blood DC compartment. Indeed, the evidence gathered here indicate that immature cells (DR^+IC) that accumulate in patients with cancer can contribute to immune suppression by means of inefficient antigen presentation, displacement of DC populations and/or generation of inadequate immune responses. It is also suggested that given the remarkable differences in functional capacity and responsiveness between DR^+IC and DC, the evaluation of blood DC broadly defined as Lin^-HLA-DR^+ cells is to be carefully assessed, particularly in patients with cancer, where DR^+IC represent a significant proportion of this compartment. More importantly, this study identifies an approach (CD40 stimulation) able to activate and differentiate these cells in vitro, thus generating more efficient T-cell responses. The finding that CD40 ligation not only boosts the antigen-presenting cell function of DC but also DR^+IC, substantiates the utilization of ex vivo conditioned APC to correct the unbalanced immunologic performance in cancer and may prove to be crucial in improving the efficacy of DC-based immunotherapies for cancer.

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