Dendritic Cell Vaccines: Release Assays and Potency Assays

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Dendritic cells (DC) captured the imagination of biomedical researchers when it became clear that these hematopoietic cells were capable of priming potent immune responses (1, 2). When methods for their in vitro culture from peripheral blood myeloid cells and murine bone marrow or splenocytes became available (3), researchers in infectious disease and cancer began testing these cells as autologous vaccines. Given their potent ability to take up antigens in their environment spontaneously, and via many pathways (pinocytosis, cell surface receptors, scavenger receptors, Fc receptors, heat shock proteins), it was clear that they are relevant in many disease settings for many antigens. Substantial data also demonstrate the unique ability of DC to integrate complex environmental cues from pathogens via toll-like receptors (TLRs) and stressful, dangerous endogenous signals (damage-associated molecular patterns (DAMPs)) to shape the subsequent immune response appropriately (4). Thus, these cells are sensing their environment, taking up antigens and presenting them to other immune cells with sufficient additional information to shape the immune response.

They are easy enough to prepare in vitro. Peripheral blood monocytes are isolated via plastic adherence, monocyte elutriation or CD14+ magnetic bead purification, most commonly. Most such trials begin with a 90 minute to 3 hour leukapheresis to prepare sufficient cells to allow delivery of 5 x 10^6 to 10^8 DC per injection, for several injections, given weekly, monthly, or by another schedule (5-9). The monocytes are cultured for 5-7 days in GM-CSF and IL-4, and then all of the clinical protocols diverge. The form of antigen chosen (synthetic peptide, purified protein, tumor or cell line protein lysate mix, viral vector, etc.), and the maturation signal delivered (inflammatory cytokine cocktail, bacterial product like LPS, others) vary broadly and have evolved with time and data (10). The schedule, dose and route of delivery are additional protocol-specific variables being tested both clinically and pre-clinically. Unfortunately, while objective clinical responses have been seen in several DC vaccine trials (10, 11), they have consistently been in a minority of patients.

How can the area of cancer vaccination move forward? A potency assay would be enormously helpful to independently determine the functional capacity of a given lot of DC. For cancer vaccines which have the goal of promoting type 1, cytotoxic T cell responses, IL-12p70 has been demonstrated to be a potent mediator produced by DC (12). This assay has been standardized (13) and used in multiple clinical trials. In an informative trial in glioblastoma multiforme, increased IL-12p70 production by DC correlated with improved progression-free survival (14). This assay is also being employed in ongoing trials to validate it as a potency assay. A more complex but unbiased approach is to test full mRNA expression profiles by transcriptome profiling (15, 16), which may hold particular promise to identify a gene expression signature of several genes that identify a functionally potent DC. Also of interest are recent
observations of structural changes in cultured DC which relate to their antigen presentation function (17). These data sets are developing in different trials and may identify gene expression profiles that help identify effective DC vaccines for cancer therapy, as well as DC capable of suppressing autoimmune responses, as in diabetes (18), or suppressing allogeneic responses to transplanted organs (19).

What is currently measured in clinical trials testing DC vaccines? There is, of course, required safety testing (sterility, mycoplasma, endotoxin). There are also identity tests, which are protocol-specific. For cancer vaccine trials, common DC phenotypic markers tested are: MHC class I (HLA - A, B, C), MHC class II (HLA-DR), costimulatory molecules (CD80, CD86, CD40), myeloid lineage markers (CD11c, CD14), maturation markers (CD83, CCR7), and markers of lymphocytes that can “contaminate” the product, particularly if plastic adherence was used to enrich monocytes (CD3, CD19, CD20, CD56). While in vitro and preclinical experiments and occasional dramatic clinical responses continue to support experiments and new trial development with DC vaccines, definitive progress in the field may depend on the identification of long-sought DC potency assays.

References


