on a subset of myeloid DCs, the CD8α$^+$ subset in mouse lymphoid tissues, for example. The latter, however, does not express TLR 5 and TLR7 (59). TLR signaling can lead to the production of cytokines with significant immune enhancing effects. For example, IL-12, whose production is enhanced by the transcription factor IRF-5 (60), acts on CD4$^+$ T cells to enhance Th1 differentiation. Type I IFNs (many IFN-αs and a single IFN-β), whose production is enhanced by the transcription factors IRF-3 and IRF-7 (61), act on CD8$^+$ T cells (62,63) and B cells (64) to enhance the development of cytotoxic T lymphocytes (CTLs), antibody formation, and memory.

The type of TLR ligand also influences the differentiation of helper T cells. CpG DNA, a TLR 9 ligand, and imiquimod, a TLR 7 ligand, can be adjuvants for Th1-type immune responses (65,66), whereas in contrast, the TLR 2 ligand, Pam3Cys, and the TLR 5 ligand, flagellin, have been reported to favor Th2 type responses (67–69).

In summary, the outcome of antigen presentation by DCs depends on the state of DC differentiation or maturation. In the steady state, immature DCs capture, process, and present a variety of environmental antigens and dying cells. The presentation of MHC-antigen complexes by immature DCs to T cells leads to tolerance, whereas mature activated DCs typically induce strong effector T-cell responses. DCs undergo terminal differentiation or maturation in response to a variety of environmental stimuli. The maturation program varies with the stimulus, and the consequences for lymphocytes are likewise different. Therefore, there is a need to dissect the immune responses that are induced by the engagement of different maturation stimuli so that vaccines can be designed to elicit responses that are appropriate to the pathogen at hand.

VACCINES COMPRISED OF DCS EXPOSED TO ANTIGENS EX VIVO

Mature DCs are Adjuvants for Immune Responses

After it became apparent that DCs were specialized and potent stimulators of T-cell mediated immunity in tissue culture, it was decided to use these cells as adjuvants in vivo in rodents. Lechler and Batchelor (70) showed that DCs were major stimulators of graft rejection in vivo and at small doses. Macatonia et al. tested hapten-modified DCs in vivo, and the cells induced contact sensitivity (71). Inaba et al. pulsed DCs ex vivo with protein antigens, reinfused the cells, and found that mice could be primed directly and specifically to the protein antigen captured by the DCs. In the latter experiments, the proteins first had to be given to the DCs in their immature or antigen-capturing state (which is the state of most DCs in vivo), and then the maturing DCs were injected (72). Investigators next considered more challenging antigenic targets. Again the DCs served as adjuvants for strong T-cell priming. DCs pulsed with tumor antigenic peptides or with viral vectors recombinant for tumor antigens were able to elicit protective immunity to tumor challenge, and in some instances caused existing tumors to undergo some regression (73,74). DCs pulsed with microbial antigens also could induce protective immunity to infection (75,76), while DCs bearing autoantigens could trigger autoimmunity (77,78). Because it is well known that proteins and preprocessed antigenic peptides are poor immunogens unless they are administered together with adjuvants, these early experiments implied that DCs could function as ''nature's adjuvants'' for inducing protective and pathogenic T cell–mediated immunity in vivo (72).

Ex Vivo-Derived, Antigen-Loaded DCs in Cancer Therapy

Once it was established that DCs could prime or sensitize T cells, after being charged with model antigens like OVA or keyhole limpet hemocyanin (KLH) and reinfused into mice, researchers decided to move this strategy into humans. To do so, one major challenge was to obtain large numbers of DCs for purposes of therapeutic vaccination or immunotherapy in the setting of cancer. Granulocyte-macrophage colony stimulating factor (GM-CSF) is a valuable cytokine for DCs (79,80), being used to expand DCs from proliferating progenitors (81–83), and for differentiating DCs from nonproliferating monocyte precursors (84,85). Much of the current research is being carried out using monocyte-derived DCs (86–88), which are potent and homogenous stimulators of immunity. It is possible to obtain populations of immature DCs by exposing monocytes to GM-CSF and IL-4 (89,90), and these can be differentiated into mature DCs by various stimuli such as TLR ligands, inflammatory cytokines, or CD40L (85,91).

Some of the first studies to assess if autologous DCs could act as immune adjuvants were carried out in healthy volunteers. DCs were pulsed with KLH or with an influenza virus peptide ex vivo and reinjected in the absence of any other adjuvant. The antigens by themselves were not immunogenic, but on DCs, T-cell immunity was induced. Interestingly, the CD4$^+$ T-cell response to KLH was Th1 in nature (92), while the memory CD8$^+$ T-cell response to influenza matrix peptide seemed to select for higher affinity T cells (93). On the other hand, when immature DCs were used, antigen specific IL-10 producing cells were generated and could suppress the response of IFN-γ producing T cells (23,92).

The first clinical trial using monocyte-derived DCs was performed by Nestle et al. in 1998 (94). These authors loaded DCs with melanoma antigens (tumor lysate) and reported that 30% of stage IV patients were able to mount a response. Thurner et al. reported that the loading of mature DCs with a melanoma-specific peptide (Mage 3A1) expanded specific cytotoxic T cells; this occasionally caused the regression of some metastases (95) and frequently primed for Th1 type CD4$^+$ T-cell immunity (96).

Besides monocyte-derived DCs, peripheral-blood DCs loaded with specific idiotype protein have been used as vaccines in patients with follicular B cell lymphoma (97). Also, DCs derived from CD34$^+$ hematopoietic progenitor cells were loaded with melanoma antigens and used to vaccinate patients. The individuals who survived longest were the ones able to mount a response against more than two melanoma antigens (98). Many phase I type safety studies have used DCs in advanced cancer patients (for a review see Ref.99). However, a major obstacle resides in the fact that the injected DCs home poorly to lymphoid tissues, thus failing to harness one of the fundamental features of DC function.

TARGETING OF TUMOR CELL VACCINES TO DCS IN VIVO

Vaccines Comprised of Irradiated Tumor Cells Transduced with Cytokines

Dranoff et al. have asked if irradiated tumor cells could acquire increased immunogenicity if transduced to express cytokine genes. Among a variety of tested cytokines, irradiated tumor cells expressing murine GM-CSF stimulated potent, long lasting, and specific antitumor immunity, requiring both CD4$^+$ and