CD8$^+$ cells (100). When tested in metastatic melanoma patients, autologous irradiated GM-CSF transduced tumor cells, known as GVAX, were immunogenic, and biopsies of the vaccine site indicated that patient DCs were being recruited and expressed high levels of B7.1 (101). Jaffee et al. used allogeneic GM-CSF-transduced tumor cell lines as a vaccine and found that antigens within the injected irradiated GVAX were being cross-presented, again suggesting that host DCs were capturing the dying tumor cells and initiating CD8$^+$ T-cell immunity (102). GVAX approaches are now in phase III trials.

Vaccines Comprised of Dying Tumor Cells and A Stimulus for NKT Lymphocytes

A critical step in vaccine efficacy is that DCs capturing vaccines must also undergo maturation to be able to induce T-cell immunity and control its quality. Evidence for DC maturation in vivo was obtained when mice were injected with lipopolysaccharide (LPS) (103) and CpG (104). Before LPS treatment, many splenic DCs were found at the margin between the red and white pulp and could process soluble proteins effectively. On the other hand, six hours after LPS administration, DCs were found in increased numbers in the T-cell area, had a reduced capacity to process proteins, but showed increased expression of B7 costimulators and T-cell stimulatory capacity. It was also shown that plasmid DNA containing CpG motifs and a transcription unit for OVA could mature plasmacytoid and myeloid DCs in vitro (105). Both DC subsets upregulated the expression of costimulatory molecules CD86 and CD40 and could produce IL-6. In vivo, however, even upon repeated vaccination with plasmid DNA, clonal expansion of OVA-specific CD8$^+$ T cells was comparable in TLR9-positive mice to TLR9-negative or MyD88-negative animals.

Fujii et al. explored the capacity of maturing DCs to present tumor cells using irradiated transplantable mouse tumor cell lines as a source of antigen and NKT lymphocytes as a maturation stimulus. The NKT cells were activated with the CD1d binding glycolipid, α-galactosyl ceramide. The maturing antigen capturing DCs in vivo were able to adoptively transfer immunity to naïve mice, which did not need to receive an additional exposure to either antigen or α-galactosyl ceramide. Interestingly, the capacity of DCs to elicit immunity required a CD40-CD40L signal that acted after the presentation of antigen and the upregulation of CD86 costimulatory molecules (106). This means that the induction of T-cell immunity by DCs in vivo is not simply dependent on ''signal one,'' which is MHC peptide and ''signal two,'' which is costimulation via B7 molecules. In addition, CD40L can be required, and this can be delivered by CD40L expressing T cells including activated NKT cells. These findings have been extended to tumor cell vaccines in mice. Liu et al. injected irradiated tumor cells intravenously, together with α-galactosyl ceramide and induced strong and long-lasting combined CD4$^+$ and CD8$^+$ T-cell immunity (107). By isolating the DCs capturing tumor antigens, they were able to link the innate NKT response to strong adaptive immunity in vivo, and this did not require that the tumor cells be modified genetically to express cytokines or other immune enhancing molecules. A more recent discovery by Shimizu et al. is that tumor cells could themselves be loaded with α-galactosyl ceramide and injected intravenously. The tumor cells could activate NKT cells and, following killing, were captured by DCs, which cross-presented the glycolipid to recruit NKT cells that matured the tumor antigen–capturing DCs; this initiated particularly strong T-cell immunity, even with a single low dose of irradiated tumor cells (108). These findings, which have only been tested in mice, suggest a path to the induction of tumor immunity in patients that harnesses the basic principles of DC biology that were outlined above. Intravenous injection of dying tumor cells leads to uptake by DCs, and these DCs can be matured to energize CD4$^+$ and CD8$^+$ adaptive T-cell immunity.

DIRECT TARGETING OF VACCINE PROTEINS TO MATURING DCS IN VIVO: THE DEC-205/CD205 EXAMPLE

A new approach to develop vaccine science and improve efficacy involves targeting of vaccine antigens to uptake receptors that are expressed by DCs in lymphoid organs. One method involves the use of antibodies to DC receptors, in which the antibodies are coupled or engineered to include vaccine proteins (Fig. 1). Antibodies target quickly and selectively to large numbers of DCs in lymphoid tissues, thus bypassing the need for DCs in the periphery to capture the vaccine and migrate to the T-cell areas. Several receptors have begun to be investigated and illustrate that receptor-based targeting of vaccines allows for greatly improved antigen presentation in vivo, by 100-fold or more relative to nontargeted vaccine protein.

Tolerance and Immunity Outcomes After Antigen Targeting to DEC-205/CD205

Hawiger et al. (44) and Bonifaz et al. (46) selectively delivered model antigens to DCs in vivo via the DEC-205/CD205 adsorptive endocytosis receptor using an anti-DEC antibody coupled to the antigen. As mentioned previously, the DEC-205 receptor is present in the CD8α-positive murine DC subset (14). In humans, this receptor is found in monocyte-derived DCs (109) as well as DCs in the T-cell areas of lymphoid tissues like lymph node (110) and spleen (111). Two different strategies were used. Hawiger et al. (44) cloned the heavy chain of the anti-DEC-205 antibody sequence and introduced in frame sequences for a hen egg lysozyme (HEL) peptide. The variable regions of both heavy and light chains of the anti-DEC-205 antibody (NLDC-145) were cloned in frame with mouse Ig kappa constant regions and IgG1 constant regions, the latter carrying mutations to reduce Fc receptors (FcR) binding (112). The antibody was produced by transient transfection of 293 cells and purified using protein G columns. When the antibody anti-DEC-HEL was administered to mice, the authors showed that the T cells were induced to expand but not polarized to produce the T helper type 1 cytokine IFN-γ, and the activation response was not sustained. Within seven days, the number of antigen-specific T cells was severely reduced, and the residual T cells became unresponsive or tolerant to systemic challenge with antigen in complete Freund's adjuvant (CFA). On the other hand, the co-injection of the DC-targeted antigen and anti-CD40 agonistic antibody changed the outcome from tolerance to prolonged T-cell activation and immunity. Therefore, the authors concluded that in the absence of additional stimuli, DCs induce transient antigen-specific T-cell activation followed by T-cell deletion and unresponsiveness (Table 1).

Interestingly, deletion was not the only mechanism by which T cells could be tolerized by targeting antigens directly to DCs under steady state. In another study (113), the targeting of the myelin oligodendrocyte glycoprotein (MOG) peptide