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Retrovirally Transduced Mouse Dendritic Cells Require $CD4^+$ T Cell Help to Elicit Antitumor Immunity: Implications for the Clinical Use of Dendritic Cells

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The identification of tumor-derived peptides presented by MHC class I molecules creates new prospects for the immunotherapy of cancer. When presented by potent antigen-presenting cells such as dendritic cellls (DCs), these peptides have been shown to elicit vigorous cytotoxic immune responses in vivo. While DCs directly activate cytotoxic T lymphocytes (CTLs), it is also well established that T cell help plays an important role in sustaining effective cytotoxic immune responses in vivo.

The aim of this thesis was therefore to examine in a murine tumor model the importance of MHC class II complexes on DCs for their use in generating MHC class I restricted CTL responses. To deliver MHC class I and class II restricted epitopes to the same cell, the entire gene encoding for chicken ovalbumin, a well characterized model antigen encoding that harbors both MHC class I and MHC class II-restricted epitopes, was expressed in DCs by retroviral-mediated gene transfer. Retroviral vectors were chosen because to achieve sustained antigen presentation using a vector that itself does not encode any viral protein.

The experiments described in this thesis first established optimized culture conditions for the generation of murine bone marrow-derived DCs and their retroviral transduction. Using a vector encoding an inert cell surface marker termed NTP, gene transfer in DCs generated in the presence of GM-CSF and IL-4 was routinely achieved in 20-30% of the cells, using a 48 hour cocultivation with ecotropic producer cells.

In subsequent experiments DCs were either transduced with the ovalbumin cDNA or pulsed with the in C57BL/6 mice MHC class I molecule K^b-presented peptide SIINFEKL. Their ability to induce CTLs was compared. A single dose of 1 x 10^5 genetically modified or peptide-pulsed DCs was sufficient to elicit potent CTL responses in vivo. However, when their ability to induce protection in tumor challenge experiments using the OVA-transfected B16 melanoma tumor cell line MO4 was tested, transduced DCs were consistently found to be more effective than peptide-pulsed DCs. All the animals receiving transduced DCs were protected whereas about half of the animals receiving peptide-pulsed DCs were protected (Wilcoxon rank sum test, $p < 0.01$).

To investigate whether the enhanced protection obtained with genetically modified DCs was conferred by the MHC class II epitope encoded by the OVA cDNA, DCs lacking MHC class II on their surface ($II^{-/-}$DCs) were transduced. Surprisingly, the activity of DCs lacking MHC class II molecules was not only reduced but abolished. The same was observed when mice were immunized with DCs pulsed with the SIINFEKL peptide. Thus, MHC class II-dependent antigen presentation, which activated $CD4^+$ T lymphocytes, was essential to achieve tumor immunity.

The importance of host $CD4^+$ T cells was further demonstrated by in vivo depletion of $CD4^+$ cells. Administration of the CD4-specific monoclonal antibody GK1.5 during the induction phase, abolished tumor protection. The same effect was achieved with genetically modified DCs and peptide-pulsed DCs. These findings implied that $CD4^+$ T lymphocytes engaged by MHC class II molecules on the immunizing DCs played a key role in establishing tumor protection.

Subsequent experiments verified whether DCs lacking MHC class II were still able to induce tumor-specific CTLs. Administration of DCs pulsed with the SIINFEKL peptide induced specifc CTLs when assayed on day 7 after immunization, whether the DCs expressed MHC class II molecules or not. However, further investigation of the SIINFEKL–specific CTL activity generated by MHC class $II^{-/-}$ DCs showed that these CTLs were short–lived compared to their counterparts induced by MHC class $II^{+/+}$ DCs. On day 30 after immunization no CTL activity was found in mice receiving MHC class $II^{-/-}$ DCs .The presence of memory CTLs correlated with tumor immunity and required $II^{+/+}$DCs.

These observations raised the complex question of what $CD4^+$ T cells are activated and what is their role in antitumor immunity? To address what kind of antigens presented by

MHC class II molecules on DCs lead to activation of $CD4^+$ T cells, the possible role of antigens present in the culture was examined. Dendritic cells are typically cultured in the presence of fetal bovine serum (FBS), a rich source of antigens. Conditions that allowed generating bone marrow-derived DCs in syngeneic C57BL/6 serum were established. DCs generated in syngeneic serum had a normal cell surface phenotype and were equally potent stimulators in primary allogeneic mixed leucocyte responses. However, they showed a significantly decreased potency in the induction of antitumor immunity compared to DCs grown in the presence of FBS. This finding showed that fetal bovine serum antigens enhance the effectiveness of cultured DCs.

To begin to address the function of the host $CD4^+$ T cells, their function to activate DCs via CD40/CD40L was investigated. Engagement of CD40 on DCs has been shown to greatly increase their potency in inducing CTL responses. The next experiments asked whether CD40 activation could enhance the function of DCs lacking MHC class II molecules, which are disabled to interact with $CD4^+$ T lymphocytes. DCs treated with the CD40 agonistic antibody IC10 were effectively activated as shown by upregulation of costimulatory molecules, the survival factor Bcl-x_L and generation of long term CTLs. However, CD40 activation of DCs lacking MHC class II did not improve their ability to induce antitumor immunity, showing that the interaction between the immunizing DCs and $CD4^+$ T helper lymphocytes in the recipient was not confined to a CD40/ CD40L interaction.

The findings presented in this thesis have important implications for the use of DCs as adjuvants in human antitumor vaccination strategies. DCs transduced with the cDNA encoding a whole tumor antigen might induce stronger immune responses than DCs pulsed with a MHC class I-restricted peptide only. Successful approaches for the induction of cytotoxic T cell responses against tumors will critically depend on the incorporation of epitopes presented on the MHC class II molecules on the immunizing DCs, leading to activation of $CD4^+$ T helper T lymphocytes. The use of DCs lacking MHC class II and the in vivo depletion of $CD4^+$ cells in the animal model used here supports this notion. Provision of $CD4^+$ helper T cell epitopes will be even more important in settings where DCs for vaccinations are generated in the presence of autologous serum or even serum free medium. Indeed, for safety concerns DCs for the use in humans are generally grown in the absence of fetal bovine serum components, which however have been show in the studies presented here, will significantly enhance the effectiveness of cultured DCs.

The experiments described investigating the role of in vitro CD40 activation of DCs lacking MHC class II prior to infusion in the host, established that this approach can improve the function of DCs in generating CTLs. However, in the animal model studied here this activation was not sufficient to replace the role of MHC class II presentation of antigens to $CD4^+$ T cells in generating protective antitumor immunity, establishing that the interaction of DCs and $CD4^+$ T cells is not confined to CD40/CD40L interactions. Other candidate molecules, like TRANCE or other yet unidentified molecules, which could enhance the function of DCs in generating CTLs in a $CD4^+$ T cell-independent manner, will be important to be evaluated.