Special Issue

Dendritic cells: Potential role in cancer therapy

Edgar G. Engleman

Stanford Medical School Blood Center, 800 Welch Road, Palo Alto, CA 94304, U.S.A.

Received 30 June 1997; accepted 18 August 1997

Key words: cancer, dendritic cells, idiotypic, immunotherapy, Malignant Lymphoma

Abstract

Dendritic cells (DC) are extremely potent antigen presenting cells, uniquely capable of sensitizing naive T cells to protein antigens and eliciting antigen specific immune responses. Studies of human DC isolated from peripheral blood indicate that these cells can be used to stimulate and expand antigen specific CD4⁺ and CD8⁺ T cells, *in vitro*. On the basis of these findings we have initiated pilot clinical studies to investigate the ability of DC pulsed *ex vivo* with tumor associated proteins to stimulate host anti-tumor immunity when re-infused as a vaccine. In the first such study DC pulsed with tumor derived idiotypic protein were infused into patients with low grade malignant B cell lymphoma who had failed conventional chemotherapy. The majority of treated patients developed T cell mediated anti-idiotypic immune responses and some of the patients experienced tumor regression. These results suggest that DC based immunotherapy is a potentially useful approach to B cell lymphoma and raises the possibility that the approach may prove useful in the treatment of other tumors as well.

Introduction

The development of an effective immune response to tumors depends on both T lymphocytes, which can lyse tumor cells in an antigen specific manner as well as secreted cytokines that activate a variety of immune cell types, and antigen presenting cells (APC), which are essential for processing antigens and presenting them to T cells. Studies carried out more than 20 yr ago, principally in mice, led to the identification of dendritic cells (DC) as particularly efficient and potent APC, uniquely capable of sensitizing naive T cells to protein antigens and eliciting antigen specific immune responses (Steinman, 1991). On the basis of these earlier studies, several years ago we decided to determine if human DC can be obtained from human blood and, if so, whether they possess antigen presentation functions similar to those of their murine counterparts.

Studies of DC, *in vitro*

Our initial efforts focussed on the development of a

method for isolating DC from peripheral blood. Briefly, single units of whole blood or leukapheresis products were subjected to ficoll-hypaque gradient centrifugation in order to remove red blood cells and granulocytes. Thereafter, monocytes were collected over percoll gradients and the monocyte depleted cells (consisting mainly of T cells, B cells, NK cells, and DC precursors) were incubated in conventional medium for 24–48 hr. During this period, the DC precursors underwent morphologic differentiation during which they developed easily visible dendrites. Then, the mature DC were separated from contaminating lymphocytes on the basis of their differences in buoyant density, by passing the cells through repeated metrizamide density gradients. This procedure resulted in dendritic cell preparations of >75% purity and, by separating the monocytes in an early step, it was possible to perform comparative studies of the APC activity of DC and monocytes obtained simultaneously from the same individual.

Studies of these cells indicated that they expressed HLA class I and II antigens, as well as accessory and co-stimulatory molecules (CD40, CD54, CD80,

Figure 1. Generation of antigen-specific CD4⁺ T cell lines from naive precursors. 1×10^5 CD4⁺ T cells were cultured for 1 week with 1×10^4 autologous DC in the presence of $25 \mu\text{g ml}^{-1}$ KLH (panel A) or SWM (panel B). Thereafter the cultures were fed weekly with antigen and monocytes. After 3 weeks the resultant CD4⁺ lines were incubated for 3 days with fresh autologous monocytes pulsed with either KLH, SWM, or no antigen, and labeled with ^3H -thymidine 16 hr prior to harvest.

CD86) at much higher density than monocytes or B cells. Moreover, they were far more efficient than fresh monocytes or B cells at stimulating T cell responses in the mixed lymphocyte reaction (MLR) (Markowicz and Engleman, 1990). Subsequently, we tested the ability of human DC to stimulate primary immune responses to nominal antigens, *in vitro* (Mehta-Damani *et al.*, 1995). Purified DC were pulsed with KLH ($25 \mu\text{g ml}^{-1}$), sperm whale myoglobin (SWM) or HIV gp160 ($1-5 \mu\text{g ml}^{-1}$) for 1 hr, and incubated in microwell cultures with autologous CD4⁺ T cells. After one week proliferative responses to these antigens were easily detected (not shown). To generate CD4⁺ T cell lines, the DC-sensitized T cells were expanded in culture in the presence of the priming antigen, either KLH or SWM. After several weeks, CD4⁺ T cell lines respond-

ing specifically to KLH or SWM were easily discernible (Figure 1). The substitution of antigen pulsed monocytes for DC in the initial week of culture failed to generate specific T helper cells (Mehta-Damani *et al.*, 1995). This suggests that most individuals have not been previously sensitized to KLH or SWM because antigen pulsed monocytes are reliable stimulators of memory T cell responses. When T cells were separated into CD45RA (naive) and CD45RO (memory) subpopulations, only the naive cells responded to KLH, SWM or HIV gp160 (Mehta-Damani *et al.*, 1995). Subsequent studies by our group have demonstrated that the costimulatory molecule CD86 (B7.2), which is expressed at high density on mature DC, plays a critical role in the generation by DC of such primary T cell responses (Fagnoni *et al.*, 1995). We also have shown that DC but not monocytes can stimulate $\gamma\delta$ T cell receptor positive T cells and that interactions between CD28 on $\gamma\delta$ cells and CD86 on DC are essential for such responses (Takamizawa *et al.*, 1995).

Despite the relative ease with which DC could be used to generate antigen-specific CD4⁺ T cells from naive precursors, the above protocol could not be used to generate antigen-specific CD8⁺ CTL, because exogenous proteins fail to cross cell membranes and enter the MHC class I processing pathway (Germain, 1994). To address this problem DC were incubated with 0.1 mg ml^{-1} KLH in hypertonic medium containing sucrose and polyethylene glycol to enhance antigen uptake by pinocytosis (Mehta-Damani *et al.*, 1994). This procedure was followed by exposure of DC to hypotonic medium in order to lyse intracellular vesicles containing antigen and to facilitate antigen processing. Freshly isolated autologous CD8⁺ T cells were then cultured with the antigen pulsed DC together with $1-2 \text{ U ml}^{-1}$ of IL1- β and rIL2. After 4 weeks of continuous culture CD8⁺ T cell lines were obtained and evaluated for cytotoxicity against unpulsed macrophages or macrophages pulsed as above with KLH. As shown in Figure 2, a CD8⁺ T cell line propagated under these conditions had potent KLH-specific lytic activity. In further studies antibodies to CD8 and MHC class I determinants blocked the observed killing, whereas anti-CD4 antibody and antibodies to MHC class II determinants did not (Figure 2).

A significant limitation to this method is its inherent toxicity (25–50% of DC die as a consequence of their exposure to hypertonic and hypotonic media), which greatly reduces its reliability and utility. Therefore, we subsequently explored the possibility of using DC pulsed with nine amino acid synthetic peptides

Figure 2. Generation of KLH specific CD8+ CTL. CD8+ T cells from a healthy blood donor were cultured with autologous DC that had been pulsed with KLH (as described in the text) and then expanded for one month prior to testing for cytotoxicity in ^{51}Cr release assays.

corresponding to known MHC class I binding epitopes to present HIV antigens to T cells obtained from healthy, HIV-1 uninfected volunteers (Mehta-Damani *et al.*, 1994). Specific sequences derived from the HIV-1 envelope and gag regions were chosen on the basis of published reports that these peptides were recognized by circulating HIV-1 specific CTL in infected individuals (Nixon and McMichael, 1991). However, because CTL recognize antigenic epitopes in association with specific HLA alleles, individuals of known HLA type were selected for the purpose of pairing a given peptide with an appropriate HLA allele. In the example shown in Figure 3, a CD3⁺,CD8⁺ T cell line generated with this method was demonstrated to specifically lyse peptide pulsed targets. Subsequent studies confirmed that HIV-specific CTL lines, generated after in vitro stimulation with peptides derived from HIV gag or env regions, can specifically lyse peptide pulsed target cells.

Results of a pilot clinical study of idiotypic pulsed DC in the treatment of Malignant Lymphoma

Our group has developed methods for isolating human DC from precursors circulating in blood. On the basis of in vitro findings indicating that human DC can be used to stimulate and expand antigen specific CD4⁺ and CD8⁺ lines, we decided to initiate pilot clinical studies to investigate the ability of autologous DC pulsed ex vivo with tumor associated proteins to stimulate host anti-tumor immunity when re-infused as a vaccine. Our first study, performed in collaboration with Drs. Frank Hsu and Ronald Levy at Stanford University, was targeted at patients with low grade malignant B cell lymphoma who had failed conventional chemotherapy. Like other B lymphocytes, the neoplastic cells in these patients express surface immunoglobulin receptors, and because B cell lymphomas are monoclonal, all the cells of a given tumor express identical immunoglobulin receptors. Moreover, these receptors are potentially immunogenic by virtue of their unique idiotypic determinants, which are formed by the combination of the variable regions of immunoglobulin heavy and light chains (Lynch *et al.*, 1979; Levy *et al.*, 1982; Maloney *et al.*, 1985). To prepare idiotypic proteins for use in our clinical trial, consenting patients underwent tumor biopsies and the immunoglobulin produced by each tumor was 'rescued' by somatic cell fusion techniques and purified from hybridoma supernatants (Carroll *et al.*, 1986). Peripheral blood

leukocyte preparations containing DC precursors were obtained from patients by leukapheresis, and monocyte depleted mononuclear cells were incubated for 36 hr in the presence of either tumor specific immunoglobulin idiotypic protein or a control protein, keyhole limpet hemocyanin (KLH). This incubation technique was similar to that used in our earlier in vitro studies, and enabled the DC precursors to take up and process the exogenous proteins, while undergoing maturation into potent APC. Following this culture period the idiotypic and KLH pulsed DC were separated from contaminating lymphocytes, on the basis of their differences in buoyant density, as described earlier. The DC were then extensively washed, resuspended in sterile saline and administered to the patients by intravenous infusion. This procedure was repeated three times at monthly intervals with a booster immunization given four to six months later. Two weeks after each DC infusion the patients received subcutaneous injections of immunoglobulin idiotypic in saline, which in the absence of DC or a chemical adjuvant would not be expected to induce an immune response (Kwak *et al.*, 1992). Throughout the trial the patients were followed for the development of an immune response to the idiotypic and their tumor burden was monitored by repeated CT scans.

To date nine patients have been treated under this protocol. A detailed report of the results obtained in the first four patients has recently been published (Hsu *et al.*, 1996). All of the treated patients, including the five not described in our published report, tolerated their infusions well and none experienced clinically significant toxicity. In addition, all of the patients developed T cell mediated anti-idiotypic and anti-KLH proliferative responses that were not observed prior to treatment initiation. An example of such a response is shown in Figure 4. The anti-idiotypic responses were specific for autologous tumor immunoglobulin when compared to irrelevant, isotype matched immunoglobulins. In addition to these proliferative responses, T cells from one patient were expanded for several weeks in vitro in the presence of idiotypic protein and shown to lyse autologous tumor hybridoma cells but not an isotype matched, unrelated hybridoma (not shown). In contrast to earlier idiotypic vaccination studies which utilized chemical adjuvants rather than DC (Kwak *et al.*, 1992; Hsu *et al.*, 1993), a humoral response to idiotypic was not detected in our patients, consistent with the interpretation that the idiotypic pulsed DC induced a T helper 1 rather than T helper 2 response. Most importantly, two of the patients experienced complete

Figure 3. Generation of HIV specific CTL from an uninfected subject. DC were isolated from healthy, HIV seronegative, HLA-B27 positive blood donors and pulsed with a control peptide ('a') or an HIV-1 gag peptide (peptide 'b') reported to serve as a target epitope for HIV-1 specific CTL present in HLA-B27 positive HIV infected patients (Nixon and McMichael, 1991). Autologous CD8⁺ T cells were cultured for one week in the presence of pulsed DC and expanded for 6 weeks before assay for cytotoxic activity against ⁵¹Cr labeled autologous monocytes that had been pulsed with the same peptides. Where indicated, the cytotoxicity assays were carried out in the presence of a monoclonal anti-CD8 antibody or a monoclonal anti-HLA framework antibody (W6/32).

Figure 4. Peripheral blood mononuclear cell (PBMC) proliferative response. PBMCs of patient D4 were cocultured *in vitro* with concentrations of KLH or idiotype (Id) protein varying from 1 μg ml⁻¹ to 100 μg ml⁻¹. Incorporation of [³H]thymidine is reported as the mean c.p.m. of quadruplicate samples above the media background with error bars representing standard deviations. PBMCs were obtained pretreatment (□), two weeks after the first infusion of DCs before the soluble protein boost (▨), and two weeks following the second infusion of DCs (■). No response was seen pretreatment. Incorporation of [³H]thymidine in response to an irrelevant, isotype matched control protein was ~2000 c.p.m. two weeks after the second infusion of DCs.

tumor regression, including one who entered the trial with bulky disease. A third patient experienced a partial response. Of the remaining patients, four have stable disease and two have experienced disease progression. These results suggest that DC based immunotherapy

is a potentially useful approach to low grade B cell lymphoma and raises the possibility that the approach may prove useful in the treatment of other tumors as well. Our group, for example, has recently initiated a trial of idiotype pulsed DC in patients with multiple

myeloma who have undergone bone marrow or peripheral blood stem cell transplantation following ablative chemotherapy. In addition, our collaborators at Activated Cell Therapy, Inc. have initiated a trial of prostate antigen pulsed DC for the treatment of advanced prostatic carcinoma.

Alternative sources of DC

Although the results to date in the lymphoma trial are provocative, the procedures we used for purifying and 'arming' DC were tedious and typically yielded fewer than ten million DC after each leukapheresis. Indeed, the observation that such a small number of APC could induce an immune response to a self-protein in tumor bearing hosts, most of whom were leukopenic as a consequence of prior chemotherapy, was surprising. On the other hand, as noted above, several of the treated patients have not experienced tumor regression, suggesting that the DC dose may have been sub-optimal. One way to increase the yields of DC from blood, which our collaborators at Activated Cell Therapy, Inc. are employing, is to reduce the number of purification steps following the in vitro culture of DC precursors. This results in cell preparations of lower purity but the yield of DC is significantly increased. In addition, extending the duration of the leukapheresis procedure and increasing the number of blood volumes processed should also significantly increase the yield of DC. Finally, a large number of DC-like cells can be generated in vitro from either CD34⁺ hematopoietic progenitors or monocyte enriched populations of PBMC following their culture in medium containing GM-CSF and IL-4 or GM-CSF and TNF- α (Caux *et al.*, 1992; Romani *et al.*, 1994; Sallusto and Lanzavecchia, 1994; Bernhard *et al.*, 1995). Such cytokine derived cells are morphologically and phenotypically similar to the 'classical' DC used in our trial and, like these cells, the cytokine derived DC are potent APC, capable of inducing strong anti-tumor responses in vivo, in experimental animals (Zitvogel *et al.*, 1996; Paglia *et al.*, 1996). Whether such cells will exhibit the same anti-tumor effects in humans remains to be determined, but clinical trials incorporating their use have already begun in patients with malignant melanoma and other tumors, so an answer should be forthcoming soon.

Availability of tumor antigens

In addition to questions regarding the best choice of DC for use in clinical trials, the choice of tumor antigen with which to 'arm' the DC is almost certainly going to have a profound influence on clinical outcome. At present, the choices are limited because only a few antigens have been identified that are either tumor or tissue restricted in their expression. On the other hand, the number of candidate antigens is increasing with time as a consequence of intensive gene cloning efforts. Moreover, several of these antigens have already been shown to be capable of stimulating T cell responses in vitro when presented by DC and in some instances T cells recognizing tumor associated antigens have been isolated from tumor bearing patients. Finally, mutant forms of self-proteins that arise in some tumors, such as p53, can behave as foreign proteins and induce strong tumor specific immune responses. Therefore, the availability of tumor associated antigens is not likely to remain a limiting factor for very long. On the other hand, because a number of these antigens are expressed on at least some normal tissues as well as by neoplastic cells, there is at least a theoretical risk that DC based presentation of these antigens may lead to autoimmune disease. This risk would be expected to be even greater if crude tumor extracts, rather than purified antigens, are used in combination with DC, although recent observations in experimental animals have suggested that such an approach may induce potent anti-tumor immunity.

Optimization of antigen delivery

In addition to questions regarding the choice of antigen and source of DC for use in immunotherapy trials, optimization of antigen delivery remains an important issue. Normally, addition of soluble proteins to DC would not be expected to result in efficient entry of the proteins into the MHC class I processing pathway, although entry into the MHC class II processing pathway is routinely observed. The latter processing pathway allows for the presentation of antigenic epitopes to CD4⁺ T cells, which are important sources of pro-inflammatory cytokines such as interferon- γ , but would not allow for presentation of antigenic epitopes to CD8⁺ CTL, which are thought to be critical effectors of anti-tumor immune responses. In this regard, it is noteworthy that B cell lymphoma is unusual among malignancies and that the neoplastic cells express both

MHC class I and II antigens and would, therefore, be susceptible to recognition and attack by either CD4⁺ or CD8⁺ T cells. Since the majority of solid tumors do not express MHC class II determinants, the generation of tumor reactive CD8⁺ T cells may be required for an effective anti-tumor response and this would necessitate that antigen be delivered to DC in a manner that assures their access to the MHC class I processing pathway. Among the approaches being developed for this purpose are gene and RNA transfer methods, both of which have been shown to result in antigen processing in the MHC class I pathway of DC. An alternative approach that we have recently developed involves the attachment of antigens to short cationic peptides that translocate proteins across cell membranes and into the class I processing pathway (Kim *et al.*, submitted for publication).

Role of DC exposed to antigen in vivo

An interesting question that has yet to be answered definitively is why administration of small numbers of antigen pulsed DC induce strong immune responses and tumor regression in patients in whom both the antigen and DC are already plentiful. One possible explanation is that the DC present in vivo in cancer patients may be defective in their capacity to take up, process and present tumor associated antigens. Recently, we performed a detailed analysis of the DC that circulate in healthy individuals and found that the vast majority of such cells are incapable of sensitizing naive T cells to protein antigens unless they are induced to differentiate, in vitro (Takamizawa *et al.*, 1997). As noted above, during ex vivo culture DC precursors undergo morphological maturation and change their buoyant density. In addition, as we have recently reported, in vitro culture of DC precursors results in their expression of critical costimulatory molecules such as CD40, CD80 and CD86 (Takamizawa *et al.*, 1997). Apparently, most DC precursors in or near malignant tissues do not become activated and fail to differentiate into competent APC, while under in vitro culture conditions the circulating DC precursors from patients (for example, patients with malignant lymphoma) mature in a manner indistinguishable from DC precursors obtained from healthy donors. This explanation is consistent with skin biopsy findings from patients with Sezary Syndrome in which few differentiated DC are observed, by comparison with normal or psoriatic skin (Nestle and Nickoloff, 1994). Similar findings have been reported in the biop-

sies of other tumors, including gastric carcinoma, with clinical outcome correlating with the number of morphologically differentiated DC (Tsujitani *et al.*, 1990). If defective DC maturation is a common occurrence in malignancy, then identification of agents that induce the expansion and maturation of DC, in vivo, may prove to be a productive strategy. In the absence of such agents, however, the approach described in this report appears to represent a promising step toward the development of effective immunotherapeutic approaches to cancer.

Acknowledgments

This work was supported in part by grants AI36608, CA71725, HL57443 and HB97075 from the National Institutes of Health.

References

- Bernhard H, Disis ML, Heimfeld S, Hand S, Gralow JR and Cheever MA (1995) Generation of immunostimulatory dendritic cells from human CD34⁺ hematopoietic progenitor cells of the bone marrow and peripheral blood. *Cancer Res* 55: 1099.
- Carroll WL, Thielemans K, Dilley J and Levy R (1986) Mouse $\times$ human heterohybridomas as fusion partners with human B cell tumors. *J Immunol Methods* 89: 61.
- Caux C, Dezutter DC, Schmitt C and Banchereau J (1992) GM-CSF and TNF- α cooperate in the generation of dendritic Langerhans cells. *Nature* 360: 258.
- Fagnoni FF, Takamizawa M, Godfrey WR, Rivas A, Azuma M, Okumura K and Engleman EG (1995) Role of B70/B7-2 in CD4⁺ T cell immune responses induced by dendritic cells. *Immunology* 85: 467-474.
- Germain RN (1994) MHC-dependent antigen processing and peptide presentation: providing ligands for T lymphocyte activation. *Cell* 76: 287-299.
- Hsu FJ, Kwak L, Campbell M, Liles T, Czerwinski D, Hart S, Syrengeles A, Miller R and Levy R (1993) Clinical trials of idiotype-specific vaccine in B-cell lymphomas. *Ann NY Acad Sci* 690: 385-387.
- Hsu FJ, Benike C, Fagnoni F, Liles TM, Czerwinski D, Taidi B, Engleman EG and Levy R (1996) Vaccination of patients with B cell lymphoma using autologous antigen pulsed dendritic cells. *Nature Medicine* 2: 52.
- Kim D, Mitchell D, Brockstedt D, Fong L, Nolan G, Fathman CG, Engleman E and Rothbard J, Introduction of soluble proteins into both MHC class I and II pathways by conjugation to a HIV tat peptide. (submitted for publication).
- Kwak LW, Campbell MJ, Czerwinski DK, Hart S, Miller RA and Levy R (1992) Induction of immune responses in patients with B-cell lymphoma against the surface-immunoglobulin idiotype expressed by their tumors. *N Engl J Med* 327: 1209-1215.
- Levy R, Hatzubia A, Brown S, Maloney D and Dilley J (1982) Immunoglobulin idiotype: A tumor-specific antigen for human

- B-cell lymphomas. In: *Malignant Lymphomas*. New York: Academic Press, Ch. 5.
- Lynch RG, Rohrer JW, Odermatt B, Gebel HM, Autry JR and Hoover RG (1979) Immunoregulation of murine myeloma cell growth and differentiation: A monoclonal model of B cell differentiation. *Immunol Rev* 48: 45–80.
- Maloney DG, Kaminski MS, Burowski D, Haimovich J and Levy R (1985) Monoclonal anti-idiotypic antibodies against the murine B cell lymphoma 38C13: Characterization and use as probes for the biology of the tumor in vivo and in vitro. *Hybridoma* 4: 191.
- Markowicz S and Engleman EG (1990) Granulocyte-macrophage colony-stimulating factor promotes differentiation and survival of human peripheral blood dendritic cells in vitro. *J Clin Invest* 85: 955–961.
- Mehta-Damani A, Markowicz S and Engleman EG (1994) Generation of antigen-specific CD8⁺ CTLs from naive precursors. *J Immunol* 153: 996–1003.
- Mehta-Damani A, Markowicz S and Engleman EG (1995) Antigen specific CD4⁺ T cells derived from naive precursors. *Eur J Immunol* 25: 1206–11.
- Nestle F and Nickoloff BJ (1994) Role of dendritic cells in benign and malignant lymphocytic infiltrates of the skin. *Dermatol Clinics* 12: 271.
- Nixon DF and McMichael AJ (1991) Cytotoxic T cell recognition of HIV proteins and peptides. *AIDS* 5: 1045–59.
- Paglia P, Chiodoni M, Rodolfo M and Colombo MP (1996) Murine dendritic cells loaded in vitro with soluble protein prime cytotoxic T lymphocytes against tumor antigen in vivo. *J Exp Med* 183: 317.
- Romani N, Gruner S, Brang D, Kampgen E, Lenz A, Trockenbacher B, Konwalinka G, Fritsch PO, Steinman RM and Schuler G (1994) Proliferating dendritic cell progenitors in human blood. *J Exp Med* 180: 83.
- Sallusto F and Lanzavecchia A (1994) Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and downregulated by tumor necrosis factor alpha. *J Exp Med* 179: 1109.
- Steinman RM (1991) The dendritic cell system and its role in immunogenicity. *Ann Rev Immunol* 9: 271.
- Takamizawa M, Fagnoni F, Mehta-Damani A, Rivas A and Engleman EG (1995) Cellular and molecular basis of human $\gamma\delta$ T cell activation. Role of accessory molecules in alloactivation. *J Clin Invest* 95: 296–303.
- Takamizawa M, Rivas A, Fagnoni F, Benike C, Kosek J, Hyakawa H and Engleman EG (1997) Dendritic cells that process and present nominal antigens to naive T lymphocytes are derived from CD2⁺ precursors. *J Immunol* 158: 2134–2142.
- Tsujitani S, Kakeji Y, Watanabe A, Kohnoe S, Maehara Y, Sugimachi K (1990) Infiltration of dendritic cells in relation to tumor invasion and lymph node metastasis in human gastric cancer. *Cancer* 66: 2012.
- Zitvogel L, Mayordomo JI, Tjandrawan T, DeLeo AB, Clarke MR, Lotze MT and Storkus WJ (1996) Therapy of murine tumors with tumor peptide-pulsed dendritic cells: dependence on T cells, B7 costimulation, and T helper cell 1-associated cytokines. *J Exp Med* 183: 87.

Address for offprints: Edgar G. Engleman, M.D., Stanford Medical School Blood Center, 800 Welch Road, Palo Alto, CA 94304, U.S.A.