Phenotypic and functional differences of dendritic cells generated under different in vitro conditions.

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1. INTRODUCTION

The immune system is capable of recognizing and rejecting autologous tumor cells. This is suggested by reported cases of spontaneous remission of various cancers (1), and the presence of infiltrating leukocytes, the majority of which consist of T cells. However, the very existence of cancer and its inevitable progression without treatment demonstrates the inefficiency of the natural immune defence in combating tumors, and the ability of neoplastic cells to evade immune-surveillance. Thus, the major objectives of immunotherapeutic approaches to the treatment of cancer rely on the ability to augment adaptive and natural immune responses against malignant cells.

During the last decade a great deal of attention has been paid to the role of dendritic cells (DCs) in the development of immune responses; the ultimate aim being to provide a novel means of cancer therapy for patients. DCs directly isolated from patients' blood have already been shown to be effective in promoting anti-tumor immunity in pilot clinical trials (2), and such approaches are likely to be effective in patients who have completed conventional anti-tumor therapies (surgery, chemotherapy, radiotherapy) and who have minimal residual disease. By reducing tumor bulk prior to immunotherapy the degree of tumor “fight back” will be reduced and the efficiency of the immune response most likely enhanced.

1.1 Prostate Cancer-Associated Antigens

Defining the expression of tumor antigens on primary and metastatic prostate cancer is the crucial first step in selecting appropriate targets for immune attack (3). Table 1 summarizes the antigens or genes over-expressed in the prostate or prostatic cancer tissues. Unfortunately, not all of the antigens expressed in cancer tissues are suitable for the development of immunotherapy. They

may, for instance, be poorly immunogenic or occur frequently in normal tissues, implying that these antigens could elicit harmful autoimmune reactions. However, some prostate cancer-associated antigens have been successfully used in dendritic cell-based immunotherapy of prostate cancer, for example with cultured autologous DCs pulsed with HLA-A2 peptide derived from prostate specific membrane antigen (PSMA) (4). The Phase I study used HLA-A2 peptide derived from PSM antigens (PSM-P1 and PSM-P2) and autologous DCs; PSM-P1- and –P2-pulsed autologous DCs were administered to fifty-one patients with advanced hormone refractory prostate cancer. Neither significant acute or chronic toxicity was observed at any of the doses used, except for mild to moderate cases of hypotension without pulse change during the time of infusion. A total of 107 patients with either local recurrence of prostate cancer after primary treatment failure or with hormone-refractory metastasis prostate cancer were treated in a Phase II clinical trial. Patients received six infusions of autologous DCs pulsed with PSM-P1 and –P2 every six weeks. The treatment was well tolerated by all participants. Approximately 30% of the trial participants had clinical responses, as defined by the modified National Prostate Cancer Project criteria including a 50% reduction in prostate specific antigen (PSA) (2, 4, 5). A population of peripheral blood-derived antigen-presenting cells, including DCs, pulsed with selected peptides from prostate acid phosphate (PAP) have been shown to induce prostate cancer-specific CTL *in vitro* (6). The use of PSA-specific peptides pulsed onto DCs to induce CTL has not been reported to date. However, Heiser and co-workers (7) observed that DCs transfected with mRNA encoding PSA could stimulate a primary PSA-specific CTL response *in vitro*.

1.2 Dendritic Cell Characteristics

DCs, first discovered in 1973 (8), originate from CD34+ progenitor cells in the bone marrow and migrate to the different lymphoid and non-lymphoid tissues. Upon exposure to inflammatory

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cytokines, DCs capture antigen and up-regulate the expression of histocompatibility and co-stimulatory molecules (9). At this point DCs are able to migrate from peripheral tissues to regional lymph nodes where they present antigen to, and promote the clonal expansion of antigen specific T lymphocytes. Final maturation is completed by the interaction of DCs with T helper cells recognizing the presented antigen (10). Indeed, evidence suggests that initial priming of cytotoxic T lymphocytes requires both presentation of antigen in the context of major histocompatibility complex class-I molecules together with an appropriate co-stimulatory signal provided by the antigen-presenting cell. Most tumors do not express co-stimulatory molecules; therefore transfer of tumor antigen from the tumor to an APC is required for the initiation of specific cytotoxic T lymphocyte responses. This transfer of antigen has been termed 'cross-priming', and evidence suggests that DCs play a central role in the process (11); failure of the majority of naturally occurring tumors to initiate an effective immune response may, in large, be due to the absence of 'cross-priming'. However, despite the various mechanisms that tumors may possess in order to evade immune attack, there are significant data to show that DC-based immunotherapy can provide a real and measurable anti-tumor effect.

DCs are therefore antigen-presenting cells capable of initiating primary immune responses (10). They display different functional repertoires of cell surface markers at different stages of their development. In the "immature" state DCs are very effective in processing native protein antigens via MHC class-II restricted as well as the MHC class-I pathways (12, 13, 14). Whole purified protein, necrotic cells or apoptotic cells given to immature DCs are then able to elicit immune responses which are class-I and class-II restricted (13, 14, 15, 16). Mature DCs are less able to capture new proteins for presentation but are much more efficient in presenting MHC associated peptides, stimulating resting $CD4^+$ and $CD8^+$ T cells to grow and differentiate. Mature

DCs pulsed with specific tumor peptide are also able to activate either $CD4^+$ or $CD8^+$ specific T-cell responses (17, 18, 19).

Difficulties in obtaining sufficient numbers of DCs have been solved, and methods have been developed to obtain substantial numbers of DCs from proliferating human bone marrow (20) and blood (21). This approach is less practical for small samples of human blood where the frequency of $CD34^+$ cells is very low (less than 1%). However enrichment and isolation of $CD34^+$ stem cells is now a standard procedure for stem cell support following high dose chemotherapy. Adequate numbers of $CD34^+$ cells can be positively selected from the blood of patients 3-4 days following treatment with G-CSF. However, the most widely used method to generate DCs to study their function and potential use in immunotherapy, is to select monocytes present in human blood and culture them *in vitro* in medium containing GM-CSF and IL-4 (21, 22, 23). Cells generated in such a manner can be characterized according to their phenotypic and functional characteristics, including morphology, cell surface marker expression, phagocytosis and ability to activate helper ($CD4^+$) and cytotoxic ($CD8^+$) T-lymphocytes.

Here we present a simple method to generate immature and mature DCs.

For clinical purposes DCs have to be generated without the use of foreign proteins, in particular xenogenic proteins present in fetal calf serum (FCS). However we, along with Pietschmann and co-workers (24), have found that DCs generated in medium containing 1% autologous serum and GM-CSF and IL-4 for 6 days and TNF-α for another two days expressed significantly lower CD40, CD1a and CD54 cell surface markers than DCs cultures in FCS-containing medium. Culturing DCs in autologous serum instead of 10% FCS induced morphological changes including the absence of aggregate formation and a reduced activity in mixed lymphocyte

DRAFT

reaction (MLR) assays. Their ability to phagocytose latex beads was comparable and both were capable of inducing T cells proliferation after incubation with 0.5 μg/mL of tetanus toxoid protein.

The following methods can be used to generate DCs and assess their phenotypic and functional properties.

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2. MATERIALS

2.1 Medium

The medium used throughout the study was RPMI 1640 supplemented with 200 mmol/L L-glutamine and with the addition of either 10% fetal calf serum (FCS) or 1% autologous serum (heat inactivated 56°C for 30 minutes).

2.2 Other Reagents

- Blocking buffer: 1 x PBS + 0.1% bovine serum albumin (BSA) and 0.02% sodium azide, stored at room temperature.
- Sheath fluid: 6.38 g NaCl + 1 g Boric acid + 0.2 g EDTA-2K + 0.2 g sodium tetraborate, made up to 1 litre with distilled water and 0.5% formaldehyde added, stored at room temperature.
- Ficoll Hypaque, stored at room temperature.
- Propidium Iodine, stored at 4ºC.
- Fluorescence Mounting Media (Dako, Cambridge, U.K), stored at 4ºC.
- Tetanus Toxoid, *Clostridium tétani* (Calbiochem, Nottingham, U.K), stored at -80ºC in aliquots after reconstitution in sterile distilled water.
- Tritium labelled thymindine (^{3}H) (Amersham, Biotech, U.K)
- 1% Paraformaldehyde, stored at 4ºC.
- FITC-labeled latex beads (Sigma, U.K), stored at room temperature.

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2.3 Cytokines

All cytokines were aliquoted using PBS + 0.1% BSA and stored at –80ºC.

- GM-CSF was obtained from R&D Systems (Oxon, U.K) and was stored at 1 x 10^7 U/mL.
- IL-4 was obtained from Prepotec (London, U.K) and was stored at 1 x 10^6 U/mL.
- TNF-α was obtained from Benderwein (Boehringer Ingelheim, Germany) and stored at 10 μg/mL.

2.4 Primary Antibodies

All antibodies were aliquoted and stored at -80ºC.

CD14 was obtained from Harlan Sera Lab (Loughborough, U.K); CD83*, CD86* and CD1a* were bought from Pharmingen International (Oxford, U.K), and CD11c, CD40, CD54, and isotype control IgG1 were bought from Diaclone (I.D.S, Tyne&Wear, U.K). All antibodies were FITC-conjugated. MHC class-II an anti-DR antibody was generated from the cell line HB-55, obtained from the ATCC.

* Non-conjugated antibodies

Secondary antibody consisted of a goat anti-mouse FITC-conjugated $F(ab)_2$ fragment and was obtained from Sigma, U.K.

Anti-tubuline antibody was obtained from Sigma, U.K. The secondary antibody consisted of an anti-IgG mouse antibody PE conjugated and was obtained from Serotec (Oxford, U.K).

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3. METHODS

3.1 Blood Separation

The blood packs used were obtained from the National Donor Centre at Sheffield, U.K, as a leukophoresis pack containing 80 mL of concentrated leukocytes obtained from 300 mL of blood. The cells were aspirated from the blood pack and mixed with 80 mL of sterile PBS in a sterile glass bottle.

The blood cells were separated using a density gradient on Ficoll Hypaque; 15 mL of diluted blood were carefully layered onto 7.5 mL of Ficoll Hypaque contained in a sterile universal. The universals were centrifuged at room temperature at 400 g for 30 minutes without brake. The plasma layer was removed, placed in a centrifuge tube and heat inactivated by incubation in a water bath at 56ºC for 30 minutes, and left to cool at 4°C for an hour or until needed. Finally, the plasma sample was centrifuged at 600 g for 10 minutes at 4ºC and the supernatant carefully aspirated off and kept at –20ºC in 5 mL aliquots.

The leukocyte layers were carefully removed with a wide ended pipette and placed into separate pre-chilled plastic sterile universals. Cold PBS was then added to fill the tubes. The universals were centrifuged at 600 g for 15 minutes at 4ºC. The supernatant was centrifuged again under the same conditions and the pellet re-suspended in 10 mL of media. The original pellets were resuspended in 10 mL of cold PBS and centrifuged at 600 g for 10 minutes at 4ºC. The supernatant from all tubes was discarded and the cells re-suspended in 10 mL of RPMI + glutamine. Cells from both tubes were pooled before counting (total of 20 mL). Cell counts were

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performed using cells diluted first in 0.6% acetic acid to lyse any remaining red blood cells and subsequently diluted in trypan blue to exclude dead cells.

3.2 *In Vitro* Generation of Immature and Mature Dendritic Cells

This method was adapted from Thurner and co-workers (23). The generation of immature and mature DCs was obtained after 6 and 8 days in culture respectively.

3.2.1 Generation of Immature Dendritic Cells

3.2.1.1 Day 0

Peripheral blood mononuclear cells (PBMC) were plated at 4-5 x 10^6 cells/mL in media containing either 10% FCS or 1% autologous serum in T75 flasks, with the volume not exceeding 40 mL. The cells were incubated at 37ºC for 2 hours, non-adherent cells were then removed, concentrated by centrifugation at 600 g for 10 minutes at 4ºC, counted and frozen at 1 x 10^7 cells/mL of freezing mixture, consisting of 60-70% FCS, 10% DMSO and media (designated T-lymphocyte fraction). The same volume of the same media as used for the 2 hours incubation was added to the adherent cells, which were incubated overnight (o/n) at 37ºC.

3.2.1.2 Day 1

The majority of the cells became non-adherent and were collected in a 50 mL tube, centrifuged at 400 g for 5 minutes, re-suspended in media and counted. The cell concentration was then adjusted to 1 x 10^6 cells/mL and 5 mL placed into each well of a 6-well plate and incubated for 45 minutes at 37ºC. The non-adherent cells were removed and discarded; the wells were washed very gently with media and 5 mL of fresh media containing the relevant serum; and 1000 U/mL

DRAFT

GM-CSF and 500 U/mL IL-4 were added to each relevant well and the plates were incubated at 37ºC.

All these steps of adherence were used to 'purify' monocyte-derived DCs. Monocytes, macrophages, and B cells can all adhere to the plastic but with different intensity and longevity. Monocytes will adhere very quickly to the plastic but will then elute off after o/n incubation but will adhere again very quickly to the plastic during the second stage of adherence. Macrophages will also adhere relatively quickly to the plastic but remain firmly adhered even after o/n incubation. B-cells may stick after the first adherence stage of 2 hours but will elute off permanently after o/n incubation.

3.2.1.3 Day 6

Immature DCs were apparent in each of the cultures (+FCS or autologous plasma), consisting of non-adherent cells showing long digits. The cells from replicate wells were pooled together and centrifuged at 400 g and counted.

Some cells were then used for flow cytometric analysis (2 x 10^5 cells for each cell surface marker to be studied) and used in functional assays as detailed below (see Subheadings 3.5 and 3.6). The concentration of the remaining cells was adjusted to 0.5 x 10^6 cells/mL and used for DC maturation.

3.2.2 Generation of Immature Dendritic Cells

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The immature DCs were re-plated at 0.5 x 10^6 cells/mL per well of a 24-well plate in fresh media containing the relevant serum and 1000 U/mL GM-CSF, 500 U/mL IL-4 and 10 ng/mL of TNF-α.

DCs can revert back to their original form when the cytokines are removed until they have fully matured; therefore cytokines need to be replenished (36).

The cells were incubated for a further two days at 37ºC. The mature cells were then harvested from the cultures and the level of expression of different cell surface marker was analyzed by flow cytometry after staining with a panel of antibodies; cultured cells were also used in two functional assays (see Subheadings 3.5 and 3.6).

Figures 1 and 2 show the morphologic and phenotypic differences between DCs generated in media containing 1% autologous plasma or 10% FCS, as demonstrated by confocal microscopy and flow cytometry.

3.3 Differential Expression of Cell Surface Markers by Dendritic Cells Generated after 6 or 8 Days of Culture (Flow Cytometry Analysis)

2 x 10^5 (immature or mature) DCs were placed into individual plastic tubes and washed once in 2-3 mL of "blocking buffer" to block any unspecific sites. The cells were centrifuged for 5 minutes at 400 g and the supernatant decanted off, and the tubes were inverted onto a tissue to clear any remaining droplets. Primary antibodies were then added to individual tubes and the cells placed on ice for 30 minutes.

The cells were then washed twice in 2 mL of blocking buffer, and centrifuged at 400 g for 5 minutes at 4ºC to remove the excess antibody. The secondary antibody was added (unless directly conjugated antibody was used) to the cells and the tubes were put on ice for a further 30 minutes. Cells were then washed twice with 2 mL of blocking buffer. The supernatant was removed and 200 µL of sheath fluid were added to each tube; the cells were analyzed by flow cytometry or covered with foil and stored at 4°C until analysis (see Figure 2).

3.4 Antigen Presentation Assay

DCs were incubated for 24-48 hours with or without tetanus toxoid (0.5 µg/mL) before co-culture with autologous or allogeneic T cells. DCs were plated in triplicate in a 96 well plate (100 µL per well), starting at a concentration of 2 x 10^5 cells/mL and then diluted down. Autologous or allogeneic T-cells were removed from liquid nitrogen, washed twice with medium, counted and made up to a concentration of $2x10^6$ cells/mL in RPMI medium + relevant serum, then 100 µL were added to relevant wells. Plates were incubated for 6 days at 37ºC. Tritiated (^{3}H) thymidine was added for the last 18 hours. The cells were harvested onto filter paper, which was transferred into scintillation tubes, followed by the addition of 2 mL of scintillation fluid. Counts per minute for each well were detected using a gamma spectrophotometer (see Figure 3).

3.5 Phagocytosis Assay

0.5 x 10^6 cells/500 µL of immature or mature DCs were seeded into chamber slides in duplicate. 10 µL of a 1/200 dilution of the FITC-labeled latex beads were added to each well and the chamber was incubated at 37ºC for 24 hours. The media was then removed and the cells gently washed with PBS. Following removal of PBS from each well, the cells were fixed with 500 µL of

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1% paraformaldehyde solution for 10 minutes at room temperature, then washed in PBS. 50 μL of anti-tubulin (1/200 dilution) antibody was then added to each well for 30 minutes and then washed twice (5 minute wash) with PBS to remove the excess of antibody. The cells were then stained with propidium iodine (10 μg/mL) for 1 minute and the wells were washed twice again with PBS. The plastic chambers on the slides were removed, and Dako immunofluorescence mounting medium added to each slide. Coverslips were placed over the chambers, which were sealed with nail varnished (see Figure 4).

4. CONCLUSIONS AND DISCUSSION

The potential role of DCs in cancer immunotherapy has been firmly established in experimental tumor-therapy models and in a number of human clinical trials (25, 26, 27, 28). DC-based cancer vaccines, formulated by loading DCs with antigenic peptides or tumor lysates or through genetically modifying DCs prior to *in vivo* administration represents a potentially powerful therapeutic strategy. Genetically modified or peptide pulsed DCs can generate effective immune responses to established tumors or subsequent tumor challenge. Autologous DCs from normal individuals or patients with prostate cancer transfected with RNA encoding PSA are effective in stimulating PSA-specific CTL *in vitro*, implying that neither natural tolerance to PSA antigens nor tumor-mediated T cell anergy represents a barriers for CTL generation against the "self-antigen" PSA (7). Delivering activated DCs (genetically modified or unmodified) directly into the tumor microenvironment was shown to enhance immunity and bypass the requirement for selective recruitment and local activation of DCs, which is usually disordered in tumors. DC inactivation has been clearly demonstrated in prostate cancer where monocyte maturation to give rise to DCs is significantly inhibited, as assessed by the expression of DC markers CD1a and CD83 and T cell proliferation (29). We have recently shown, using a murine CT26 tumor model,

that combined intra-tumor injection of disabled infectious single cycle HSV virus encoding mGM-CSF (DISC-mGM-CSF) and syngeneic bone marrow derived DCs reduced the tumor growth of both primary and distant tumor implants (25). A period of 4-6 hours between the injection of DISC-mGM-CSF virus and DCs was required to enhance the therapeutic effect. Similar therapeutic efficacy was also reported by others using intratumor injection of DCs engineered to secrete IL-12 or modified to express CD40L (30, 31, 32). In order to select the appropriate conditions for DCs in therapy the following criteria need to be addressed: antigen selection, methods for generation and activation of DCs, introducing (targeting) the antigen into either the MHC class-I or class-II processing pathways, and route of administration.

In the present study culturing human adherent PBMC for 6 days in the presence of GM-CSF and IL-4 successfully generated DCs using media containing either FCS or autologous serum supplements. DCs have to be matured in order to become potent T-cell stimulators. During the maturation process a variety of different, specific genes are expressed including genes encoding co-stimulatory molecules, MHC class-I and class-II antigens (9); in addition, maturation induces the rearrangement of cytoskeleton proteins, adhesion molecules and cytokine receptors (9). A series of tests was conducted to evaluate the phenotypic and functional status of the activated DCs including MLR, cell surface markers expression, latex beads uptake and antigen processing. DCs at Day 6 were considered immature since they were efficient in taking up latex particles, necrotic cells and tetanus protein; they expressed low levels of cell surface markers and induced a weak stimulation of allogeneic T cells in MLR (results not given). Culturing DCs for a further 2 days in the presence of TNF-α induced maturation, which was manifested by potent stimulation of allogeneic T-cells in MLR, increased expression of CD86, CD40 and CD83, and down regulation of bead uptake.

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Generation of DCs in medium containing human plasma (preferably autologous) is required if they are to be used for human therapy. Significant differences in the immunnophenotype, macropinocytosis, endocytosis and activity in MLR have been reported for DCs generated in FCS versus human plasma (33, 34). In our hands, high yields, increased expression of co-stimulatory molecules and cell clustering during the first 3 days of culture were observed when PBMC were cultured in media supplemented with 10% FCS but not in 1% autologous plasma. A significant increase in the expression of CD1a, CD40 and CD54 was observed in DCs cultured in the presence of 10% FCS for 10 days in comparison to those cultured in autologous plasma; in addition, similar differences were noticed in functional assays using MLR.

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DRAFT

TABLE 1: Antigens or genes over-expressed in the prostate or prostate cancer tissues

Antigen	**Brief description**
PSA	Prostate specific antigen; 34 kDa glycoprotein
PSMA	Prostate specific membrane antigen; 100 kDa membrane glycoprotein
PSCA	Prostate stem cell antigen; 123 amino acid protein
PAP	Prostate acid phosphatase; 343 amino acid, 41 kDa
PCTA-1	Prostate carcinoma tumor antigen; 35 kDa
Ep-CAM	Transmembrane glycoprotein; 40 kDa
MUC-1 and 2	Mucin antigens
GM-2	Ganglioside antigen
Thy-1	Transmembrane glycoprotein with 30% homology to PSCA; 110 kDa
Bcl-2	Anti-apoptosis protein
Tn, sTn, TF	Blood group antigens
hCG beta	Human chorionic gonadotrophin
PAGE-1	X chromosome-linked GAGE-like gene
NY-ESO-1	CT antigen
p53	Tumor suppressor protein
Survivin	Anti-apoptosis protein

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Figure legends:

Figure 1: Dendritic cells generated in media containing either 10% FCS (A) or autologous (B) serum

Adherent PBMC were cultured for 6 days in RPMI containing 1000 U/mL of GM-CSF and 500 U/mL of IL-4 eith either 10% FCS (A) or 1% autologous serum (B). Cells were observed under different magnifications (x 10, x 20 and x 40). Cells cultured with FCS showed clear cluster formation.

Figure 2: Cell surface marker expression on immature dendritic cells (A) and mature dendritic cells (B)

Cell surface marker expression was detected on immature (A) or mature (B) dendritic cells generated with either 10% FCS or 1% autologous serum using an FITC-conjugated antibody (CD11c, CD54) or non-conjugated antibody (CD1a, CD83, CD86, HLA-DR) followed by an FITC-conjugated goat anti-mouse F(ab)' fragment. Analysis of the samples was by flow cytometry.

Figure 3: T cell proliferation in response to allogeneic and autologous dendritic cells

Dendritic cells generated with either 10% FCS or 1% autologous serum, were co-cultured with autologous or allogeneic T cells for 6 days. 20 µL of 0.37 Mbq/mL of Tritiated thymadine was then added to each well for the last 18 hours of culture. Both DC populations were able to induce an allogeneic T cell proliferation response but no or very little autologous T cell proliferation.

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Figure 4: T cell proliferation in response to dendritic cells incubated with 0.5 μg/mL tetanus toxoid

Dendritic cells generated with either 10% FCS or 1% autologous serum were incubated o/n with 0.5 μg/mL of tetanus toxoid protein and then co-cultured with increasing numbers of autologous T cells for 6 days. 20 μL of 0.37 Mbq/mL of Tritiated thymadine was then added to each well for the last 18 hours of culture. Both DC populations were able to induce autologous T cell proliferation when 0.5 μg/mL tetanus toxoid was used.

Figure 5: Beads uptake by immature dendritic cells (A and B) or mature dendritic cells (C)

Dendritic cells cultured for 6 days (A & B) or 10 days (C) were incubated o/n at 37°C with FITC-labeled latex beads (0.2 μm). Nuclei were stained with ethidium bromide and cytoskeleton with a mouse anti-tubulin antibody followed by a PE-conjugated anti-mouse antibody. Examination by confocal microscopy showed the beads (in green) inside the majority of immature dendritic cells (A & B) but only in very few matured DC (C).

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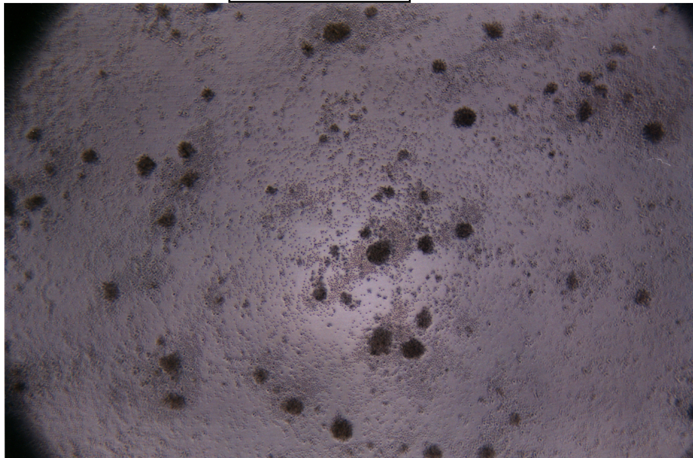
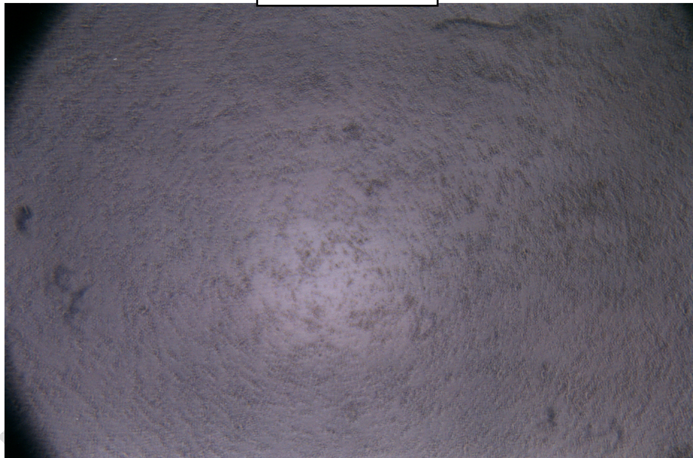
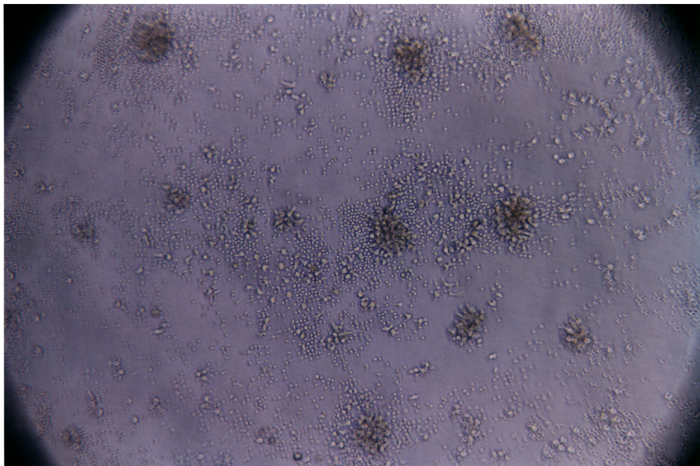
Figure 1: Dendritic cells generated in media containing either 10% FCS (A) or autologous serum (B)

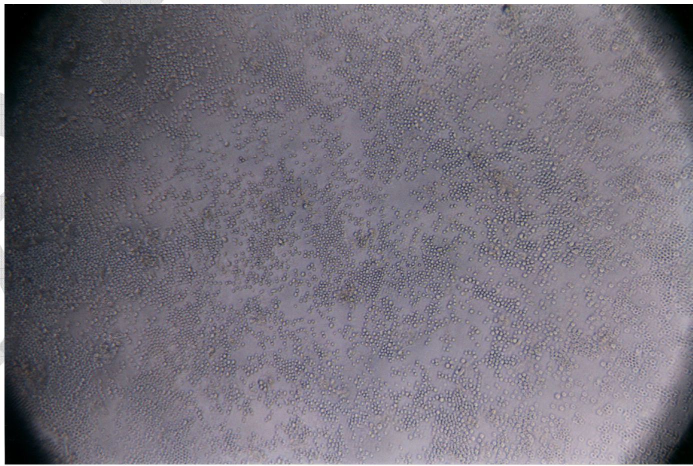
Figure A

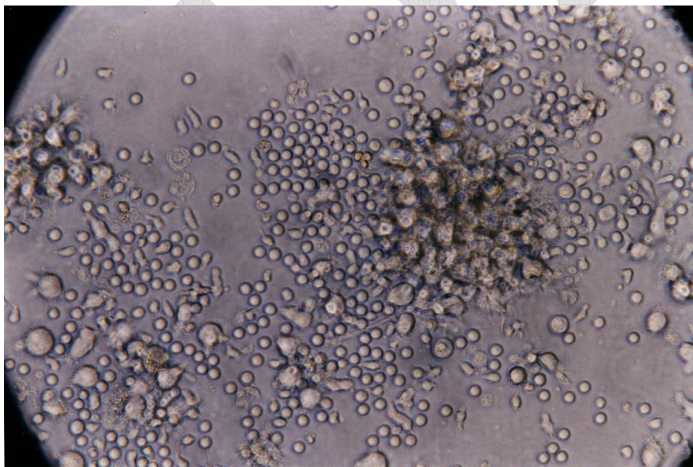
Figure B











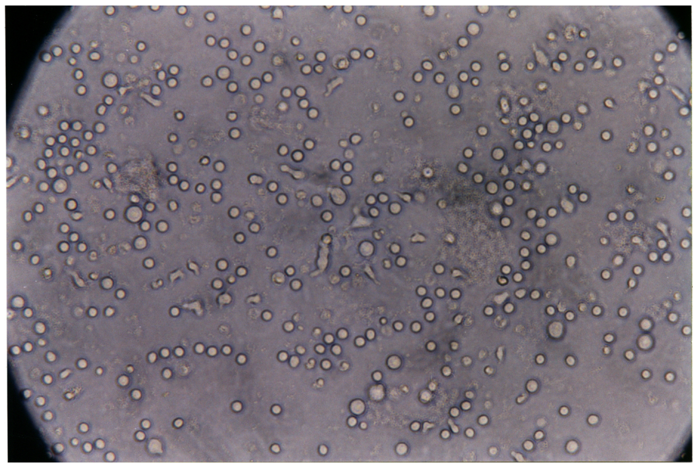
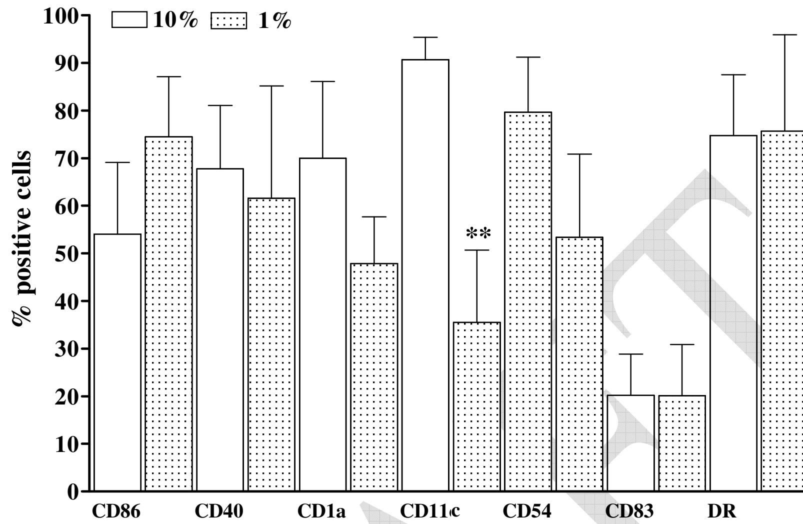


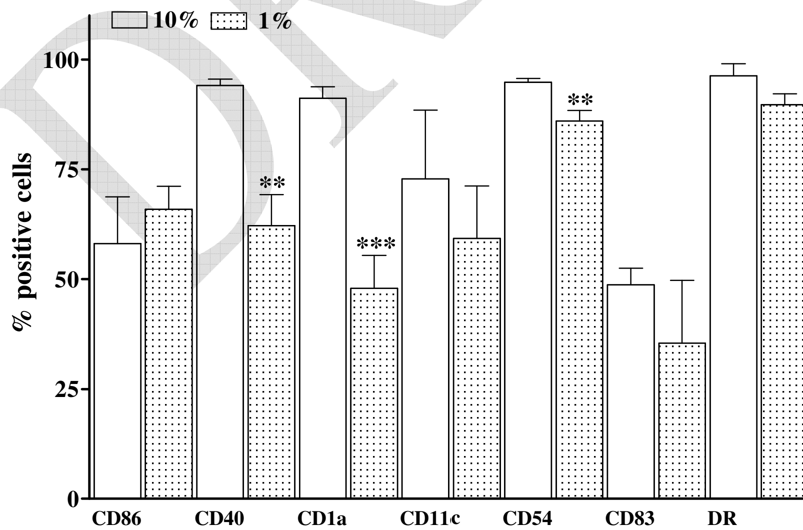
Figure 2: Cell Surface Marker Expression on Immature Dendritic Cells (A) and Mature Dendritic Cells (B)

A



** P<0.01 versus 10%
n=3-5

B



** P<0.01 versus 10%
***P<0.001 versus 10%
n=3-5

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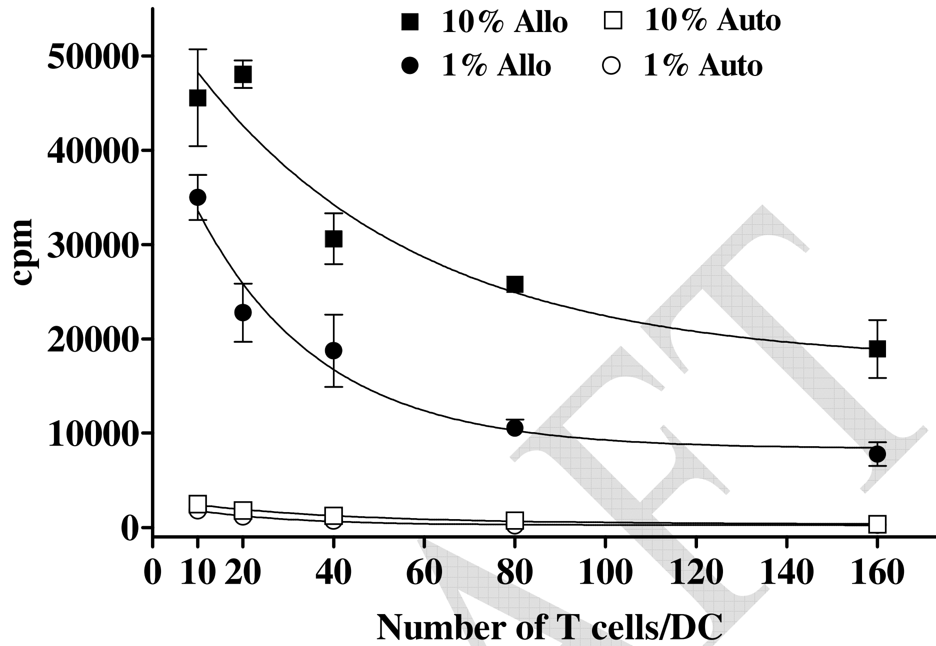
Figure 3: T Cell Proliferation in Response to Allogeneic and Autologous Dendritic Cells



n=3-5	10% Auto	10% Allo
10% Auto	/	** P<0.01
1% Auto	NS	** P<0.01
10% Allo	** P<0.01	/
1% Allo	* P<0.05	* P<0.05

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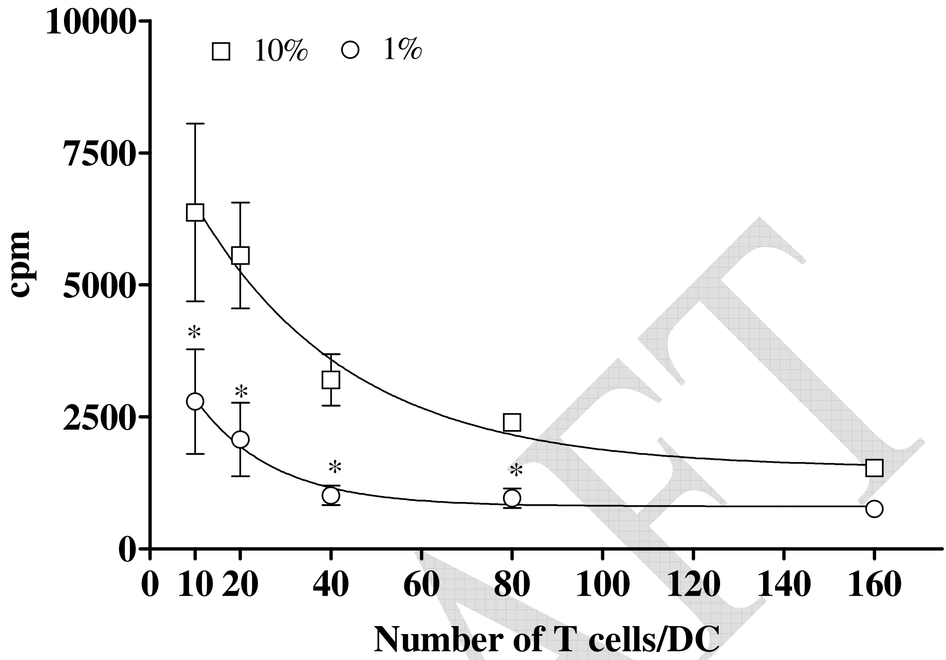
Figure 4: T Cell Proliferation in Response to DC Incubated with 0.5mg/ml Tetanus Toxoid



* P<0.05 versus 10%
n=3

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Figure 5: FITC-labelled latex beads uptake by immature (A&B) or mature (C) dendritic cells

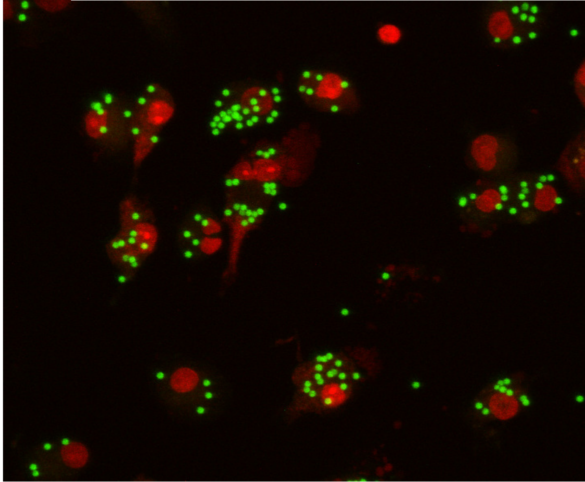

Figure A: Day 7 1% Blood pack

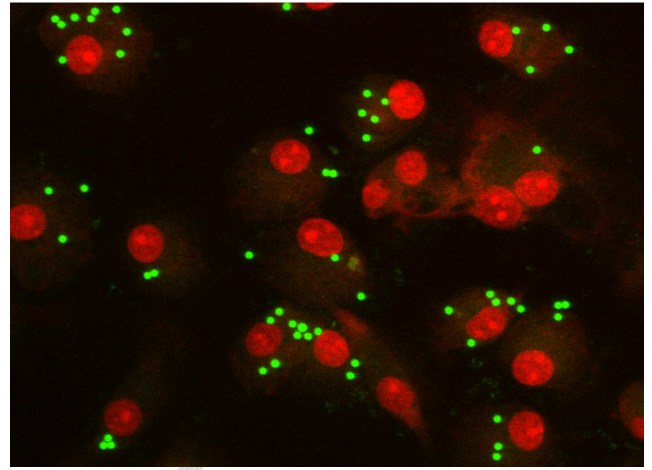

Figure B: Day 7 10% Blood pack

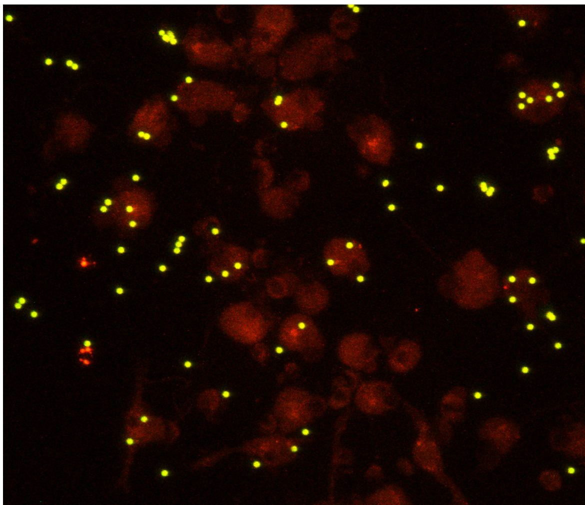

Figure C: Day 10 Matured cells fresh blood 10%

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