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Generation of large numbers of human dendritic cells from whole blood passaged through leukocyte removal filters: an alternative to standard buffy coats

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Abstract

Many blood banks now use whole blood inline filtration to produce leukocyte-depleted blood products. As a result, a common source of large numbers of human dendritic cells (DC) for research purposes, namely standard buffy coats, has been lost. Therefore, we have adapted our conventional method for growing DC from $CD14^+$ precursors in order to make use of these filter units. A dextran solution containing human serum albumin was used to flush back the filters. After pelleting, mononuclear cells were obtained by standard density gradient centrifugation (Lymphoprep™). To eliminate T cells, we used rosetting with sheep red blood cells. In addition to the classical PBMC, the cell population obtained after Lymphoprep™ centrifugation was found to contain high numbers of $CD14^+$ granulocytes which could be depleted by separation on an additional Percoll gradient.

At this stage, FACS analysis revealed a cell population that resembled the $CD14^+$ monocyte-enriched population, obtained from traditional buffy coat preparations after Lymphoprep™ centrifugation and T cell elimination. Culture of the cells and the induction of maturation was identical to the previously described procedures, except that the culture time was reduced from 7 to 5 days and the maturation time from 3 to 2 days. Analyses of the major molecules indicative of DC maturation (CD83, CD86, CD208/DC-LAMP) and functional analyses of the T cell-stimulatory capacity of the DC population (using the MLR assay with normal peripheral T cells and naive T cells) revealed no major differences from buffy coat-derived DC preparations.

Keywords: Dendritic cells; Leukocyte removal filters; Blood

1. Introduction

Dendritic cells (DC) are of bone marrow origin and differentiate into cells specialised in the initiation of primary immune responses. In a first differentiation step, they populate most organs and tissues,

Abbreviations: DC, dendritic cells; PBMC, peripheral blood mononuclear cells; DC-LAMP, dendritic cell–lysosome-asociated membrane glycoprotein

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where they reside in order to monitor their surroundings for antigen. At this stage, we refer to the cells as immature DC. These cells are very effective in capturing and processing antigen, but only when further immunostimulatory (i.e., inflammatory) signals have reached the DC do they leave the periphery to migrate into the secondary lymphoid organs. This migration is accompanied by further differentiation with morphological and functional changes, leading to the mature DC. This cell is now a very potent stimulator of T cells. In the T cell areas of lymph nodes and spleen, DC select the antigen-specific T cells for activation and initiation of cellular immune responses (Banchereau et al., 2000; Banchereau and Steinman, 1998; Bell et al., 1999; Steinman et al., 1999). Both stages of DC maturation are characterised by the expression of different surface and intracellular molecules, and these may be used to monitor the maturation stage of DC. Mature DC express CD83 and enhanced levels of CD86 and lose CD115 expression when compared to immature DC (Bender et al., 1996; Romani et al., 1996). Furthermore, a lysosome-associated membrane glycoprotein (CD208/DC-LAMP) has been described as a new, selective marker for terminal DC maturation (De Saint-Vis et al., 1998).

Many features of DC function and maturation were elucidated when methods became available to grow DC from precursors to both stages of maturation. These precursors are either dividing $CD34^+$ progenitors, which are very rare in peripheral blood (Caux et al., 1992), or $CD14^+$ non-dividing precursors (i.e., monocytes) which are grown in GM-CSF and IL-4 for 5–7 days to generate immature DC (Romani et al., 1994; Sallusto and Lanzavecchia, 1994). Further stimuli, such as monocyte-conditioned medium (Bender et al., 1996; Romani et al., 1996), defined cytokine cocktails (Jonuleit et al., 1997), TNFα + PGE2 (Rieser et al., 1997), LPS or double-stranded DNA (Cella et al., 1999), are needed to induce further differentiation or terminal maturation of DC to the T cell stimulatory stage. We have mainly used the former approaches to generate mature DC for research purposes and have used standard buffy coats from the local blood bank as a source when large numbers of DC have been needed.

When our local blood bank introduced whole blood inline filtration as a standard procedure, we were obliged to adapt the initial processing of blood, in order to ensure a similar cell population to that obtained from standard buffy coats, for use as progenitors in the generation of DC (this applied only to DC for research purposes; DC for in vivo clinical use are best generated from leukapheresis products; Schuler-Thurner et al., 2000; Thurner et al., 1999). Leukocyte depletion of all blood products was totally implemented in Canada, Switzerland, UK and Ireland in 1999. It is presently being introduced in Austria, and will become a standard procedure worldwide (Expert Panel on Blood Transfusion and Immunohematology, 2000). Due to the superior quality of blood products obtained by the use of leukocyte removal filters (Bowden et al., 1995; Pamphilon et al., 1999; Rapaille et al., 1997; Riggert et al., 1997), it is likely that buffy coats, which were often obtained as a by-product and discarded, will soon be no longer available for the research community.

2. Materials and methods

2.1. Media and reagents

The following culture media were used: RPMI-1640 (PAA Laboratories, Linz, Austria, or Biological Industries, Kibbutz Beit Haemek, Israel), supplemented with 50 μg/ml of gentamycin, 10% FCS (Biological Industries or Seromed Biochrom, Berlin, Germany); Medium 199 (Gibco BRL, Gaithersburg, MD). Human GM-CSF (Leukomax™, specific activity 1.1×10^6 U/mg) was purchased from Novartis, Basel, Switzerland; human IL-4 was used in the form of a culture supernatant (5% v/v) of a IL-4 transfected cell line (IL-4-62 cells, kindly provided by A. Lanzavecchia, Basel, CH); human TNFα was a generous gift of Dr. G.R. Adolf (Bender, Vienna, Austria; specific activity 6×10^7 U/mg); human IL-1β (specific activity 1×10^7 U/mg) and IL-6 (specific activity 1×10^7 U/mg) were from PeproTech, London, UK; prostaglandin E2 (Prostin E™) was obtained from Pharmacia-Upjohn, Uppsala, Sweden.

2.2. Filter units

Leukocyte removal filter units (Cat. No. WBF2, Pall-Gelman, Ann Arbor, MI), as used by our local

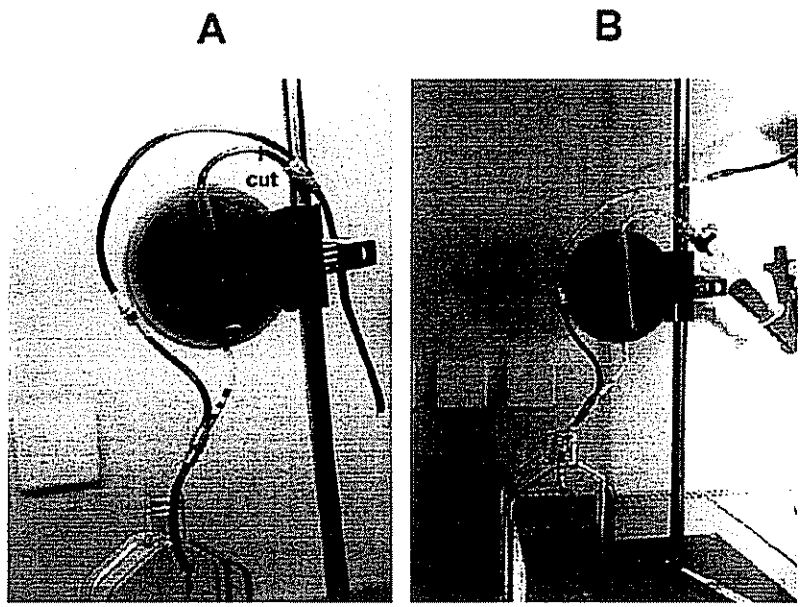

Fig 1. Leukocyte removal filter. A leukocyte removal filter is shown as supplied from the local blood bank and mounted for back flushing (A). The positioning of the syringe is indicated in panel B.

blood bank to prepare plasma for clinical use, were the source of leukocytes. During a standard procedure, 450 ml ± 10% of whole blood were passed through one filter unit, which was then discarded. We collected these filter units for removal of the leukocytes.

Table 1
Monoclonal antibodies used for phenotypic analysis by flow cytometry

Specificity	Clone/name	Ig class	Source
HLA-DR-FITC	L243	mouse IgG2a	BDIS[a]
HLA-DR-PE	L243	mouse IgG2a	BDIS[a]
CD1a	OKT-6	mouse IgG1	ATCC[b]
CD3	SK7	mouse IgG1	BDIS[a]
CD14	MΦP9	mouse IgG2b	BDIS[a]
CD19	4G7	mouse IgG1	BDIS[a]
CD45RA	HI100	mouse IgG2b	BD-Pharmingen[c]
CD45RO	UCHL1	mouse IgG2a	BD-Pharmingen[c]
CD56	MY31	mouse IgG1	BDIS[a]
CD65w	88H7	mouse IgM	Immunotech/Coulter[d]
CD80	L307 4	mouse IgG1	BD-Pharmingen[c]
CD83	HB-15a	mouse IgG2b	Immunotech/Coulter[d]
CD86	IT2 2	mouse IgG2b	BD-Pharmingen[c]
CD115/c-fms	Ab-1	rat IgG1	Oncogene[e]
CD208/DC-LAMP	104 G4	mouse IgG1	Immunotech/Coulter[d]

[a]Becton–Dickinson Immunocytometry Systems, Mountain View, CA.
[b]American Type Culture Collection, Manassas, VA.
[c]BD-PharMingen, San Diego, CA.
[d]Immunotech/Coulter/Beckman, Fullerton, CA.
[e]Oncogene Science, Cambridge, MA.

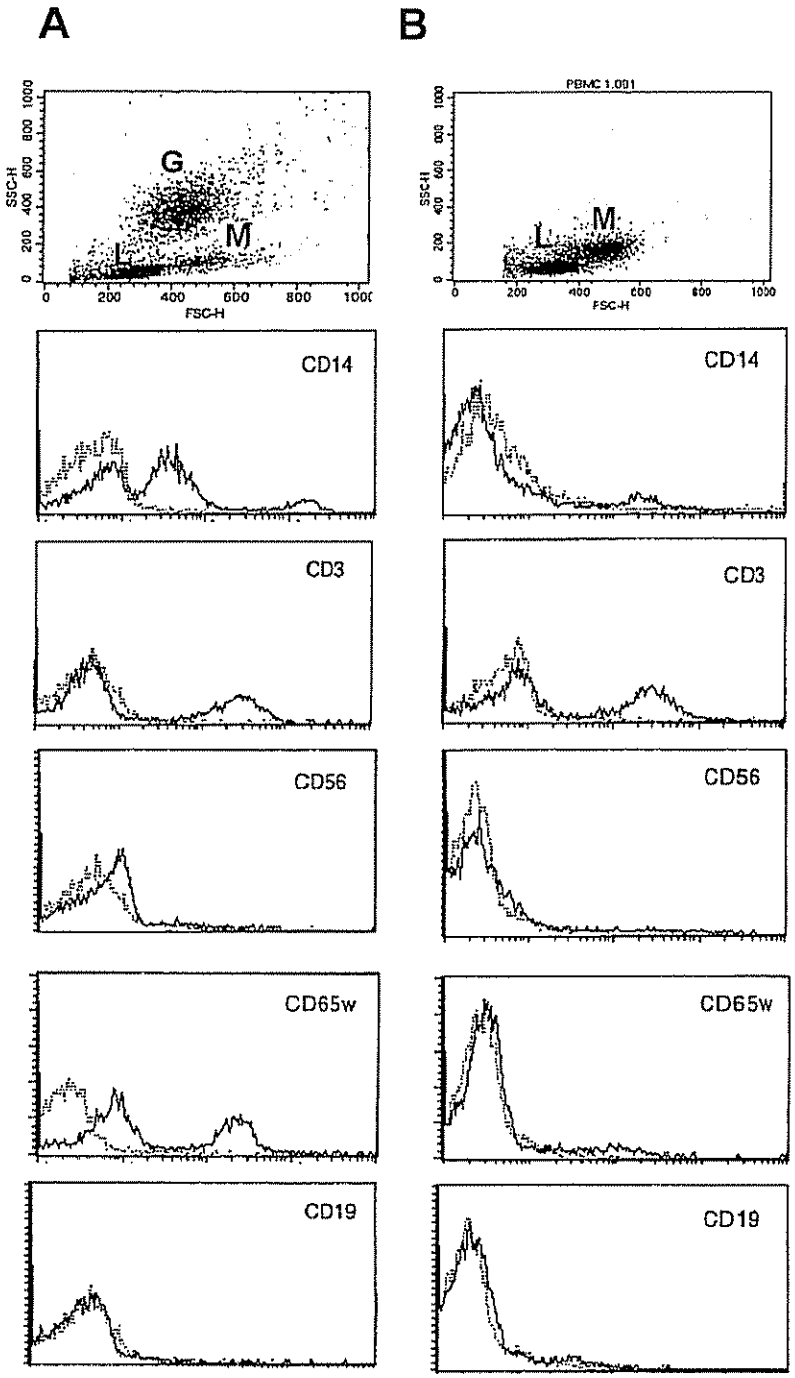

Fig 2. FACS analyses of PBMC populations. PBMC populations, as obtained after back-flushing a leukocyte removal filter (A) or by processing a standard buffy coat preparation (B), are shown. The cell populations are labelled M (monocytes), L (lymphocytes) and G (granulocytes). The additional cell population appearing on the side scatter/forward scatter plot in the top panel in A (G) is a $CD14^+$ low granulocyte population, as identified by staining with CD65w. Histograms show staining of ungated populations. Dotted line indicates staining by isotype-matched irrelevant control antibodies.

2.3. Elution of blood cells by back-flushing of filter units

Filter units obtained from the local blood bank were wiped with an ethanol-soaked tissue paper. All further steps were performed on a clean bench. The dextran solution used to flush back the filter units was that recommended by the manufacturer of the units, namely: 9% dextran, MW 70 000 (Cat. 31390 Fluka, Buchs, Switzerland), 2.5% saccharose (Cat. No. 7651, Merck, Darmstadt, Germany), 3% human serum albumin (albumin "SRK" 20%, Cat. No. 2-00203, Biochemie, Vienna, Austria) in phosphate-buffered saline.

On the plastic housing of the filter units, an arrow indicated the direction of blood flow during plasma preparation. Using 150 ml of the above-described dextran solution in 20-ml portions and a sterile 20-ml syringe, we flushed the filter units in the opposite direction. To achieve this, the outlet tubing was cut off before the bypass connection at the position indicated in Fig. 1A. The syringe was connected via a two-way valve to the filter unit (Fig. 1B). The solution was initially applied slowly to allow the whole unit to be filled with dextran solution. Once this was achieved, the back flush solution was introduced at about 20 ml/min and collected in a tissue culture bottle. An additional 150-ml volume of PBS was applied to the filter unit, collected in the same tissue culture bottle and mixed, in order to prevent the high dextran concentration from damaging the cells. For unknown reasons, from about 10% of the filter units, we were only able to isolate damaged cells. Therefore, we developed a simple test to detect and discard these filter units as early as possible: 12 ml of the cell suspension obtained after back-flushing with the dextran solution (prior to the additional back flush with PBS) were transferred to a 15-ml Falcon tube and spun at $300 \times g$ for 15 min at 4°C. Only those flush-back cells yielding clear supernatants were processed further. When red supernatants (i.e., lysed erythrocytes) were obtained, the cells were discarded.

2.4. Processing of back-flushed blood cells to obtain lymphocyte-depleted PBMC

To reduce the volume and thereby the number of Lymphoprep™ gradients needed to further process

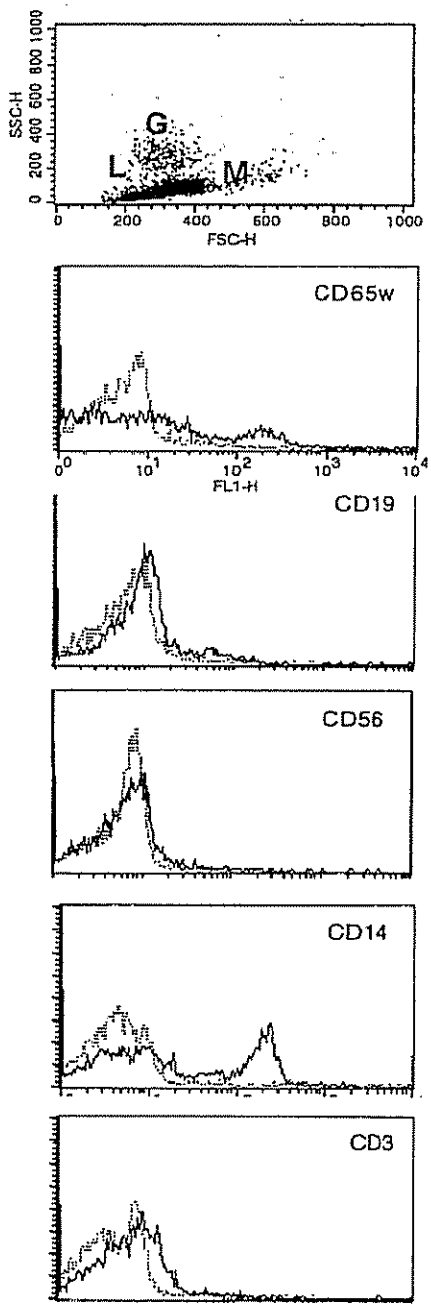

Fig. 3. FACS analyses of granulocyte-depleted PBMC from leukocyte removal filter. Granulocytes were depleted by a Percoll gradient; the remaining cell population (recovered between fractions 2 and 3 of the Percoll gradient) resembled the lymphocyte-depleted PBMC, as obtained from standard buffy coats. A small population of granulocytes (G) was still visible on the dot plot (top panel). The histograms show the staining of ungated populations. The dotted line indicates staining by isotype-matched irrelevant control antibodies.

blood cells, 100 ml of the collected dextran/PBS mixture containing blood cells were spun in two 50-ml Falcon tubes at $300 \times g$ for 20 min at 4°C. The supernatant was discarded and the cell pellets were resuspended with the remaining 200-ml blood cell suspension in dextran/PBS solution. This cell suspension was then subjected to gradient centrifugation by overlaying 25–30-ml volumes on 10 ml Lymphoprep™ (1.077 g/ml; Nycomed Pharma, Oslo, Norway) and spinning at $400 \times g$ for 30 min at RT as recommended by the manufacturer. The PBMC were collected from the interface and washed twice with PBS by resuspending and spinning at $300 \times g$ for 8 min at 4°C. At this point, samples are taken for further analyses. After counting, PBMC were subjected to rosetting with a 5% solution of neuraminidase-treated (0.01 U/ml, 1 h, 37°C) sheep red blood cells by incubation for 1 h on ice, followed by another Lymphoprep™ centrifugation to remove rosetted T cells and NK cells from the PBMC population.

The remaining cell population containing monocytes, B cells and granulocytes was washed twice in PBS, and further processed on a Percoll gradient to remove the granulocytes; $2–3 \times 10^7$ cells were resuspended in 1.8 ml Medium 199 (Cat. No. 31153-026, Gibco BRL, Life Technology, Paisley, Scotland) and mixed with 3.2 ml isotonic Percoll stock solution [10 ml $10 \times$ Medium 199 (Cat. No. 21183-025, Gibco BRL), 2 ml 1 M HEPES-buffer (Cat.No. L-1613, Seromed, Biochrom), 90 ml Percoll (Cat. No. 17-0891-01 Pharmacia) pH 7.2–7.4] to produce Per-

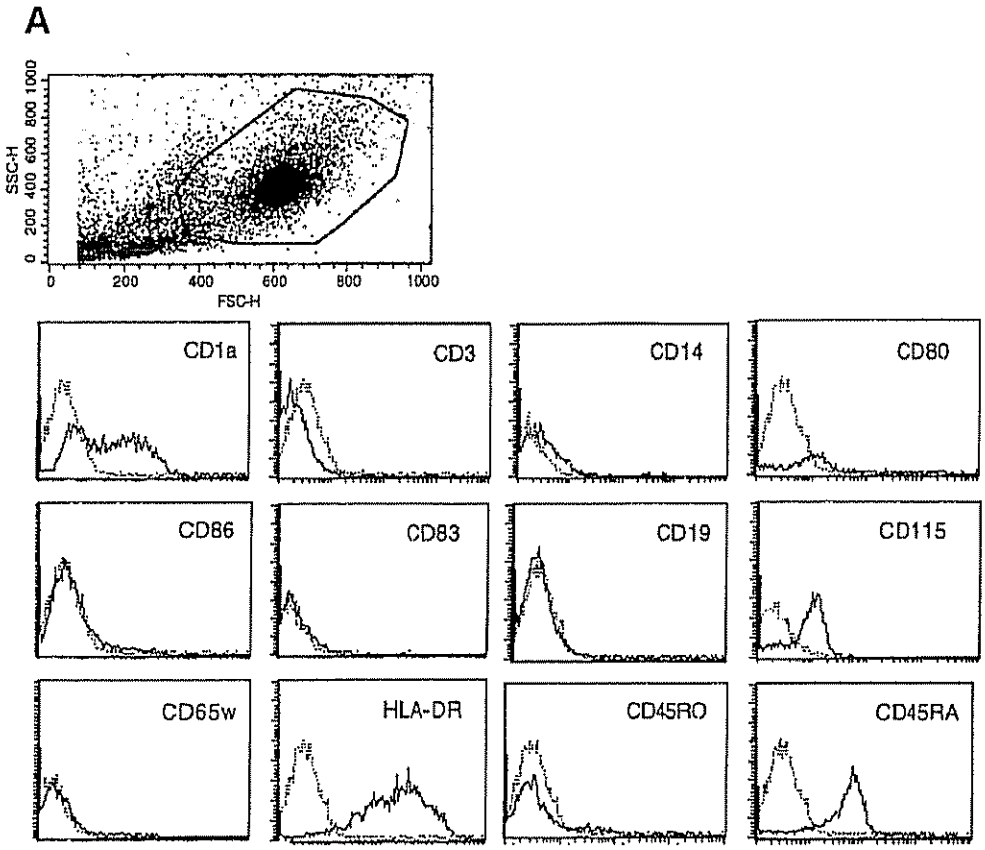

Fig 4. Comparative FACS analyses of immature (A) and mature (B) DC derived from lymphocyte- and granulocyte-depleted PBMC from leukocyte removal filters. Expression of CD115 was lost during maturation, while CD80, CD83 and CD86 expression levels were enhanced. The histograms show staining of gated populations, as depicted in the light scatter plots (top panels). The dotted line indicates staining by isotype-matched irrelevant control antibodies.

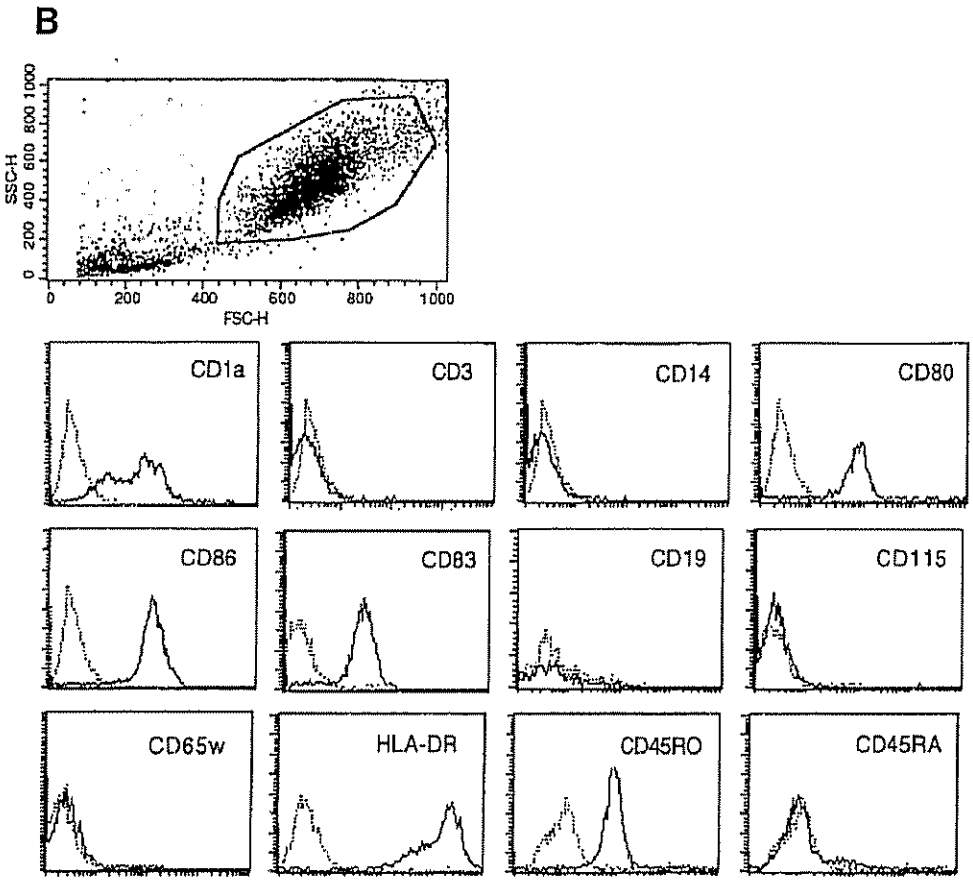

Fig 4 (*continued*).

coll fraction 1, with a density of 1.080. Each 5-ml Percoll fraction was transferred into a 15-ml polystyrene conical tube (Cat. No. 35-2095, Falcon, Becton–Dickinson Labware, Meylan, France) and overlaid with 5 ml Percoll gradient fraction 2 (2.45 ml Medium 199, 2.55 ml Percoll stock solution, density: 1.064). This was again overlaid with 3 ml fraction 3 Percoll gradient solution (Medium 199, density: 1.004). The gradient was spun at $1000 \times g$ for 20 min at RT. From two interphases appearing after centrifugation, the upper one between fractions 2 and 3 was collected, washed twice with PBS and cultured to produce DC.

2.5. Culture technique

Monocyte-enriched PBMC were cultured essentially as described (Bender et al., 1996; Romani et al., 1994, 1996). Briefly, 2×10^6 cells/well were plated in six-well tissue culture plates, in 3 ml of complete culture medium containing 800 U/ml GM-CSF and 1000 U/ml IL-4. Culture medium was renewed every other day by removing 1 ml of the medium and adding back 1.5 ml of fresh medium containing 1600 U/ml GM-CSF and 1000 U/ml IL-4. On day 5, non-adherent cells were harvested and analysed, or fed again in the six-well plates for a further culture period of 2 or 3 days, in the presence or absence of 1.5 ml/well of monocyte-conditioned medium (Bender et al., 1996; Romani et al., 1996) supplemented with TNFα and PGE2, or a defined cytokine cocktail as described by Jonuleit et al. (1997), consisting of TNFα (10 ng/ml), IL-1β (2 ng/ml), IL-6 (1000 U/ml) and PGE2 (1 μg/ml) as maturation stimuli.

2.6. Flow cytometry and immunocytochemistry

Specimens were analyzed on a FACScalibur instrument using CellQuest software (BD Immunocytometry Systems, San Jose, CA). The primary antibodies used are listed in Table 1. The intracellular antigen DC-LAMP/CD208 (De Saint-Vis et al., 1998) was visualized on acetone-fixed cytospin preparations using a biotinylated anti-mouse Ig (Amersham-Pharmacia; Amersham, UK) followed by Texas red-conjugated streptavidin (Amersham). After blocking of residual binding sites with an excess of mouse gamma globulin (100 μg/ml), DC were counterstained with an FITC-conjugated anti-HLA-DR mAb (clone L243; BD Immunocytometry Systems).

2.7. Mixed leukocyte reaction

Graded doses of DC derived from monocytes, isolated either from leukocyte removal filters or directly from blood (1×10^4, 3×10^3, 1×10^3, 3×10^2, 1×10^2), were cocultured with 2×10^5 allogeneic bulk peripheral blood T cells or with naive $CD45RA^+$ T cells [6 days, proliferation measured by ^3H thymidine incorporation at 4 μCi–148 kBq/ml of [^{3}H]TdR (specific activity 247.9 GBq/mmol = 6.7 Ci/mmol; New England Nuclear, Boston, MA) over the last 16 h] in flat-bottom 96-well microtiter plates (#3072; Falcon Labware, Oxnard, CA) in 200 μl of culture medium.

T cells were isolated from the rosettes that had formed with neuraminidase-treated sheep red blood cells during the monocyte isolation procedure (see above) by lysing the sheep red blood cells with ammonium chloride as described (Romani et al., 1997). $CD45RA^+$ naive T cells were enriched from this population using negative selection by panning with antibodies against CD8, CD14, CD16, CD19, CD45RO and CD56. FACS staining with anti CD45RA of the enriched population revealed an average of 80% positive cells.

3. Results

3.1. PBMC flushed from filter units

When we first attempted to elute blood cells from filter units, we processed them in the same way as blood cells in standard buffy coat preparations: Lymphoprep™ centrifugation followed by adherence to tissue culture dishes, to enrich for $CD14^+$ monocytes. Monocytes from filters, however, did not adhere as well as monocytes isolated from standard buffy coat preparations. Moreover, FACS analyses revealed an additional cell population. As shown in Fig. 2A, an additional $CD14^+$ population was pre-

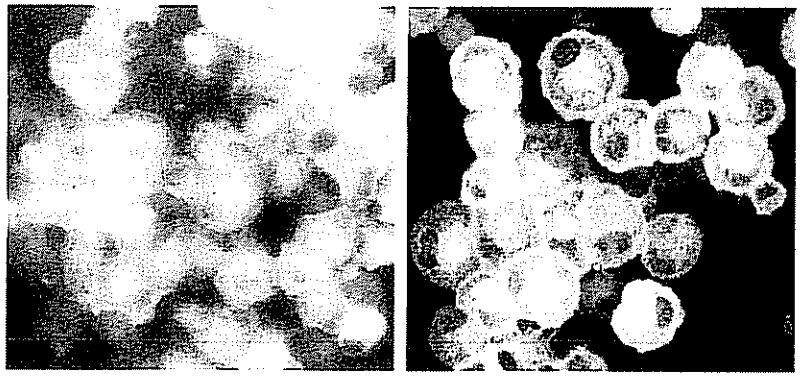

Fig. 5. Expression of DC-LAMP/CD208 on immature and mature filter-derived DC. Cells were cultured in the presence of GM-CSF and IL-4 for 5 days (immature DC), followed by another 2 days in the presence of monocyte-conditioned medium as a maturation stimulus (mature DC). Fluorescence panels show double-labeling for DC-LAMP and HLA-DR. Note that no DC-LAMP-positive cells can be found in the population of immature DC (left panel); only diffuse intracellular staining for HLA-DR is evident. In contrast, mature DC (right panel) have strongly upregulated DC-LAMP (perinuclear fluorescent spot) in virtually all HLA-DR-positive cells. The staining pattern for HLA-DR (peripheral fluorescent rim) is typical for mature DC. Magnification: 400×

sent in comparison with PBMC obtained from buffy coat preparations (Fig. 2B). This cell population was identified as granulocytes by staining with anti-CD65w antibodies.

3.2. PBMC after initial processing

To remove lymphocytes, we incubated the PBMC with neuraminidase-treated sheep red blood cells which form rosettes with T cells and NK cells. These rosettes were then separated from single cells by Lymphoprep™ centrifugation. To eliminate the additional cell population consisting of granulocytes, we used an additional Percoll gradient. Fig. 3 shows FACS analyses performed with the cell population recovered between fractions 2 and 3 of the Percoll gradient. According to the surface markers used to define the PBMC, the population was identical to the lymphocyte-depleted PBMC population isolated from buffy coats.

3.3. DC after 7 days of culture with and without maturation stimuli added during the last 2 days

Initial experiments using the original time schedule, i.e., 7 days of differentiation culture followed by another 3 days of maturation culture in the presence of maturation stimuli, such as monocyte-conditioned media (Bender et al., 1996; Romani et al., 1996) or a cytokine cocktail (Jonuleit et al., 1997), showed that the population of immature DC was not homogeneous with regards to the degree of immaturity: some mature DC consistently "contaminated" the cultures. Therefore, the initial culture period was shortened. After 5 days of culture in the presence of GM-CSF and IL-4, the non-adherent cell population was harvested and analysed by flow cytometry. It should be noted that very few cells adhered to the bottom of the wells by days 5–7.

Flow cytometry showed that the cell population on day 5 displayed the typical marker profile of immature DC (Fig. 4A): $CD1a^+$, $CD3^-$, $CD14^-$, $CD80^-$, $CD86^-$, $CD83^-$, $CD19^-$, $CD115^+$, $CD65w^-$, HLA-DR^+, $CD45RO^-$, $CD45RA^+$. This is identical to the phenotype of DC obtained from buffy coats after a standard 7-day culture. In contrast, cells that were subjected to maturation stimuli from days 5–7 expressed markers of mature DC: $CD1a^+$, $CD3^-$, $CD14^-$, $CD80^+$, $CD86^+$, $CD83^+$, $CD19^-$, $CD115^-$, $CD65w^-$, HLA-DR^{++}, $CD45RO^+$, $CD45RA^-$ (Fig. 4B). The intracellular lysosome-associated membrane glycoprotein DC-LAMP/CD208, which is exclusively expressed by terminally mature DC (De Saint-Vis et al., 1998), was absent from immature DC, but expressed de novo on virtually all mature DC (Fig. 5).

3.4. Functional analyses using the allogeneic mixed leukocyte reaction

As shown in Fig. 6, the ability to stimulate bulk T cells (Fig. 6A), as well as naive $CD45RA^+$ T cells

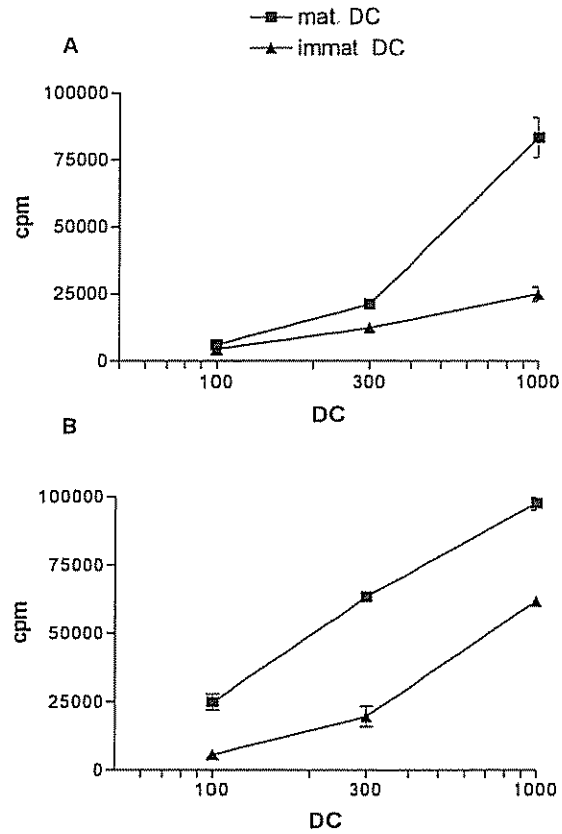

Fig. 6. Immunostimulatory function of mature and immature DC. A mixed leukocyte reaction was performed with both immature and mature DC as indicated. The maturation stimulus comprised a cytokine cocktail containing IL-1β, IL-6, TNFα and PGE2. Different numbers of DC were cocultured with 1.5×10^5 allogeneic T cells (A) and $CD45RA^+$ naive allogeneic T cells (B) for 6 days. Proliferation was measured by ^3H thymidine incorporation in counts per minute (cpm). Background proliferation of T cells alone was 300 cpm.

Table 2
Yields of dendritic cells (DC) recovered from leukocyte removal filters (A) and standard buffy coat preparations (B)

(A) Filter-derived cells					(B) Buffy coat-derived cells				
Experiment	PBMC	Mono	DC	DC in percent Mono	Experiment	PBMC	Mono	DC	DC in percent Mono
1	805	17 0	7 0	41	1	106	8 3	2 3	28
2	1200	130 0	64 0	49	2	583	78 1	32 0	41
3	1250	120 0	30 0	25	3	529	59 2	17 4	29
4	1300	55 0	14 6	27	4	319	50 0	10 5	21
5	865	32 0	9 0	28	5	481	96 2	21 1	22
6	885	31 0	8 0	26	6	625	136 8	14 3	10
7	1200	110 0	38 8	35	7	828	192 0	22 3	12
8	1200	40 0	9 5	24	8	417	78 0	12 1	16
9	1000	44 0	10 6	24	9	531	29 2	12 2	42
10	915	80 3	14 0	17	10	460	104 0	32 7	31
	1062 ± 186 a	66 ± 41 b	20 6 ± 18 5 c	29 6 ± 9 4 d		488 ± 191 e	83 ± 53 f	17 7 ± 9 6 g	25 2 ± 11 1 h

Cell numbers are given in millions Both filters and buffy coats contained cells from a blood volume of 400–450 ml PBMC: peripheral blood mononuclear cells immediately after Lymphoprep™ centrifugation; Mono: monocytes, after removal of T cells by rosetting, or removal of T cells and granulocytes by rosetting and Percoll gradient DC: mature dendritic cells, obtained after the culturing and maturation period of 5–7 days DC in percent Mono: numbers of mature dendritic cells expressed relative to the percentage of monocytes at the beginning of the culture The data in columns a and e differed significantly (Student's t-test; $p < 0.0001$), but comparisons of columns b vs f, c vs g, and d vs h revealed no significant differences

(Fig. 6B), was acquired during the maturation period induced by either monocyte-conditioned medium supplemented with TNFα and PGE2, or with the maturation cocktail consisting of IL-1β, TNFα, IL-6 and PGE2. The stimulatory capacity of filter-derived DC for T cells was comparable to that obtained with buffy coat-derived DC.

3.5. DC yields

Table 2 shows the yields of DC obtained from leukocyte removal filters (Table 2A) and standard buffy coat preparations (Table 2B). The initial volume of blood was equal for both filters and buffy coats, i.e., approximately 450 ml. The absolute numbers of PBMC recovered from the filters ($1060 \pm 186 \times 10^6$, range 805–1300, $n = 10$) were substantially greater than those of PBMC from buffy coats (490 ± 190, range 106–828, $n = 10$), mainly because granulocytes were still present.

The absolute numbers of monocytes (on day 0) and mature DC (on day 7) recovered per filter or standard buffy coat preparation differed considerably from donor to donor. In a series of 10 comparative experiments, no statistically significant differences in yield were found between filter- and buffy coat-derived monocytes and DC (Table 2). Analysis of relative recoveries shows that the numbers of mature DC (on day 7), in relation to the percentage of monocytes (on day 0), was similar for filters and buffy coats (Table 2). This suggests that the filter procedure neither increases nor diminishes the capacity of monocytes to develop into DC.

4. Discussion

To study DC biology, especially at the molecular level, the generation of large numbers of DC is essential. For many years, most researchers have used standard buffy coats from local blood banks as the major source of $CD14^+$ precursors to generate DC. Since buffy coats are no longer available, we have adapted the procedure to isolate PBMC populations, enriched for $CD14^+$ monocytes, from the cell population trapped in the leukocyte removal filter during the preparation of plasma.

The PBMC population, isolated by back-flushing the filter unit followed by Lymphoprep™ centrifugation, contains an additional cell population account-

ing for about 40% of all cells. Therefore, the yields of PBMC after the first Lymphoprep™ centrifugation obtained from the filter units are higher than from standard buffy coats. These additional cells, which were identified as granulocytes, are usually depleted from the PBMC population during Lymphoprep™ centrifugation. We hypothesise that, since these cells have been exposed to substantial physical forces, the physical properties of some of the cells may have been changed, and as a consequence, they changed their behaviour on the gradient. Applying an additional Percoll gradient, however, removes this cell population and the PBMC obtained are virtually indistinguishable from those isolated from standard buffy coats. Once both T cells and granulocytes are removed by rosetting and Percoll gradient separation, respectively, the numbers before (i.e., monocytes) and after (i.e., mature DC) the culture periods are comparable. The yields differ considerably from donor to donor, but the range is again comparable to that obtained from buffy coats (Table 2). One substantial difference in the DC, generated by backflushing the filter units, is that both the culture period and the maturation period are shorter. To obtain comparable DC populations, we culture the PBMC for 5 days, allowing an additional 2 days for maturation, as opposed to 7 days plus an additional 3 days for buffy coat-derived DC (Bender et al., 1996; Romani et al., 1996). A similar phenomenon was observed with DC that were cultured from PBMC obtained from leukapheresis products. Here, too, the maturation period was shorter than for standard buffy coat-derived DC (Thurner et al., 1999). The functional analyses obtained using the mixed leukocyte reaction are again comparable to those obtained with buffy coat-derived DC.

Although this new method to recover $CD14^+$ precursors from leukocyte removal filters needs an additional step for the elimination of granulocytes, the quality and quantity of DC obtained is acceptable and similar to the conventional method using standard buffy coat preparation as a source. Since most blood banks have changed, or will probably change soon, their standard procedure for plasma preparation to leukocyte removal filters (Expert Panel on Blood Transfusion and Immunohematology, 2000), this method may become a standard procedure for the preparation of large numbers of DC.

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