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# MHC II$^+$ CD45$^+$ cells from synovium-rich tissues of normal rats: phenotype, comparison with macrophage and dendritic cell lineages and differentiation into mature dendritic cells *in vitro*

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## Abstract

**Synovial tissues are frequent sites of inflammatory disorders in which dendritic cells (DCs) may play an important role. This study examines potential antigen-presenting cells obtained from synovium-rich tissues (SRTs) by vascular perfusion of rat hind limbs with collagenase and further enzymatic digestion of the disarticulated hind paws *in vitro*. The three sub-populations of interest were: CD45$^+$MHC II$^{hi}$, mainly CD11c$^+$ and CD163$^-$; CD45$^+$MHC II$^{lo}$, mainly CD11c$^-$ and CD163$^+$ and CD45$^+$MHC II$^-$, mainly CD11c$^-$ and CD163$^+$. Expression of CD11c and CD163 correlated with ruffled cell-surface (CD11c$^+$CD163$^-$) and highly vacuolated cytoplasm (CD11c$^-$CD163$^+$), respectively. Culture of the CD45$^+$CD163$^-$ sub-population in granulocyte macrophage colony-stimulating factor (GM-CSF) yielded CD45$^+$MHC II$^{hi}$ CD11c$^+$CD163$^-$ cells with veiled morphology, while the large vacuolated cells that expressed CD163 resembled type A synoviocytes in both surface antigen phenotype and morphology. These results demonstrate that SRTs contain indeterminate cells that can differentiate into mature DCs *in vitro* in response to GM-CSF, plus mature synovial lining macrophages.**



## Introduction

Dendritic cells (DCs) are the principle accessory cells of the immune system. Typically, mature DCs have dendritic or veiled morphology, abundant expression of MHC class II and a unique capacity to activate naive T cells (1, 2). Immature DCs derived *in vitro* from bone marrow or monocyte precursors are actively endocytic but they do not express high levels of surface MHC class II molecules, present processed antigens effectively or provide co-stimulation to naive T cells (1, 3). Such cells may be important in the maintenance of peripheral tolerance (4). However, the nature and the functional characteristics of immature DCs within normal tissue microenvironments are less well understood. In particular, resident DCs in a variety of normal tissues deviate from the *in vitro* paradigm by expressing high levels of MHC class II molecules (5, 6). Synovial DCs are of special interest because they are likely to play an important role in inflammatory joint diseases. Increased numbers of DCs have been reported in the peripheral blood, synovial fluid and synovial tissue in rheumatoid arthritis (RA) (7, 8) and the DCs in rheumatoid synovium have both activated phenotype and increased activity as antigen-presenting cells (APCs) (9, 10). In RA, DCs may present peptides from synovial- or cartilage-derived arthritogens to pathogenic T cells (11, 12). There is poor understanding of how DC phenotype and function differs between normal and rheumatoid synovium, what changes occur during the evolution of the disease process or whether such changes are of primary or secondary importance to the establishment and progression of the autoimmune process. Furthermore, since macrophages also belong to the APC system (13) and they share many surface antigens with monocytes and DCs, it is a considerable problem to distinguish these cells phenotypically or to trace their lineages with certainty (13, 14).

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A major advantage of animal models of autoimmunity is that they provide access to the inductive stages of the disease process. We have approached the task of studying the role of synovial APCs in the pathogenesis of adjuvant-induced arthritis (AA) by first characterizing putative DCs and macrophages in normal rat synovial tissues. An enzyme-digestion method was developed to recover viable cells from the synovium-rich tissues (SRTs) of the hind paws of rats. $CD45^+MHC\ II^+$ cells from SRTs have been studied and compared with blood monocytes, peritoneal macrophages, DCs from pseudo-afferent mesenteric lymph and synoviocytes lavaged from the knee joint, utilizing two key lineage markers—CD11c (expressed by DCs) and CD163 (a marker of macrophages in the rat) (15). Sub-populations of these cells have been examined for their ability to generate typical DCs when cultured with granulocyte macrophage colony-stimulating factor (GM-CSF). The study provides the basis for phenotypic and functional studies on synovial APCs during the pathogenesis of experimental polyarthritis.

## Methods

### *Animals*

Inbred specific pathogen-free female Dark Agouti (DA) rats were obtained at 8–10 weeks of age from the Gilles Plains Animal Resource Centre (Adelaide, Australia). The rats were maintained at the animal facility of the Institute of Medical and Veterinary Science (IMVS) and had free access to standard food pellets and water. Experiments were conducted according to a protocol approved by the institutional Ethics Committees of the IMVS and the University of Adelaide.

### *Reagents*

Mouse recombinant granulocyte macrophage colony-stimulating factor (rGM-CSF), produced using a baculovirus expression system, was provided kindly by T Hercus (Human Immunology, IMVS, Adelaide, Australia). The following mouse anti-rat mAbs have been described elsewhere (16) and were used as neat hybridoma culture supernatants: R73 (anti-TCR $\alpha/\beta$), W3/25 (anti-CD4), OX33 (anti-CD45, B cell-specific isoform), OX6 (anti-MHC class II, RT1B) and WT5 (anti-CD11b, Mac-1). mAbs OX1 (anti-CD45) (17), V65 (TCR $\gamma/\delta$) (18), 3H5 (CD80/B7.1) (19), 8A2 (CD11c; Serotec, Oxford, England) and UA016 (anti-endothelial cell, probable PECAM-1/CD31; G.M., unpublished results) were also used as neat hybridoma supernatants. The mAb 24F (CD86/B7.2) (19) was affinity purified from hybridoma supernatant and used at 10 $\mu g\ ml^{-1}$. The following purified mouse anti-rat mAbs were purchased and used at the concentrations recommended by the manufacturer. RECA-1 (pan endothelial marker), ED1 (CD68, monocytes, macrophages and DCs) and ED2 (CD163, pan tissue macrophage) were obtained from Serotec. RP1 (granulocyte marker), D34-485 (CD32/Fc$\gamma$IIR) and the PE-conjugated mAbs OX1, OX6 and OX38 (anti-CD4) were purchased from PharMingen (San Diego, CA, USA) and used at the recommended concentrations. Isotype-matched control mAbs were 1B5 (IgG1, anti-giardia; G.M., unpublished results) and ID4.5 (IgG2a, anti-*Salmonella typhimurium*) (20). The antibodies were used to stain cells on cytospins using the indirect immunoperoxidase technique, or to label cells in suspension for flow cytometric analysis (direct or indirect immunofluorescence). Secondary antibodies were FITC-conjugated goat anti-mouse Ig, biotin-labelled goat anti-mouse Ig and goat anti-IgG2a (PharMingen), HRP-conjugated sheep $F(ab')_2$ anti-mouse Ig (Amersham Life Science, Sydney, Australia) and goat anti-mouse Ig conjugated to peroxidase-labelled dextran polymer (Envision+ system, Dako, CA, USA). The biotinylated secondary antibodies were detected by a streptavidin–PE conjugate (Southern Biotechnology Associates, Birmingham, AL, USA).

### *Isolation of mononuclear cells from hind paws*

Rats were anaesthetized by isoflurane inhalation. The abdominal cavity was opened through a midline incision and the aorta and posterior vena cava were ligated caudal to the renal vessels. A 22-gauge and a 16-gauge intravenous catheter (Becton Dickinson, San Jose, CA, USA) was inserted into the distal aorta (antegrade) and the vena cava (retrograde), respectively. The aorta was perfused manually for 2 min with ~5 ml of PBS in order to flush blood from the hind limbs. The solution was then replaced with 20 ml of pre-warmed complete medium (CM) (RPMI 1640 medium; GIBCO, Rockville, MD, USA; containing 2% heat-inactivated foetal calf serum, 100 $U\ ml^{-1}$ penicillin, 20 $\mu g\ ml^{-1}$ gentamycin, 2 mM L-glutamine and 10 mM HEPES buffer), containing collagenase 250 $U\ ml^{-1}$ (collagenase type 1; Sigma Chemical Co., St Louis, MO, USA) and DNAse 10 $\mu g\ ml^{-1}$ (Worthington Biochemical Corp., Lakewood, NJ, USA). This medium was perfused over ~8 min after which the lower limbs were excised at the knee. The nails were removed, the skin was incised from the knee to the toes along the dorsal surface of the foot and the skin was then stripped with gentle dissection to separate it from the subcutaneous connective tissues around the ankle joint. The tendons were divided above the ankle and the muscles of the leg were removed from the tibia. The soft tissue around the ankles was then removed and diced into small pieces in CM containing collagenase and DNAse. Each paw was then disarticulated at the ankle. The calcaneus, cuboid and navicular bones were disarticulated from the remaining cuneiform bones and the metatarsals and phalanges were also separated, thus exposing the synovium of the joints to the enzymes. The tissue was digested for 2 h at 37°C (20 ml of enzyme-containing CM for each rat), with constant gentle rocking. The material released by digestion was disrupted further using a loose-fitting glass homogenizer, followed by passage through a 19-gauge needle. The resulting suspension was filtered through a metal mesh and a nylon cell strainer (70-$\mu$m pore size; Becton Dickinson), centrifuged and washed twice in CM. The cells were then prepared for analysis by immunocytochemistry and/or flow cytometry. Control experiments showed that incubation of blood monocytes or peritoneal macrophages with collagenase for 2 h at 37°C did not alter the surface antigen phenotype of the cells (data not shown).

### *Cell culture with GM-CSF*

The washed cells were centrifuged over a density gradient of Lymphoprep medium (Nycomed Pharma AS, Oslo, Norway) to

remove debris, erythrocytes and vascular segments. The cells at the interface were harvested, washed in CM and counted. After removing samples (day 0) for cytocentrifugation (1–2 $\times$ $10^5$ cells per slide), the remaining cells were cultured in 24-well dishes (Costar, Cambridge, MA, USA) in 1 ml of RPMI 1640 medium supplemented with 10% FCS (CM) and 500 U $ml^{-1}$ mouse rGM-CSF, at 1 $\times$ $10^6$ cells per well. Cultures were incubated at 37°C in a humidified atmosphere of 5% $CO_2$ in air. The cells were harvested at daily intervals (1–3 days), the majority being dislodged easily by pipetting. They were cytocentrifuged and used subsequently for staining with May–Grunwald–Giemsa stain (BDH Chemicals, Victoria, Australia) or by immunocytochemistry.

*Preparation of synovial cells*

Cells lining the synovium of the knee joint were obtained by lavage, using CM containing 250 U $ml^{-1}$ of collagenase. Lavage was accomplished by introducing a 26-gauge needle, attached to a 1-ml syringe containing ~0.4 ml of collagenase solution, into the joint space from the lateral border of the patella. The joint space was lavaged for ~2 min by repeated injection and withdrawal of 50–100 μl of medium. The aggregate yield of cells from lavage of the knees of three rats was ~2.5 $\times$ $10^5$ cells. The harvested cells were analysed by immunocytochemistry and flow cytometry.

*Collection of mesenteric pseudo-afferent lymph DCs*

Mesenteric lymphadenectomy and thoracic duct cannulation were performed as described previously (21). Thoracic duct cells were centrifuged through a Nycodenz gradient (Nycomed Pharma; 30.55 g per 100 ml of water). This stock was diluted with EDTA solution to a density of 1.068 g $cm^{-3}$ and osmolarity of 340 mOsm at 4°C (16). The low-density fraction contained lymphoblasts and DCs, with a purity of 60–80% DCs (as assessed by the presence of cells with veiled morphology) and a viability of >90%.

*Preparation of peritoneal cells*

Peritoneal cells were harvested by lavage of the peritoneal cavity with 5 ml of ice-cold PBS containing 50 U $ml^{-1}$ heparin. The cell suspension was centrifuged, washed with PBS and analysed using immunocytochemistry and flow cytometry.

*Preparation of PBMCs*

Heparinized blood (6–10 ml) was obtained by cardiac puncture. Dextran 500 (2% w/v, Pharmacia Biotech, AB, Uppsala, Sweden) in PBS was mixed with blood at the ratio of 1 : 1 and allowed to stand for 30 min at room temperature to sediment most of the erythrocytes. Mononuclear cells were separated from erythrocytes and granulocytes by density-gradient centrifugation over Lymphoprep. Cells at the interface were harvested for flow cytometry analysis.

*Immunocytochemistry*

Cytospin preparations were made by loading a cytocentrifuge (Thermo Shandon Inc, PA, USA) with 1–2 $\times$ $10^5$ cells per slide and centrifugation at 200 r.p.m. for 5 min. The slides were air-dried and stored with desiccant at −80°C until required for immunocytochemistry. After fixation in acetone for 10 min, the cytospin preparations were incubated with primary antibodies for 1 h, washed three times with PBS, incubated with secondary antibody for a further 1 h and then washed again. The preparations were then incubated with hydrogen peroxide and diaminobenzidine (Sigma FAST DAB Tablet; Sigma) as described elsewhere (5) and counterstained lightly with haematoxylin. In negative control preparations, specific mAbs were replaced with isotype-matched irrelevant control mouse mAbs.

*Flow cytometry*

Cells (1–2 $\times$ $10^5$ cells per tube) were stained by either single-color indirect immunofluorescence or by dual-color staining, using a combination of indirect and direct immunofluorescence. For single-color labelling, primary mAbs were applied for 1 h on ice, followed after washing by a further 1-h incubation with FITC-conjugated goat anti-mouse Ig. Mouse mAbs with irrelevant specificity and appropriate isotype were used as negative controls. In some cases, where the secondary antibody was biotinylated, the cells were further incubated with a streptavidin–PE conjugate for 1 h. After a final wash, the cells were fixed with 1% PFA and stored in the dark at 4°C. For dual-color flow cytometry, the cells were labelled first for indirect immunofluorescence, as described above. After two washes, they were incubated with 20 μl of neat normal mouse serum for 30 min, to block free valencies of the secondary antibody. Without further washing, a PE-conjugated mAb (OX1, OX6 or OX38) was applied, incubation was continued for a further 1 h and after washing the cells were fixed with 1% PFA. Cells were analysed using a Coulter EPICS XL-MCL flow cytometer and Coulter XL software. Events were gated to exclude dead cells and debris and at least 10 000 events were acquired from each analysis.

*Detection of intracellular antigens*

To detect intracellular MHC class II molecules, cells were fixed for 20 min in 3% PFA and then permeabilized for 30 min in PBS containing 1% saponin and 5% BSA (essentially as described by Regnault *et al.*) (22). Cell-surface plus intracellular MHC class II molecules were then detected by direct immunofluorescence, followed by incubation with the mAb OX6-PE conjugate. The CD68 antigen, which has an intracellular distribution, was detected by indirect immunofluorescence in permeabilized cells, by essentially the same method.

*FACS*

Cells labelled indirectly with mAb ED2 (anti-CD163) and directly with PE-conjugated mAb OX1 (anti-CD45) were resuspended in CM supplemented with 2% FCS. $CD45^+CD163^-$ and $CD45^+CD163^+$ cells were sorted into separate populations using a $FACStar^{plus}$ FACS and CellQuest software (Becton Dickinson). Cells were sorted at a rate of 3000 events $s^{-1}$ and were kept at 4°C throughout the procedure.

## Results

*General characteristics of cells from SRT digests*

After collagenase perfusion, complete disarticulation and further collagenase digestion (see Methods), a pair of hind

paws from normal rats yielded ~5.5 × $10^6$ ($n$ = 29, SD ± 1.2 × $10^6$) viable cells. Giemsa-stained cytospin preparations of SRT digests contained a mixture of cells, vascular segments and some debris (Fig. 1A). Many of the cells were mononuclear and of these some were large and possessed simple oval-shaped nuclei and numerous cytoplasmic vacuoles. These resembled mature macrophages, while smaller round cells with oval or slightly indented nuclei had indeterminate morphology but were similar to monocytes. A few mononuclear cells were medium to large, with smooth or ruffled borders and bean-shaped eccentric nuclei. Morphologically, these appeared to be DCs. Eosinophils, neutrophils, mast cells and lymphocytes were present in small numbers (data not shown).

Cytospin preparations stained by mAb OX1 revealed many $CD45^+$ cells (data not shown). The majority of the $CD45^-$ cells had morphology similar to those stained by the anti-endothelial mAbs UA016 and RECA-1 (data not shown) and the remaining $CD45^-$ cells were presumed to be fibroblasts. mAb OX6 (MHC class II) stained many of the small- and medium-sized mononuclear cells strongly (Fig. 1B) and also stained large cells that had ruffled or veiled cytoplasmic membranes (Fig. 1B, inset). Staining of the large macrophage-like cells was variable and in most cases weak to moderate in intensity (Fig. 1B). The aggregates of endothelial cells were stained weakly by mAb OX6 (data not shown).

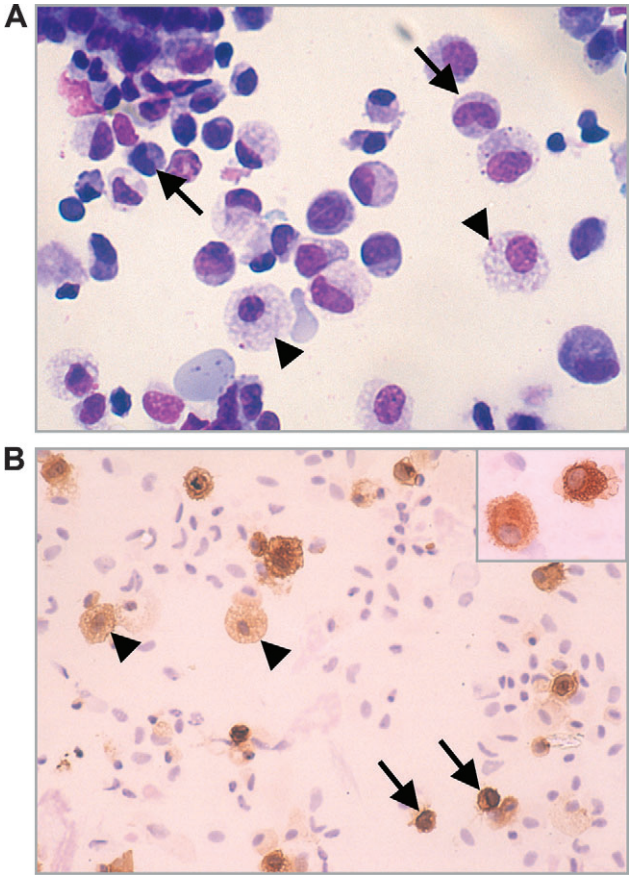


**Fig. 1.** Cytospin preparations of cell suspension from enzymatic digest of hind paw SRT. (A) Giemsa stain. Arrows indicate small- to medium-sized round cells with indented nuclei. Arrowheads indicate large cells with cytoplasmic vacuoles and simple oval-shaped nuclei (macrophage-like cells). (B) Indirect immunoperoxidase staining with mAb OX6 to detect MHC class II molecules. Arrows show small cells with eccentric indented nuclei that express high level of MHC class II. Arrowheads show macrophage-like cells that express low levels of MHC class II molecules. Inset shows medium to large cells with ruffled borders and eccentric indented nuclei that express moderate levels of MHC class II molecules. Original magnifications: (A) ×165; (B) ×83; inset ×412.

### *Flow cytometric analysis of surface and intracellular antigens expressed by cells from SRTs*

The cells in digests of SRTs exhibited great diversity of forward scatter (FSC) and side scatter (SSC) of light (Fig. 2A). A population containing putative APCs was gated for analysis (Fig. 2A), based on the light-scatter characteristics of DCs in mesenteric pseudo-afferent lymph (Fig. 2B). mAb against CD45 (OX1) stained ~25% of the cells in this gate (Fig. 2C). In permeabilized preparations, ~16% was stained by mAb against CD68 (ED1, presumptive mononuclear phagocytes) and ~4% was neutrophils (identified by mAb RP1) (data not shown). No cells in this gate were stained by mAbs OX33, R73 or V65 (B cell-specific isoform of CD45, TCR α/β and TCR γ/δ, respectively).

Co-expression of surface antigens by $CD45^+$ cells within the gate was studied by dual fluorochrome immunofluorescence (Fig. 3). Expression of the intracellular antigen CD68 is also included. Most $CD45^+$ cells expressed intermediate levels of CD32 (80%) and CD11b (77%), while mAbs against CD68 and CD163 (ED1 and ED2) stained 65 and 45% of $CD45^+$ cells, respectively. Approximately 55% of the $CD45^+$ cells expressed CD4. The proportions of cells expressing MHC class II, CD11c, CD80 and CD86 ranged from 35 to 50%.

### *Detection of intracellular MHC class II in permeabilized cells from SRTs*

We focused on cells that express MHC class II molecules. Using appropriate negative control mAbs (isotype matched), the MHC class $II^+$ cells could be divided into three subpopulations: $CD45^+MHC\ II^{hi}$, $CD45^+MHC\ II^{lo}$ and $CD45^+MHC\ II^-$. Of the 41% of $CD45^+$ cells that expressed surface MHC

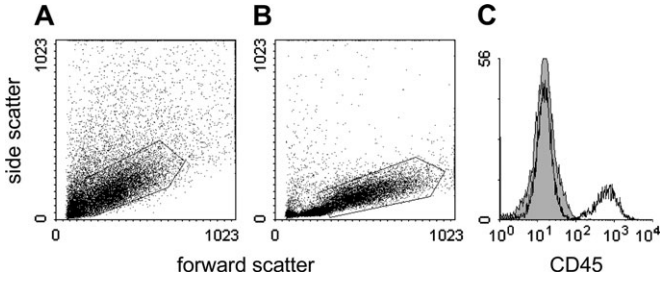


**Fig. 2.** Analysis of $CD45^+$ cells from SRTs by flow cytometry. FSC and SSC of putative APCs recovered from enzymatic digests of hind paw SRT (A) are gated, based on the light-scatter characteristics of DCs in mesenteric pseudo-afferent lymph (B). The histogram (C) shows $CD45^+$ cells within the putative APC gate, stained with mAb OX1. The filled histogram depicts staining with the isotype control mAb.

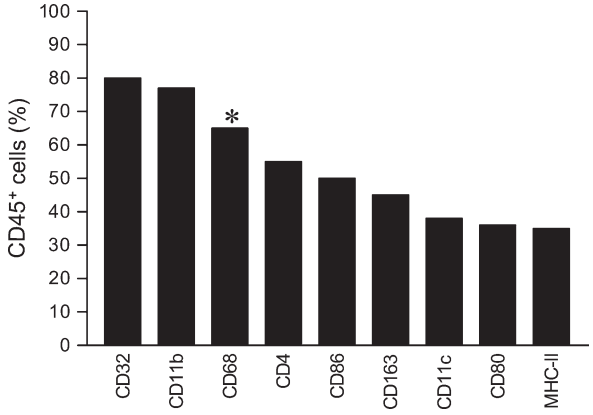


**Fig. 3.** Surface expression of specified molecules by CD45-positive cells recovered from SRTs by enzymatic digestion, as assessed by dual-color flow cytometry. Where indicated by asterisk, cells were fixed and permeabilized before labelling with antibody.

class II molecules, 29% expressed relatively high levels and 12% expressed low levels (Fig. 4A). We then examined whole-cell (cell-surface plus cytoplasmic) expression of MHC class II molecules by CD45$^+$ cells. The cells were labelled with mAb OX1 against CD45 (indirect, FITC) and then fixed, permeabilized and labelled with mAb OX6 against MHC class II (direct, PE) to detect surface plus cytoplasmic MHC class II molecules (see Methods). Permeabilization increased the proportion of CD45$^+$MHC II$^+$ cells from 41 (fresh cells, Fig. 4A) to ~60% (fixed cells, Fig. 4B). The proportion of MHC II$^{hi}$ cells increased only slightly. However, the proportion of CD45$^+$MHC II$^{lo}$ cells was increased from 12 to 23% by permeabilization (Fig. 4B), indicating the presence of cells in SRTs that contain cytoplasmic MHC class II molecules but lack surface MHC II.

The co-expression of other cellular antigen markers was studied in permeabilized cells stained to demonstrate MHC class II. The MHC II$^{hi}$ cells are simple to study because they all express CD45 (Fig. 4A and B). The surface antigen phenotype of the MHC II$^{hi}$ cells (as defined in Fig. 4B) is shown in Fig. 4(C–H). The majority of the MHC II$^{hi}$ cells expressed CD11b and/or CD32 (Fig. 4C and D), and many expressed CD4 and/or CD11c (Fig. 4F and G). A small proportion of MHC II$^{hi}$ cells expressed CD163 (Fig. 4H), but surprisingly, 55% of the cells expressed CD68, another macrophage-associated marker (Fig. 4E). Approximately half of the cells expressed CD86 (data not shown). The proportions of the MHC II$^{hi}$ cells expressing each molecule are shown in Fig. 5(A). Importantly, 40% of the CD45$^+$ cells expressed CD11c and only 18% expressed CD163.

It is calculated that CD45$^+$MHC II$^{lo}$ cells constituted 40% of the total CD45$^+$MHC II$^+$ population (Fig. 4B). This population was only about half of the total MHC II$^{lo}$ cells (Fig. 4B), the remainder of which were CD45$^-$ (non-haematopoietic origin). However, because all cells that express CD11b, CD32, CD86, CD68, CD4, CD11c and CD163 also express CD45 (data not shown), the cells expressing these markers can be calculated as a proportion of the total CD45$^+$MHC II$^{lo}$ cells (Fig. 5B). A total of 60% of the MHC II$^{lo}$ cells expressed CD163, while between 66 and 77% expressed CD11b, CD32, CD86, CD68 and CD4. Approximately 34% of the MHC II$^{lo}$ cells expressed CD11c.

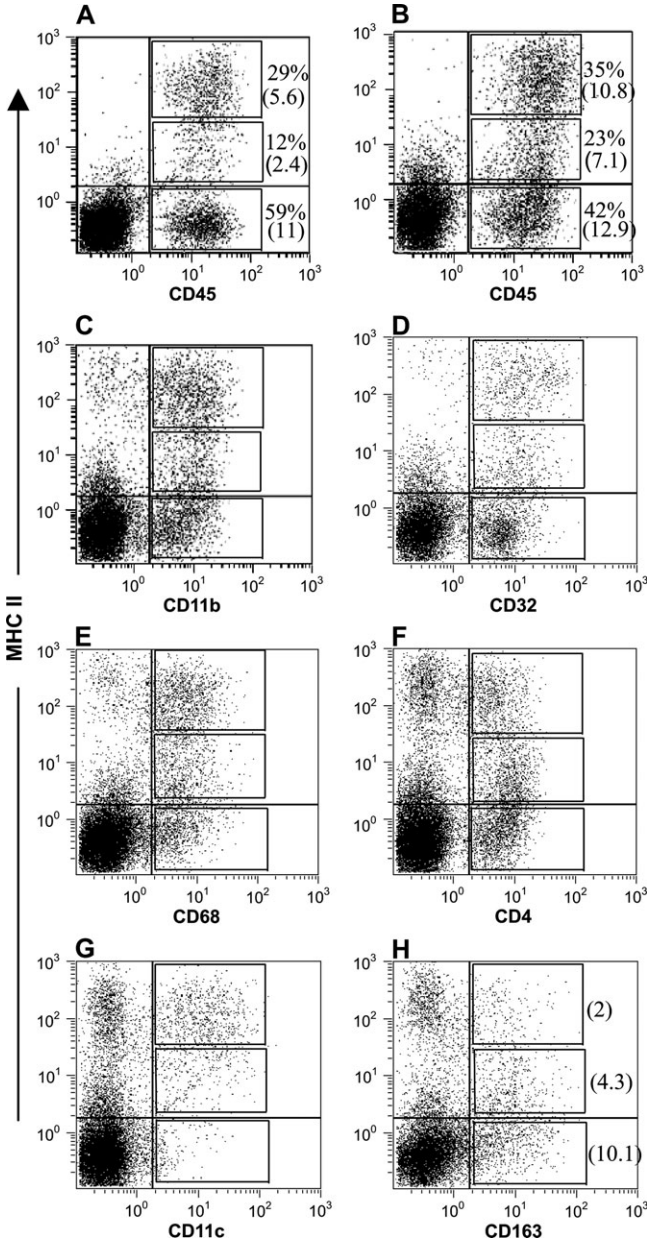


**Fig. 4.** Double-color flow cytometric analysis of single-cell suspensions prepared from enzymatic digests of hind paw SRT. The population analysed by flow cytometry was obtained by gating on the basis of light-scatter characteristics of DCs in mesenteric pseudo-afferent lymph (Fig. 2B). The cells were stained live (A) or after fixation with 4% PFA and permeabilization with saponin (B–H). The percentages of CD45$^+$MHC II$^{hi}$, CD45$^+$MHC II$^{lo}$ and CD45$^+$MHC II$^-$ cells are shown for unpermeabilized cells (A) and permeabilized cells (B). Percentages of CD45$^+$ cells that express MHC II$^{hi}$, MHC II$^{lo}$ and MHC II$^-$ are indicated in A and B. Bracketed numbers are the percentages of all events within the respective gated sub-populations (A, B and H). (C–H) The expression of CD11b (C), CD32 (D), CD68 (E), CD4 (F), CD11c (G) and CD163 (H) by the CD45$^+$MHC II$^{hi}$ and CD45$^+$MHC II$^{lo/-}$ cells.

The remaining 42% of CD45$^+$ cells were MHC II$^-$ (Fig. 4B). Moreover, there was a population of MHC II$^-$ cells that

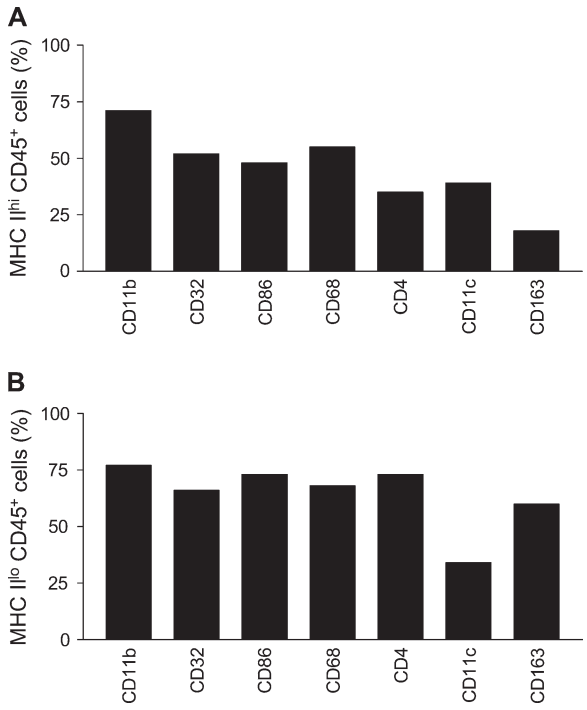


**Fig. 5.** Surface antigen phenotype of permeabilized MHC class II$^{+}$ cells prepared from enzymatic digests of hind paw SRT. Surface antigens were labelled on viable cells, followed by fixation, permeabilization and staining of surface and cytoplasmic MHC class II molecules (see Methods). The histogram shows the proportion of MHC II$^{hi}$ cells (A) and MHC II$^{lo}$ cells (B) that express other surface antigens. The data were obtained by expressing the proportion of MHC II$^{hi}$ and MHC II$^{lo}$ cells that express each marker (Fig. 4C–H), relative to the total CD45$^{+}$MHC II$^{hi}$ and CD45$^{+}$MHC II$^{lo}$ cells (Fig. 4B).

expressed CD11b, CD32, CD68, CD4 and CD163 (Fig. 4C–F and H). These cells also expressed CD45 (see above), allowing the proportions of the CD45$^{+}$MHC II$^{-}$ cells to be calculated as 75% CD11b$^{+}$, 85% CD32$^{+}$, 45% CD68$^{+}$, 60% CD4$^{+}$ and 78% CD163$^{+}$, respectively. Very few CD45$^{+}$MHC II$^{-}$ cells expressed CD11c (Fig. 4G).

We have concluded, therefore, that CD45$^{+}$ cells within the gated population can be divided into three sub-populations. The first is an indeterminate population of MHC II$^{hi}$ cells, the majority of which expresses CD11b, CD32 and/or CD68 and approximately half expresses CD4, CD11c and/or CD86 (Fig. 5A). Only 18% of these indeterminate cells express CD163. In the second population (MHC II$^{lo}$ cells), the majority (60%) expresses CD163 and/or CD11b, CD32, CD86, CD68 and CD4 (Fig. 5B), while one-third of these cells express CD11c. The third population is MHC II$^{-}$ and most of these cells express CD11b, CD32, CD4 and/or CD163. Approximately 50% expresses CD68 and very few express CD11c.

### *Comparison of cells in SRTs with monocytes, macrophages and pseudo-afferent lymph DCs*

Some cells from SRTs (Fig. 6A) expressed MHC class II molecules at levels comparable with mesenteric pseudo-afferent lymph DCs (Fig. 6B). Most pseudo-afferent lymph DCs expressed CD11b at intermediate levels and CD11c at high levels but none expressed CD163. Resident peritoneal macrophages expressed intermediate levels of surface MHC class II and high levels of CD11b (Fig. 6C). Most peritoneal macrophages expressed CD163 weakly but a sub-population expressed levels comparable to some SRT mononuclear cells. No cells were observed to express CD11c. Mononuclear cells in SRTs were also compared with peripheral blood monocytes (Fig. 6D). Most monocytes did not express surface MHC II molecules but a small sub-population expressed these molecules at high levels. Some monocytes expressed levels of CD11b that were similar to pseudo-afferent lymph DCs and SRT mononuclear cells. CD11c was expressed at levels similar to SRT cells but lower than those expressed by most pseudo-afferent lymph DCs. Monocytes expressed CD163 weakly, at levels comparable with those expressed by the majority of SRT mononuclear cells and most peritoneal macrophages.

### *Morphology of mononuclear cell subsets from SRTs*

To correlate morphology and surface antigen phenotype of the CD45$^{+}$ cells, the MHC II$^{hi}$, MHC II$^{lo}$ and MHC II$^{-}$ sub-populations were sorted, using dual-color immunofluorescence. Due to the low yield of sorted cells, re-analysis of the sorted cells by flow cytometry was not feasible. However, Giemsa-stained cytospin preparations of the sorted CD45$^{+}$MHC II$^{hi}$ cells showed small- to medium-sized cells with smooth or ruffled borders, eccentric kidney-shaped nuclei and cytoplasm without vacuoles (Fig. 7A). Most of these cells were stained strongly by the anti-CD11c mAb (Fig. 7B), whereas most did not express CD163 (Fig. 7C). The CD45$^{+}$MHC II$^{-}$ population contained medium to large round cells with simple oval nuclei and cytoplasm containing many vacuoles, giving them a foamy appearance (Fig. 7D). Most of these cells did not express CD11c (Fig. 7E) but were stained strongly by antibody against CD163 (Fig. 7F). The CD45$^{+}$MHC II$^{lo}$ population contained a mixture of the morphologies seen in the MHC II$^{hi}$ and MHC II$^{-}$ populations (data not shown).

### *In vitro differentiation of DCs from SRT cells*

Although some cells from SRTs have an antigenic phenotype consistent with rat DCs (i.e. MHC II$^{+}$, CD11b$^{lo}$, CD32$^{lo}$, CD68$^{+}$, CD11c$^{+}$ and CD163$^{-}$), as described by Talmor *et al.* (23), only a few had the morphology of mature DCs. We asked, therefore, whether some of the indeterminate cells described above have the potential to differentiate into mature DCs *in vitro* in the presence of GM-CSF. Unseparated SRT cells were cultured in CM containing mouse rGM-CSF (500 U ml$^{-1}$) for 24–72 h. The non-adherent cells were then harvested and viable cells were counted. The recovery of viable cells was 40% at 24 h, 33% at 48 h and 40% at 96 h. Giemsa-stained cytospin preparations from cells cultured for 24 h contained large cells with lobulated nuclei that had the morphological features of DCs (Fig. 8A). CD45$^{+}$ cells with veils and/or cytoplasmic processes, irregular-shaped eccentric nuclei and MHC II$^{+}$ perinuclear granules appeared as early as 48 h and increased in number by 72 h. These cells expressed high levels of MHC class II molecules (Fig. 8B). The proportion of MHC II$^{+}$ DCs increased with time in culture (1% at 24 h, 6% at 48 h and 40% at 72 h) and many of them expressed CD11c

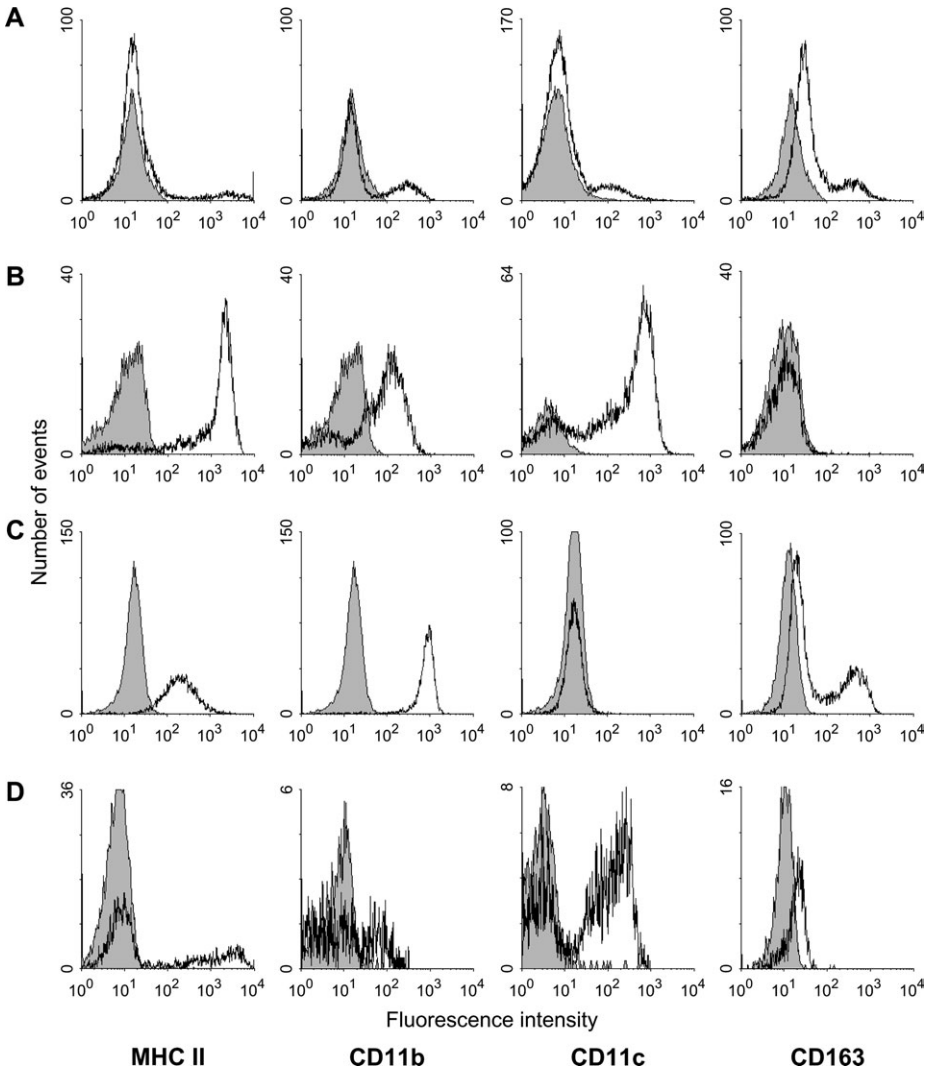


**Fig. 6.** Flow cytometric analysis of surface antigens by cells obtained from enzymatic digestion of hind paw SRT (A), mesenteric pseudo-afferent lymph DCs (B), peritoneal macrophages (C) and blood monocytes (D). Viable cells were labelled to detect MHC II, CD11b, CD11c and CD163 using indirect immunofluorescence. Unfilled histograms, test mAb. Grey-filled histograms, staining by isotype control mAb.

(Fig. 8C). While the macrophage-like cells and the mononuclear cells with smooth or ruffled borders and bean-shaped eccentric nuclei (DC-like cells) both expressed CD32 at the commencement of incubation with GM-CSF, most of the veiled cells that developed in the culture did not express CD32 (Fig. 8D, arrow). In contrast, macrophage-like cells continued to express CD32 throughout the culture period (Fig. 8D, arrowheads). These findings indicated that SRTs contain a population of DC precursors.

### *DC precursors are contained in the CD45$^+$CD163$^-$ subset of SRT cells*

To examine which cells in the CD45$^+$ population from SRTs contain DC precursors, expression of CD163 was used to distinguish cells committed to the macrophage lineage from presumptive DC precursors. CD45$^+$CD163$^+$ and CD45$^+$CD163$^-$ cells were separated by dual fluorochrome cell sorting (Fig. 9A). From $6 \times 10^6$ SRT cells (two pairs of hind paws), the recovery of viable CD163$^-$ and CD163$^+$ cells was ~$4 \times 10^5$ and $3 \times 10^5$, respectively. Of the 103 cells counted in Giemsa-stained cytospin preparations of the CD163$^-$ population, 100 had monocyte-like morphology and 3 had macrophage morphology. In contrast, of the 107 CD163$^+$ cells counted, 103 had macrophage morphology and only 4 had monocyte-like morphology. Examples of the CD163$^-$ population are shown in (Fig. 9B). The cells were small to medium in size, rounded with smooth or ruffled borders and contained eccentric nuclei that were often indented. The majority of these cells expressed surface MHC class II molecules (Fig. 9C) and/or CD11c (Fig. 9D). In contrast, the macrophage-like cells in the CD163$^+$ population were medium to large in size, with simple oval nuclei and smooth borders. The cytoplasm contained abundant cytoplasmic vacuoles (Fig. 9E). Most of the CD163$^+$ cells did not express surface MHC class II molecules (Fig. 9F) or CD11c (Fig. 9G).

The sorted populations of CD163$^+$ and CD163$^-$ cells were cultured in CM containing mouse rGM-CSF (500 U ml$^{-1}$) for 72 h.

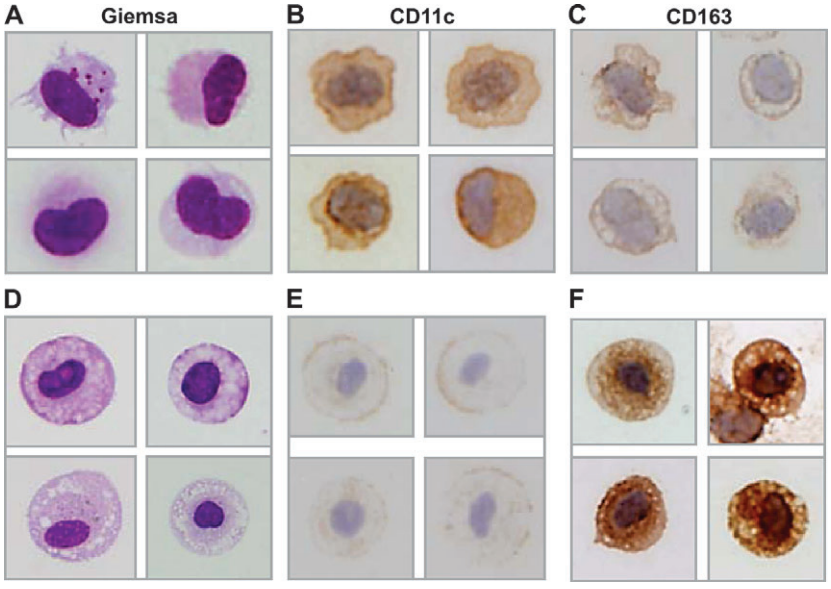


**Fig. 7.** Cytospin preparations of sorted CD45$^+$ MHC class II$^{hi}$ (A–C) and CD45$^+$ MHC class II$^-$ (D–F) cells from enzymatic digests of hind paw SRT. Giemsa-stained preparations of CD45$^+$ MHC class II$^{hi}$ and CD45$^+$ MHC class II$^-$ cells show cells with eccenteric kidney-shaped nuclei and smooth or ruffled borders (A) and round cells with cytoplasmic vacuoles and simple oval nuclei (D), respectively. Indirect immunoperoxidase staining shows that MHC II$^{hi}$ cells (B), but not the MHC II$^-$ cells (E) express CD11c. The MHC II$^{hi}$ cells did not express CD163 (C), while this antigen is expressed strongly by the macrophage-like MHC II$^-$ cells (F). The low level of staining observed in C (MHC II$^{hi}$ cells stained for CD163) and E (MHC II$^-$ cells stained for CD11c) was not significantly different from background staining by the isotype control mAb 1B5 (data not shown). Original magnification: ×60 objective.

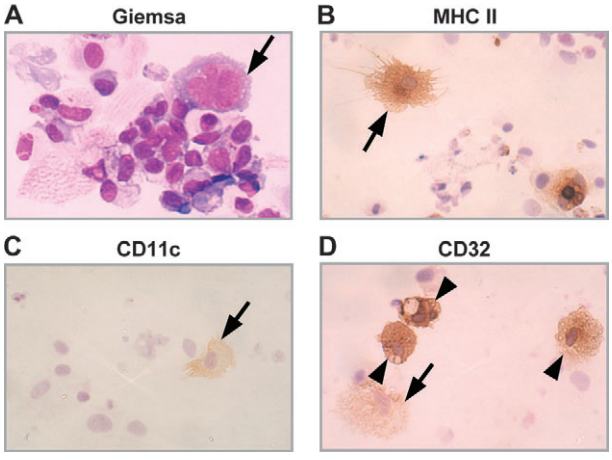


**Fig. 8.** Response of cells from SRTs to culture with GM-CSF. Giemsa-stained cytospin of cell suspension from SRTs cultured with GM-CSF for 24 h, showing a large cell with lobulated nucleus (A, arrow). Indirect immunoperoxidase staining of SRT cells after culture with GM-CSF for 72 h. Veiled cells expressed MHC class II molecules (B, arrow) and CD11c (C, arrow). Veiled cells (arrow) expressed CD32 weakly, while macrophage-like cells (arrowheads) were stained strongly (D). Original magnification: ×165.

The yields of viable cells after culture with GM-CSF for 72 h were ~16 and 25% of the starting numbers, respectively. Giemsa-stained cytospin preparations of non-adherent cells from cultured CD163$^-$ cells revealed small clusters composed of three to six cells. Of the 127 cells counted, 33 (25%) had veiled morphology, including cytoplasmic processes and irregularly shaped eccentric nuclei (Fig. 10A). There were very few macrophage-like cells. Most of the cells expressed MHC class II molecules (Fig. 10B) and CD11c (Fig. 10C). Compared with occasional macrophage-like cells in these cultures, expression of CD32 was weak or absent on the DC-like cells (Fig. 10D). The few macrophage-like cells expressed CD32 strongly (Fig. 10D) but they did not express either MHC class II molecules or CD11c (data not shown).

Giemsa-stained cytospin preparations of sorted CD163$^+$ cells that had been cultured with GM-CSF for 72 h revealed mainly macrophage-like cells with oval-shape nuclei (Fig. 10E). Of the 182 cells counted in the Giemsa-stained cytospin preparation, only 3 had veiled morphology (~2%). The majority did not express MHC class II molecules, although there was an increase in the proportion expressing MHC class II molecules (Fig. 10F), relative to the starting population of sorted cells (increased from 1 to 6%, respectively). The macrophage-like cells expressed CD32 but did not express CD11c (data not shown). No staining was observed when preparations were stained with the isotype-matched negative control mAb (Fig. 10G).

Taken together, the results depicted in Fig. 10 show that the CD45$^+$CD163$^-$ subset is enriched in precursors that differentiate into cells with typical DC morphology and phenotype in the presence of GM-CSF.

### *Macrophage-like cells in SRTs resemble type A synoviocytes*

In order to identify a source of the macrophage-like cells in SRTs, we compared them with synoviocytes obtained by lavage of rat knee joints with collagenase-containing medium. Geimsa-stained cytospin preparations of lavage cells revealed cells with

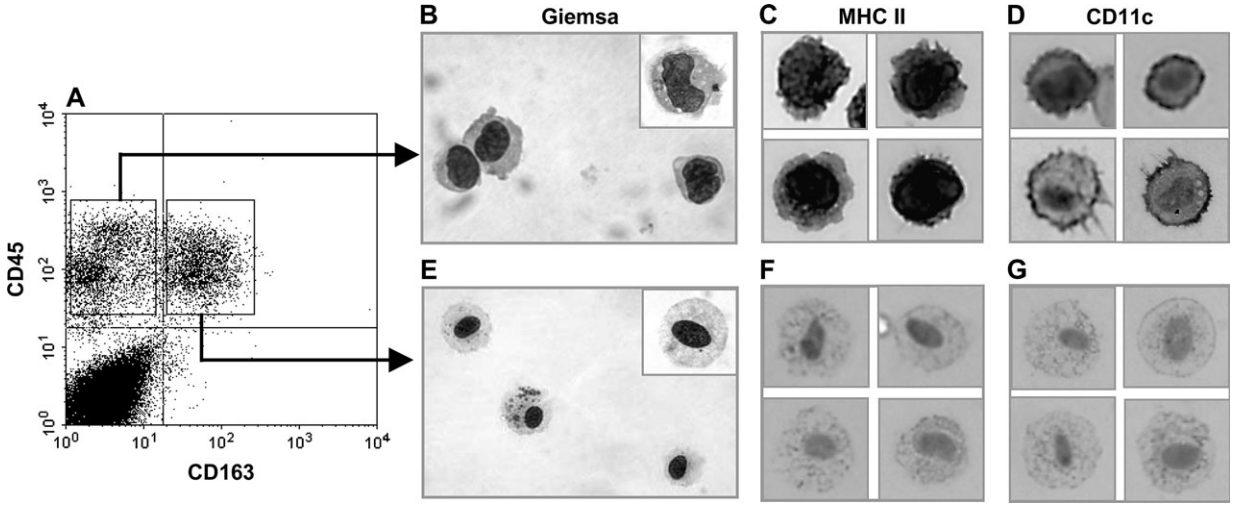


**Fig. 9.** Separation of CD45$^+$CD163$^-$ and CD45$^+$ CD163$^+$ cells from enzymatic digests of hind paw SRT, using FACS. Dual-colour immunofluorescence demonstrates CD45$^+$CD163$^-$ and CD45$^+$ CD163$^+$ sub-populations (A). Cytospin preparations of CD45$^+$CD163$^-$ (B–D) and CD45$^+$CD163$^+$ (E–G) sub-populations purified by cell sorting. Giemsa staining of the CD45$^+$ CD163$^-$ sub-population shows small to medium cells with smooth or ruffled borders and eccentric indented nuclei (B and inset). Indirect immunoperoxidase staining shows that these cells express MHC class II (C) and CD11c (D). Giemsa-stained preparations of the CD45$^+$ CD163$^+$ sub-population show macrophage-like cells (E and inset). Many of these cells did not express MHC class II (F) and none expressed CD11c (G). Original magnifications: (B, E) ×165; insets ×412; (C, D, F, G) ×60 objective.

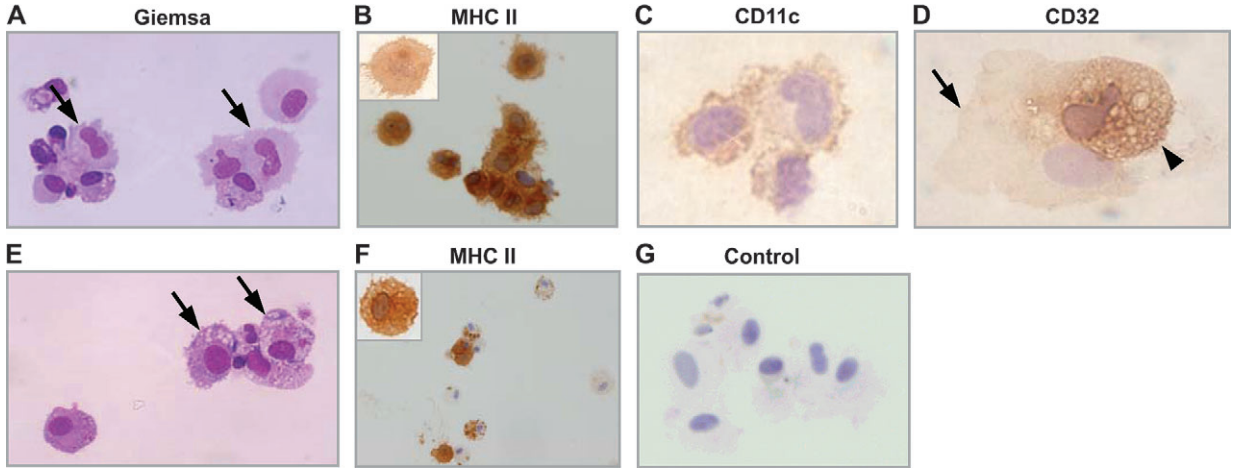


**Fig. 10.** Cytospin preparations of CD45$^+$CD163$^-$ (A–D) and CD45$^+$CD163$^+$ (E–G) cells sorted from SRTs and cultured for 72 h in the presence of GM-CSF. CD45$^+$CD163$^-$ cultured cells stained with Giemsa (A) show small clusters of cells, some of which have veiled morphology (arrows). Indirect immunoperoxidase staining shows that the veiled cells express MHC class II molecules (B) and CD11c (C). Expression of CD32 (D) by these cells (arrow) is weak, compared with that of a minority population of macrophage-like cells (arrowhead). Cultured CD45$^+$CD163$^+$ cells stained with Giemsa (E) contain predominance of macrophage-like cells (arrows). Most of the macrophage-like cells do not express MHC class II molecules (F) but some express moderate levels (inset). Negative isotype control (G). Original magnifications: (A, E) ×165; (B, F) ×40 objective; insets ×412; (C, D) ×412; (G) ×60 objective.

macrophage morphology that had foamy cytoplasm and centrally placed round or oval nuclei (Fig. 11A). Immunoperoxidase staining of these preparations showed that all of the cells with macrophage morphology expressed CD45 (data not shown) and that most of them expressed CD11b (Fig. 11B), CD4 (data not shown) and CD163 (Fig. 11C). Expression of MHC class II molecules was heterogeneous, with some cells stained strongly by the anti-MHC class II mAb OX6, while others were either stained weakly or were unstained (Fig. 11D). The macrophage-like cells did not express CD11c (data not shown). These cells resemble, therefore, the macrophage-like synovial lining cells (type A) that have been shown to express CD68, CD163 and CD4 antigens in sections of normal rat knee joints (24). Cells with ruffled borders and eccentric kidney-shaped nuclei were rare in the lavage fluid. The CD45$^-$ cells that were present were round, with oval or round nuclei and without cytoplasmic vacuoles (data not shown). The latter probably represent type B synoviocytes (25).

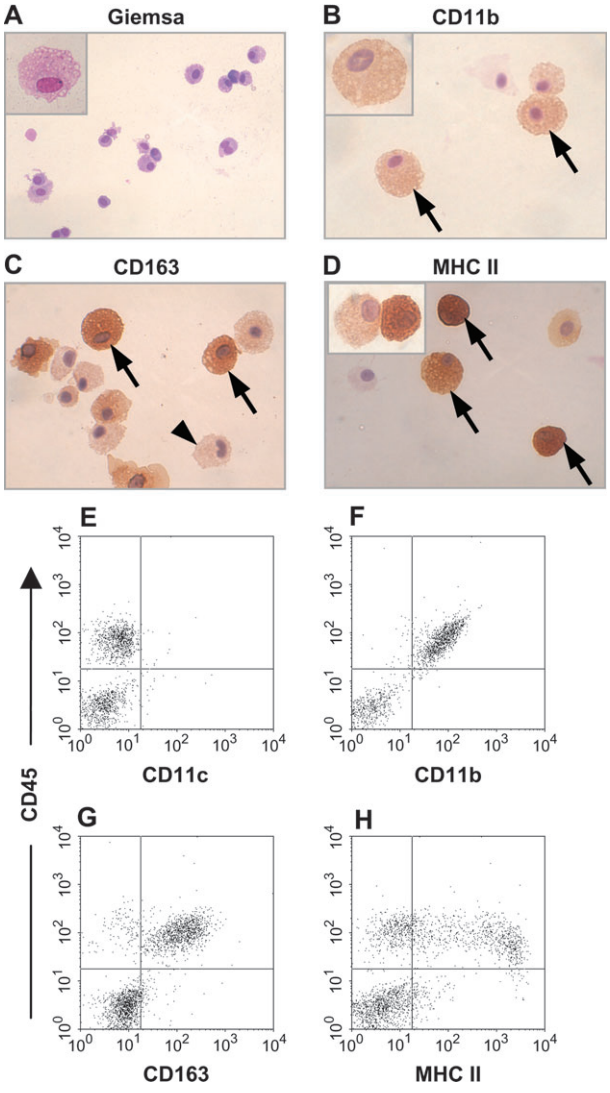


**Fig. 11.** Studies on synoviocytes obtained by lavage of knee joints. Cytospin preparation of synoviocytes (A–D). Giemsa staining (A). Most of the cells are large with foamy cytoplasm and eccentric oval or round nuclei (A, and inset). Indirect immunoperoxidase staining (B–D). Most macrophage-like cells (arrows) express CD11b (B) and CD163 (C). Occasional cells with eccentric and kidney-shape nuclei do not express CD163 (C, arrowhead). Expression of MHC class II molecules by macrophages is heterogeneous (D) with some expressing high levels (arrows and inset) and others staining weakly or unstained (inset). Flow cytometric analysis of surface antigens expressed by synoviocytes (E–H). Cells were gated on the basis of the FSC and SSC characteristics of mesenteric pseudo-afferent lymph DCs (see Fig. 2B). Dual-color staining for CD45 and CD11c (E), CD11b (F), CD163 (G) and MHC II molecules (H). Original magnifications: (A) ×83, inset ×165; (B) ×165, inset ×412; (C) ×165; (D) ×165, inset ×412.

Lavage cells were analysed further by flow cytometry, using the gate of FSC and SSC set for pseudo-afferent lymph DCs (Fig. 2B). Approximately 40% of the synoviocytes within this gate expressed CD45 (data not shown). Dual fluorochrome studies revealed that <5% of CD45$^+$ synoviocytes expressed CD11c (Fig. 11E), almost all expressed CD11b (Fig. 11F) and ~90% expressed CD163 (Fig. 11G). Approximately 60% of the CD45$^+$ cells expressed MHC class II molecules (Fig. 11H).

## Discussion

Several studies suggest that DCs play a role in the pathogenesis of RA (7, 8, 10, 11, 26–28). Recently, it has been shown that bone marrow-derived DCs pulsed with type 2 collagen (CL) can induce arthritis in DBA/1 mice (29). Furthermore, *ex vivo*-differentiated HLA-DR4 human DCs and macrophages can present antigens from rheumatoid synovial fluid to HLA-DR4-restricted mouse T hybridoma cells specific for either type 2 CL or human cartilage gp39 peptides (12). These findings suggest that the presentation of synovial auto-antigens by local APCs to T lymphocytes could orchestrate the inflammation that is characteristic of RA. It is of interest, therefore, that in the rat AA model of RA (30), the disease can be transferred adoptively to naive syngeneic recipients with purified activated CD4$^+$ T cells from arthritic donors (16, 31), independently of any requirement for co-transfer of donor-derived APC or adjuvant-derived antigen (16). This suggests that arthritogenic T cells recruited to normal synovium respond to processed auto-antigens associated with resident APCs.

To investigate the nature of the APCs in normal synovium, we have isolated cells by enzymatic digestion from rat tissues that are rich in synovial linings of joints and tendon sheaths. Flow cytometry showed that ~25% of the cells within a gate designed to include large mononuclear cells expressed CD45. Approximately 60% of these CD45$^+$ cells expressed surface and/or cytoplasmic MHC class II molecules (Fig. 4B). One pair of normal rat hind paws contained ~$7.5 \times 10^5$ CD45$^+$ MHC class II$^+$ cells and of these approximately one-third expressed only cytoplasmic MHC class II molecules (extra MHC class II$^+$ cells revealed by permeabilization). Based on the expression of MHC class II molecules (surface and/or cytoplasmic), three subsets of putative APCs were identified: CD45$^+$MHC II$^{hi}$, CD45$^+$MHC II$^{lo}$ and CD45$^+$MHC II$^-$. The size of the MHC II$^{hi}$ sub-population was increased 6% by permeabilization, and together with a small increase in the intensity of staining, this suggests that some of the cells have an intracellular compartment of MHC class II molecules. In contrast, some MHC II$^{lo}$ cells were detected only after permeabilization, indicating that in these cells MHC class II molecules are mainly intracellular.

The CD45$^+$MHC II$^+$ cells were examined for expression of other markers, including the key lineage markers CD11c and CD163. The MHC II$^{hi}$ cells have the phenotype CD11c$^+$, CD163$^-$ and CD68$^+$, similar to that of rat DCs (23). In cytospin preparations of unsorted SRT cells, the MHC II$^+$ population varied in morphology from small- to medium-sized cells, with oval or slightly indented eccentric nuclei, to large cells with vacuolated cytoplasm and macrophage-like morphology. When sorted, the CD45$^+$MHC II$^{hi}$ cells were found to consist mainly of small- to medium-size cells, with either smooth or ruffled borders, eccentric indented nuclei and non-vacuolated cytoplasm. Most of the MHC II$^+$ cells did not express CD163 and of the MHC II$^{hi}$ cells, most expressed CD11b, CD32 and/or the CD68 antigen, while ~40% expressed CD4, CD86 and/or CD11c.

We calculate that ~18% of the MHC II$^{hi}$ cells express CD163, indicating that they are macrophages with up-regulated expression of MHC class II molecules. When

compared with other cell types, most of the other MHC II$^{hi}$ cells resemble monocytes, both morphologically and phenotypically (32, 33), with the exception that typical blood monocytes express low levels of CD163 and most do not express surface MHC class II molecules (Fig. 6D; 32, 33). However, the proportion of MHC II$^{+}$ monocytes has been found to increase dramatically in response to an ongoing immune response or to the administration of IFNγ (33). The CD45$^{+}$MHC II$^{hi}$ cells from SRTs also differ from rat monocytes (32, 33) by expressing CD86, while only a sub-population expresses CD4. Nevertheless, it is noteworthy that culture of rat monocytes with GM-CSF plus IL-4 up-regulates expression of CD86 (32), while exposure to IFNγ *in vivo* has been shown to down-regulate expression of CD4 (33). Furthermore, we have found that 80% of monocytes from normal DA rats express low levels of CD86 (Gillman, Moghaddami and Mayrhofer, unpublished results), in contrast to the findings of Richters *et al.* (32) in Lewis rats.

It appears, therefore, that normal SRTs contain a population of CD45$^{+}$MHC II$^{hi}$ cells that shares many characteristics with blood monocytes and in particular with monocytes that have been activated by either an ongoing immune response (33) or by exposure *in vivo* or *in vitro* to certain pro-inflammatory cytokines (32, 33). However, the CD45$^{+}$MHC II$^{hi}$ cells also resemble DCs from pseudo-afferent lymph (Fig. 6). They express similar levels of surface MHC class II molecules and CD11b but pseudo-afferent lymph DCs express higher levels of CD11c than either blood monocytes or CD45$^{+}$MHC II$^{hi}$ cells. Resident peritoneal macrophages were least similar to the CD45$^{+}$MHC II$^{hi}$ cells from SRTs. They expressed uniformly low levels of MHC II molecules, they did not express CD11c, expression of CD11b was high and a sub-population expressed high levels of CD163. In view of the extensive overlap of differentiation markers between the CD45$^{+}$MHC II$^{hi}$ cells, monocytes, macrophages and DCs, it seems appropriate at this stage to consider most of the CD45$^{+}$MHC II$^{hi}$ population as cells that are indeterminate in both origin and potential for differentiation, notwithstanding recent evidence that blood monocytes can differentiate into DCs *in vitro* (34) and *in vivo* (35) and that at least some resident macrophages have this potential also (36). Significantly, the indeterminate cells differ from the orthodox description of *in vitro*-derived immature DCs in rat (23) and other species (1, 3) by expressing high levels of surface MHC class II molecules, while only a sub-population expresses CD11c.

The CD45$^{+}$MHC II$^{lo}$ and CD45$^{+}$MHC II$^{-}$ populations are complex (Figs 4B and 5B), because they contain significant proportions of cells that express CD163 at low (presumptive monocytes) to high (macrophages) levels (Fig. 4H). Of the total surface-labelled SRTs, the proportions of CD163$^{+}$ cells in the CD45$^{+}$MHC II$^{hi}$, CD45$^{+}$MHC II$^{lo}$ and CD45$^{+}$MHC II$^{-}$ subsets were 1.5, 2.3 and 15%, respectively (data not shown), while the respective proportions in permeabilized cells were 2, 4.3 and 10.1% (Fig. 4H). The changes in proportions of MHC II$^{-}$ and MHC II$^{lo}$ cells following permeabilization indicate that some CD163$^{+}$ cells have cytoplasmic MHC class II molecules. It can be seen that the proportion of MHC II$^{lo}$ CD163$^{+}$ cells is equivalent to the proportion of MHC II$^{lo}$ CD45$^{+}$ cells (Fig. 4A), showing that this proportion contains insignificant numbers of indeterminate cells. However, in permeabilized SRT cells, it can be calculated that of the CD45$^{+}$MHC II$^{lo}$ cells (which constitute 7.1% of the total gated SRTs, Fig. 4B), only 4.3% (Fig. 4H) express CD163 (presumptive monocytes or macrophages), while the remaining 2.8% do not express CD163 (indeterminate cells). Thus, permeabilization has revealed a small population of surface MHC II$^{-}$ CD45$^{+}$ cells that contain cytoplasmic MHC class II molecules. Interestingly, permeabilization resulted in loss of a similar proportion of surface MHC$^{-}$ CD11c$^{+}$ cells (data not shown) and the appearance of MHC II$^{lo}$ CD11c$^{+}$ cells (Fig. 4G). These cells are possible conterparts of the immature monocyte-derived DCs that have been described *in vitro* (1, 2).

We used GM-CSF to investigate whether indeterminate cells can indeed differentiate into mature DCs (23, 37). The large MHC II$^{+}$ CD11c$^{+}$ cells that appeared in GM-CSF-stimulated cultures of crude SRT cells had down-regulated CD32, a molecule expressed by immature antigen capturing DCs (34) and down-regulated by splenic DCs, Langerhans cells and DCs derived by culture of blood monocytes with GM-CSF (34, 38, 39). Further investigation indicated that the CD45$^{+}$CD163$^{-}$ sub-population in SRTs had the greatest ability to produce mature DCs in response to GM-CSF. Higher expression of MHC class II molecules by the CD45$^{+}$CD163$^{+}$ cells after culture with GM-CSF was consistent with the reported behaviour of rat monocytes and macrophages (33, 40) and like inflammatory macrophages, these cells appear unable to differentiate into DCs when exposed to pro-inflammatory cytokines (36). It appears that DCs produced from human synovial fluid in response to IL-4 and GM-CSF may have arisen from indeterminate cells rather than from macrophages, as suggested by Komi *et al.* (41).

In conclusion, we have characterized mononuclear cells of hematopoietic origin in normal SRTs. Some of these cells have the morphology and immunophenotype of macrophages and they resemble type A synoviocytes. They probably originate from monocytes that migrate into the tissues constitutively and differentiate under inductive influences that may be located, at least in part, at the synovial interface with the joint space. The population of indeterminate cells has similarities to both monocytes and DCs, raising questions as to their origin. Indeterminate cells could arise either from monocytes or from 'immature DCs' in blood (42). This distinction is difficult to assess *in vivo*, because the monocyte immunophenotype can change in response to the prevailing cytokine environment (33) and also to the process of extravasation (35). Thus, the presumptive DC precursors in blood could themselves represent monocytes that have undergone 'precocious' development, as suggested by the work of Geissmann *et al.* (43). Our findings are compatible with either origin, especially as a subset of monocytes has been described that can enter non-inflamed tissues and differentiate into DCs (43). The surface MHC II$^{-}$ indeterminate cells appear to conform to the description of monocyte-derived immature tissue DCs (1, 2), but the majority of the indeterminate cells expresses high levels of MHC class II molecules. This raises important questions about the state of activation of the MHC II$^{hi}$ cells, their place in the 'life cycle' of DCs in normal tissues and their function in normal homeostasis of the immune system. Further studies are required to define the precise cellular origins of DCs and macrophages in SRTs, the factors responsible for

the differentiation of these cells in the synovial microenvironment and the potential of the cell types that we have defined in presentation of auto-antigens in normal synovium and during the pathogenesis of polyarthritis.

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## Abbreviations

| | |
|---|---|
| AA | adjuvant-induced arthritis |
| APC | antigen-presenting cell |
| CL | collagen |
| CM | complete medium |
| DA | Dark Agouti |
| DC | dendritic cell |
| FSC | forward scatter |
| GM-CSF | granulocyte macrophage colony-stimulating factor |
| IMVS | Institute of Medical and Veterinary Science |
| RA | rheumatoid arthritis |
| rGM-CSF | recombinant granulocyte macrophage colony-stimulating factor |
| SRT | synovium-rich tissue |
| SSC | side scatter |

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