

Dendritic cells with a potent accessory activity are present in human exudative malignant pleural effusions

M. Gjomarkaj*, E. Pace*, M. Melis*, M. Spatafora**, D. D'Amico*, G.B. Toews+

Dendritic cells with a potent accessory activity are present in human exudative malignant pleural effusions. M. Gjomarkaj, E. Pace, M. Melis, M. Spatafora, D. D'Amico, G.B. Toews. ©ERS Journals Ltd 1997.

ABSTRACT: Dendritic cells are human leucocyte antigen (HLA)-DR positive accessory cells, that play a critical role in the development of cell-mediated immune reactions. Since the pleural space is frequently involved in cell-mediated immune reactions, we sought to isolate dendritic cells from pleural fluid.

Pleural effusion mononuclear cells (PEMCs) were obtained by Ficoll centrifugation of exudative pleural effusions recovered from 19 patients with malignant pleurisy. After double-step adherence, firmly-adherent mononuclear cells (FAMs) and loosely-adherent mononuclear cells (LAMs) were recovered. The latter cells were centrifuged on a bovine plasma albumin gradient to obtain a loosely-adherent low density fraction. Nonadherent cells were rosetted with sheep red blood cells (SRBC) to obtain a nonadherent nonrosetting (NANR) cell fraction. Mitomycin C-treated PEMCs, NANRs, FAMs, and LAMs served as stimulatory cells in mixed leucocyte reaction experiments. ³H-thymidine incorporation by purified normal allogeneic blood T-lymphocytes was assessed as an index of accessory cell function. The phenotype of NANR, FAM and LAM cells was characterized using single and double stainings with a panel of monoclonal antibodies (MoAbs).

Accessory cell (AC) activity (counts per minute (cpm) $\times 10^3(\pm SE)$; 2.5×10^4 AC-well⁻¹) of LAM (148 ± 24) and NANR (108.4 ± 11.2) was greater than that observed for FAM (59.3 ± 9.4) and for unfractionated PEMC (13.8 ± 4.9). The FAM fraction was virtually entirely composed of CD68+ HLA-DR+ mononuclear phagocytes. NANR and LAM contained $51 \pm 12\%$ and $65 \pm 6\%$ HLA-DR+ cells, respectively, and most HLA-DR positive cells were negative for CD3, CD19, and CD68, markers for T-, B-lymphocytes and mononuclear phagocytes. Moreover, both NANR and LAM fractions contained significant numbers of cells bearing the RFD1 surface marker, expressed on dendritic cells.

These results suggest that dendritic cells are present in exudative pleural effusions, and that they may be involved in the development of cell-mediated immune reactions in the pleural space.

Eur Respir J 1997; 10: 592–597.

The development of exudative malignant pleural effusions is a common event in patients with advanced bronchogenic carcinomas. Whilst, in some instances, the accumulation of fluid results from the impairment of lymphatic drainage from the pleural space, in other instances, the metastatic involvement of the pleural surfaces leads to the development of compartmentalized inflammatory and immune reactions [1]. Exudative malignant pleural effusions contain increased proportions of T-lymphocytes (mostly of the CD4+ helper/inducer phenotype), compared to autologous peripheral blood and transudative effusions [2]. In addition, T-lymphocytes isolated from exudative malignant pleural effusions, but not autologous peripheral blood T-lymphocytes, vigorously proliferate in response to autologous non-T-cells [3]. These findings suggest that reactive T-lymphocytes accumulate in the pleural space and that local proliferation of reactive T-lymphocytes may be the expression of compartmentalized inflammatory and immune reactions against tumour antigens.

Accessory cells are heterogeneous human leucocyte antigen (HLA)-DR-positive cells, capable of stimulating the proliferation of autologous or allogeneic T-lymphocytes in the presence or absence of a specific antigen [4]. The cell type responsible for accessory cell activity in the pleural space has not been determined and, in particular, it is unknown whether dendritic cells, the most powerful accessory cells described so far [5], are present in exudative malignant pleural effusions.

In order to characterize the phenotype of the accessory cells and to ascertain whether dendritic cells are present in exudative malignant pleural effusions, we used a cell fractionation protocol that has previously allowed the isolation of enriched accessory cell fractions from peripheral blood and parenchymal organs [6, 7]. We tested the accessory activity of different cell fractions enriched from exudative malignant pleural effusions, and evaluated the surface phenotype of these fractions with a panel of monoclonal antibodies (MoAbs) by immunohistochemical staining and flow cytometry.

*Istituto di Fisiopatologia Respiratoria, Consiglio Nazionale delle Ricerche, Palermo, Italy. **Istituto di Medicina Generale e Pneumologia, Università degli Studi, Palermo, Italy. +Division of Pulmonary and Critical Care Medicine, Dept of Internal Medicine, University of Michigan, Ann Arbor, MI, USA.

Correspondence: M. Gjomarkaj
CNR Istituto di Fisiopatologia Respiratoria
Via Trabucco 180
90146 Palermo
Italy

Keywords: Antigen-presenting cells
dendritic cells
pleural effusion

Received: July 31 1995
Accepted after revision October 31 1996

This work was supported by the Italian National Research Council.

This study suggests that dendritic cells are present in exudative malignant pleural effusions and that these cells are potent accessory cells.

Materials and methods

Isolation of cells from pleural effusions

Exudative malignant pleural effusions were collected by therapeutic thoracentesis in sterile polyethylene bags containing heparin (10–20 international units (IU)·mL⁻¹) from 19 patients (13 males and 6 females aged 46–84 yrs) with primary bronchogenic carcinoma. All effusions contained tumour cells and were exudative, as confirmed by conventional biochemical parameters, *i.e.*: 1) pleural fluid protein/serum protein ratio >0.5; 2) pleural fluid lactate dehydrogenase (LDH)/serum LDH >0.6; and 3) pleural fluid LDH >2/3 of the upper normal limit for normal serum LDH [8].

After filtration through double layers of sterile surgical gauze, the pleural fluid was centrifuged (300×g for 10 min) in 50 mL tubes (Sterilin, Teddington, UK). The cells recovered were washed three times in Hank's balanced salt solution (HBSS) and resuspended in complete medium (RPMI 1640 + 10% foetal calf serum + 25 mM hydroxyethylpiperazine ethanesulphonic acid (HEPES) + 2 mM glutamine + 100 ng·mL⁻¹ penicillin + 100 ng·mL⁻¹ streptomycin) (all from Gibco, Paisley, UK). Pleural effusion mononuclear cells (PEMCs) were obtained by Ficoll-Hypaque (Pharmacia, Uppsala, Sweden) centrifugation (400×g for 25 min), and resuspended in complete medium at a concentration of 10×10⁶ cells·mL⁻¹.

Ten to fifteen millilitre aliquots of PEMC suspensions were allowed to adhere to plastic dishes (Sterilin) at 37°C, 5% CO₂. After 2 h, nonadherent cells were collected with five rinses of HBSS, washed three times with HBSS, and rosetted at 4°C with 2-aminoethylisothiuronium bromide (AET)-treated sheep red blood cells (SRBC). After 14 h, the cells were spun through Ficoll-Hypaque and nonrosetting cells were collected from the interface of the gradient, washed three times with HBSS, and resuspended in complete medium. These cells will, hereafter, be referred to as nonadherent nonrosetting mononuclear cells (NANR).

The adherent cells were cultured (37°C, 5% CO₂) for an additional 18 hours. The cells that remained adherent to dishes after 18 h were recovered with a rubber policeman, washed three times with HBSS, and resuspended in complete medium. These cells will, hereafter, be referred to as firmly adherent mononuclear cells (FAMs).

The cells that failed to remain adherent to dishes after 18 h were collected with five rinses of warm (37°C) HBSS, resuspended in light bovine plasma albumin (Sigma, St. Louis, MO, USA) solution (density 1.048 g·mL⁻¹) and carefully layered on the top of dense bovine plasma albumin solution (density 1.085 g·mL⁻¹). After centrifugation at 10,000×g (30 min at 4°C), the cells located at the interface were harvested, washed three times with HBSS, and resuspended in complete medium. These cells will, hereafter, be referred to as loosely adherent low density mononuclear cells (LAMs).

PEMCs, NANRs, FAMs, and LAMs were resuspended in complete medium at a concentration of 2×10⁶ cells·mL⁻¹ for subsequent use in mixed leucocyte reaction experiments and characterization with monoclonal antibodies (MoAbs).

Isolation of peripheral blood T-lymphocytes

Mononuclear cells were obtained from normal donor peripheral blood buffy-coats by means of a Ficoll-Hypaque centrifugation, washed three times in HBSS, resuspended in complete medium and counted. Lymphocyte-enriched fractions were obtained from mononuclear cells by fractionation on a one-step Percoll (Pharmacia) gradient centrifugation, as described previously [9]. The lymphocyte-enriched fraction was incubated for 1 h (37°C, 5% CO₂) on plastic dishes to deplete adherent cells. Nonadherent cells were collected after gently washing the plates with HBSS, centrifuged, resuspended in RPMI 1640 + 20% AB human serum, and rosetted with AET-treated SRBC. After overnight incubation at 4°C, the cells were spun through a Ficoll-Hypaque gradient and rosetting cells were collected at the bottom of the tube. The rosetting cells were treated with ammonium chloride solution to lyse SRBC, resuspended in complete medium, and passed through a nylon wool column to further purify the T-cell population (1 h at 37°C, 5% CO₂) [10]. The recovered T-cell fraction was >95% CD3+.

Mixed leucocyte reaction

PEMCs, NANRs, FAMs and LAMs were incubated (37°C, 5% CO₂) with mitomycin C (25 µg·mL⁻¹; Sigma). After 20 min, cells were washed three times in complete medium and resuspended at a concentration of 5×10⁵ cells·mL⁻¹. Purified T-lymphocytes (5×10⁵) were cultured alone or with various numbers of PEMCs, NANRs, FAMs and LAMs. Cultures were performed in quadruplicate in round-bottomed microtitre plates (Sterilin) in 0.2 mL of complete medium. Cultures were incubated for 6 days (37°C, 5% CO₂) and 18 h before termination, 0.5 µCi·well⁻¹ of 3H-thymidine (5.0 Ci·mM⁻¹; Amersham Corp., UK) was added to the cultures. Cells were collected with an automated cell harvester (Flow, Laboratories, Norway), and counted in a scintillation counter (LS 1801; Beckman Instruments, Irvine, CA, USA). Data are expressed as mean counts per minute (cpm) of triplicate cultures.

Characterization of NANR, LAM and FAM fractions with MoAbs

The following MoAbs were used in this study: Dako-macrophage (anti-CD68, from the cell line EBM 11; Dakopatts, Glostrup, Denmark) specific for mononuclear phagocytes; UCHM1 (anti-CD14, from the homonymous cell line; Sera-lab, Crawley Down, Sussex, UK) specific for peripheral blood monocytes and granulocytes; Leu4 (anti-CD3, from the cell line SK 7; Becton Dickinson, Mountain View, CA, USA) specific for T-lymphocytes; Dako CD19 (anti-CD19, from the cell line HD37;

Dakopatts), and Dako L26 (anti-CD20, from the homonymous cell line; Dakopatts) both specific for B-lymphocytes; Dako cytokeratin (anti-cytokeratins 6 and 18, from the cell line LP 34; Dakopatts) specific for epithelial and mesothelial cells; anti-HLA-DR (from the cell line L 243; Dakopatts); Ortho-mune OK-NK (anti-CD16; Ortho Pharmaceuticals, Raritan, NJ, USA), specific for natural killer (NK) cells and neutrophils. Additional stainings were made with the anti-RFD1 MoAb (kindly provided by L.W. Poulter, Royal Free Hospital, London UK); in previous studies this molecule was shown to be expressed on dendritic cells and on some B-cells [11].

Cytopreparations of the NANRs, FAMs and LAMs were made, fixed in cold acetone (5 min), and allowed to air dry for 2 h. Immunocytochemical stainings were made using the immunalkaline phosphatase antialkaline phosphatase (APAAP) method, as described in detail previously [12]. The alkaline phosphatase reaction product was visualized by using as substrate a Naphthol AS-BI-fast red (Sigma) solution. For each specimen, a minimum of 300 cells·slide⁻¹ was counted with a ×40 objective. Negative and positive control slides were included in each staining session by using an irrelevant MoAb (anti-smooth muscle; Ortho Pharmaceuticals, Raritan, NJ, USA) or the antileucocyte common antigen MoAb (Dako LC), respectively.

NANR and LAM cells were immunostained by double immunofluorescence technique, as described previously, with minor modifications [13]. Briefly, cells were resuspended in complete medium at a concentration of 10⁶·mL⁻¹ and 1 mL aliquots were incubated in the dark (30 min at 4°C) with 10 ng·mL⁻¹ of phycoerythrin (PE)-conjugated anti-HLA-DR MoAb (Becton-Dickinson), and one of either fluorescein-isothiocyanate (FITC)-conjugated anti-CD3 (Ortho), or anti-CD19 (Dakopatts) MoAbs. The double stainings with PE-conjugated anti-HLA-DR MoAb and anti-CD68 (Dakopatts) MoAb (subclass immunoglobulin G1 (IgG1)) were made by an indirect immunofluorescence technique, using a FITC-conjugated rabbit antimouse MoAb (F(ab')₂ fragment of IgG) (Dakopatts); this technique was used because a FITC-conjugated anti-CD68 MoAb was not available. Control FITC-conjugated and PE-conjugated MoAbs of the appropriate isotype, but of irrelevant antigen specificity, were used to evaluate nonspecific binding. After three washes (300×g for 10 min) with phosphate buffer solution (PBS) containing 2% bovine serum albumin, cells were analysed on an Ortho Cyturon flow cytometer, setting laser excitation at 488 nm. Specific binding of MoAbs was determined by subtraction of nonspecific binding of the respective control MoAb.

Results

Cell recovery in PEMC, FAM, LAM and NANR fractions

The volume of pleural fluid recovered was variable (1300±496.6 mL; range 800–1900 mL). After filtration and Ficoll-Hypaque centrifugation 153.2±169.3×10⁶ cells·mL⁻¹ PEMCs were recovered. After subsequent purification procedures, the total counts of FAM, LAM and NANR cells were 4.4±2.3, 4.2±2.9 and 12.2±2.2 ×10⁶ cells·mL⁻¹, respectively.

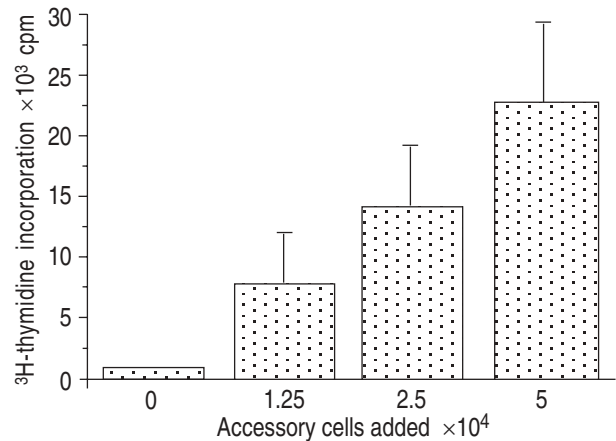


Fig. 1. – Accessory function of pleural effusion mononuclear cells (PEMC). PEMCs were isolated as described in Materials and Methods and co-incubated for 6 days with allogeneic normal peripheral T-lymphocytes. The ³H-thymidine incorporation into proliferating T-lymphocytes was determined as an index of accessory activity of PEMC. ³H-thymidine incorporation in cultures containing PEMC only was always less than 500 counts per minute (cpm). Data represent mean±SD of six patients.

Accessory function of enriched cell populations

The ability of PEMCs to function as accessory cells for allogeneic T-lymphocytes was tested by utilizing a mixed leucocyte reaction (fig. 1). PEMCs stimulated a mixed leucocyte reaction in a dose-dependent manner, confirming that efficient accessory cells are present in exudative malignant pleural effusions.

LAM and NANR fractions exerted a very potent accessory function at all concentrations in a dose-dependent manner (fig. 2). In contrast, FAMs exerted a weaker accessory activity, as compared to NANR and LAM fractions, and decreasing levels of T-cell proliferation were observed at the highest stimulatory:responder cell ratios.

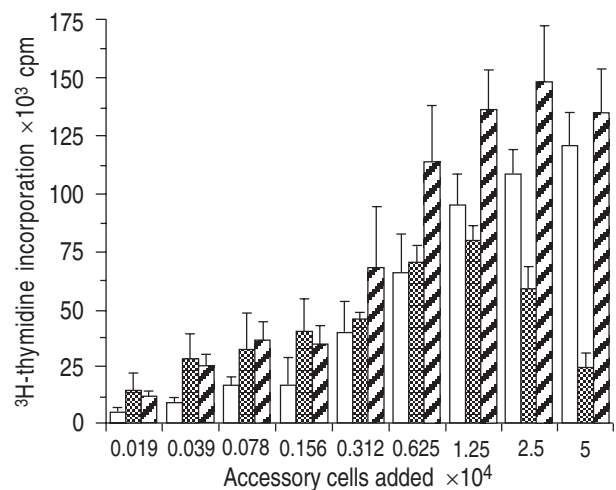


Fig. 2. – Accessory function of NANRs, LAMs and FAMs. NANRs, LAMs and FAMs were isolated as described in Materials and Methods and co-incubated for 6 days with allogeneic normal peripheral T-lymphocytes. The incorporation of ³H-thymidine into proliferating T-lymphocytes was determined as an index of accessory activity of NANRs, LAMs and FAMs. ³H-thymidine incorporation in cultures containing stimulatory or responder cells only was always less than 500 counts per minute (cpm). Data represent mean±SD of five patients. □: NANR; ▨: FAM; ■: LAM. NANR: nonadherent nonrosetting cells; LAM: loosely-adherent mononuclear cells; FAM: firmly-adherent mononuclear cells.

Phenotypic characterization of NANRs, LAMs and FAMs

A panel of MoAbs was used to characterize the phenotype of FAMs, LAMs and NANRs. Since the expression of Class II major histocompatibility antigens on the cell surface is critical for accessory cell activity, we determined the number of HLA-DR positive cells with an anti-HLA-DR MoAb. Surprisingly, whilst nearly 90% of FAMs were HLA-DR+, the percentage of NANR and LAM cells expressing this marker was $51\pm 12\%$ and $65\pm 6\%$, respectively (table 1). Thus, the ability of enriched cell fractions isolated from exudative malignant pleural effusions to function as accessory cells in mixed leucocyte reaction experiments was not related to the percentage of HLA-DR+ cells, but rather to the relative potency of the different HLA-DR+ accessory cells present in each fraction.

The identity of the cells present in each fraction was determined using MoAbs. The FAM population was virtually entirely composed of CD68+ cells; in addition, $52\pm 7\%$ of FAMs expressed CD14, a marker found on mononuclear phagocytes at early stages of differentiation and very few expressed the RFD1 marker, known to be expressed on dendritic cells and on some B-cells [11, 14] (table 1). In contrast, low percentages of CD68+ and CD14+ cells were present in LAM and NANR fractions, suggesting that the powerful accessory activity of NANR and LAM populations was unrelated to the presence of mononuclear phagocytes.

The NANR fraction contained $21\pm 7\%$ CD3+ T-lymphocytes and very low proportions of B-lymphocytes, NK cells, and mesothelial cells (table 1). The LAM fraction did not contain B-lymphocytes and NK cells; $14\pm 2\%$ of the cells were CD3+ T-lymphocytes and $11\pm 3\%$ of the cells expressed cytokeratin (table 1). These data suggested that the potent accessory cell present in NANR and LAM fractions was a cell other than macrophages, B-lymphocytes, or NK cells.

Since dendritic cells are HLA-DR+ cells that do not express markers specific for mononuclear phagocytes, T-lymphocytes, and B-lymphocytes [6], the LAM and NANR populations were double stained to evaluate the percentage of HLA-DR+/CD68-, HLA-DR+/CD3-, and HLA-DR+/CD19- cells. Among the HLA-DR+ NANR cells ($51\pm 12\%$ of NANRs, see above), $4\pm 2\%$ expressed CD68, $5\pm 6\%$ expressed CD3, and $12\pm 21\%$ expressed CD19. Hence, $79\pm 25\%$ of HLA-DR+ NANR cells were not mononuclear phagocytes, T-cells or B-cells. Among the HLA-DR+ LAM cells ($65\pm 6\%$ of LAMs, see above),

$4\pm 2\%$ expressed CD68, $5\pm 2\%$ expressed CD3, and $0.4\pm 0.4\%$ expressed CD19. Hence, $91\pm 1\%$ of HLA-DR+ LAM cells were not mononuclear phagocytes, T-cells or B-cells (fig. 3). Moreover, both NANR and LAM fractions contained significant numbers of cells bearing the RFD1 surface marker, expressed on dendritic cells and on some B-cells [11, 14] (table 1). Because the RFD1 antigen is a specific determinant of the HLA-DR molecule [14], we did not perform experiments in which cells were stained with HLA-DR and RFD1 MoAbs. In both fractions, the majority of the HLA-DR+ cells were not mononuclear phagocytes, T- or B-lymphocytes, and most of them expressed the RFD1 marker, suggesting that the powerful accessory cells present in NANR and LAM fractions were dendritic cells.

At a morphological level, dendritic cells appeared on light-microscopy observation as a cell intermediate in size between a lymphocyte and a macrophage, did not

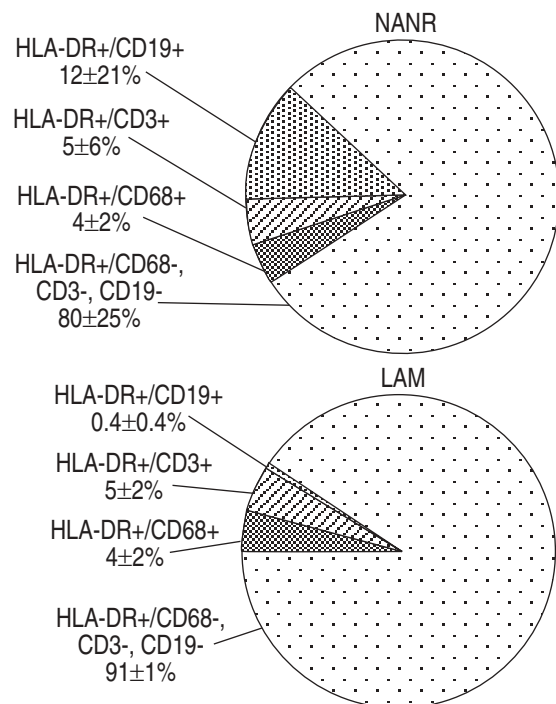


Fig. 3. – Phenotype of NANRs and LAMs. NANRs and LAMs were immunostained by double immunofluorescence technique (see Materials and Methods for details) to evaluate the percentage of HLA-DR+/CD19-, HLA-DR+/CD3-, and HLA-DR+/CD68- cells. Data represent mean \pm SD of four patients. HLA: human leucocyte antigen. For further definition see legend to figure 2.

Table 1. – Phenotype of NANRs, LAMs and FAMs isolated from pleural effusion

MoAb	Specificity	NANR %	LAM %	FAM %
HLA-DR	Class II antigen-bearing cells	51 ± 12	65 ± 6	87 ± 5
Dako-macrophage (anti-CD68)	Mononuclear phagocytes	3 ± 3	23 ± 1	89 ± 4
UCHM1 (anti-CD14)	Monocytes, granulocytes	4 ± 3	11 ± 6	52 ± 7
L26 (anti-CD20)	B-lymphocytes	5 ± 2	0	0
OK-NK (anti-CD16)	Natural killer cells	0	0	0
Dako-cytokeratin	Epithelial and mesothelial cells	2 ± 0.3	11 ± 3	2 ± 0.6
Leu4 (anti-CD3)	T-lymphocytes	21 ± 7	14 ± 2	4 ± 1
RFD1	Dendritic cells and some B-cells	18 ± 9	16 ± 4	3 ± 3

Cytopentrifuge preparations of NANRs, LAMs and FAMs were stained using the APAAP immunocytochemical method (see Materials and Methods for details). Results are expressed as percentage of positive cells (mean \pm SD of five patients). MoAb: monoclonal antibody; NANR: nonadherent neurosetting cell fraction; LAM: loosely-adherent mononuclear cell fraction; FAM: firmly-adherent mononuclear cell fraction; HLA: human leucocyte antigen.

contain intracytoplasmic inclusions and displayed an eccentric nucleus. These morphological characteristics were confirmed by flow cytometry, and resembled the previously described characteristics of dendritic cells isolated from peripheral blood and parenchymal organs, including the lung [15].

Discussion

Accessory cells play a pivotal role in the development of cell-mediated immune reactions. In this study, we have provided evidence that several accessory cell populations are present in exudative malignant pleural effusions recovered from patients with bronchogenic carcinomas. Whilst mononuclear phagocytes contained in the FAM fraction were relatively poor accessory cells, dendritic cells present in the NANR and LAM fractions were potent accessory cells for inducing T-lymphocyte proliferation. NANR and LAM enriched cell populations contained significant numbers of HLA-DR positive cells, the majority of which did not express markers specific for B- and T-lymphocytes, and mononuclear phagocytes. Moreover, a significant number of them stained positively with the anti-RFD1 MoAb expressed on dendritic cells and some B-cells [11, 14]. To our knowledge, this is the first study suggesting the presence of dendritic cells in exudative malignant pleural effusions.

The mechanisms leading to the generation of an inflammatory response during metastatic involvement of the pleural space are unclear. The presence of inflammatory cells in the pleural space could be the consequence of local proliferation and/or migration of blood precursors under chemotactic signals. In this respect, 52% of FAMs express CD14, a marker of monocyte-like "young" mononuclear phagocytes, suggesting that a significant proportion of mononuclear phagocytes present in the FAM fraction has been recently recruited from the blood. However, we have previously demonstrated that mononuclear phagocytes isolated from transudative pleural effusions express this (and other) monocyte-lineage markers similarly to FAMs isolated from exudative malignant pleural effusions [16], suggesting that "young" monocyte-like mononuclear phagocytes are also present in the pleural space in the absence of an ongoing inflammatory reaction.

The phenotype of pleural FAMs is remarkably different from that of alveolar macrophages, but closely resembles the phenotype of peritoneal macrophages [17, 18]. Whilst the origin and fate of resident and inflammatory pleural macrophages are unknown, previous studies have evaluated cell turnover of peritoneal macrophages. In normal conditions, the peritoneal macrophage population is maintained for at least 49 days without replacement by recruited cells from the blood [19]; in contrast, the accumulation of macrophages in the peritoneal space during inflammatory reactions, is mainly related to mobilization of marginated peritoneal macrophages, rather than to the recruitment of monocytes from the blood [20]. Hence, it is possible that a pool of marginated macrophages is also present in the pleural compartment, and that the mobilization of these marginated cells plays a role in the accumulation of cells in pleural fluid during tumour-associated inflammation.

Interestingly, FAMs displayed a bell-shaped dose-response curve when used as stimulatory cells in mixed leucocyte reaction experiments. Two mechanisms could explain this phenomenon: FAMs might produce a soluble factor which inhibits T-lymphocyte proliferation at high ratios of FAM:T-cells; alternatively, the initiation of specific T-cell proliferation might require a cell-cell physical interaction that occurs optimally at a certain stimulatory-responder cell ratio [21].

Another important cell type in exudative malignant effusions is represented by T-lymphocytes, whose accumulation in the pleural space may be a consequence of specific activation and proliferation at sites of disease and/or to mechanisms of "passive" accumulation, such as chemotactic gradients, lymphatic obstruction by tumour cells, or differences in recirculation kinetics [1]. In malignant effusions, several authors have suggested that the increased CD4+/CD8+ cell ratio is the expression of the host reaction against tumour antigens [22]. This hypothesis is supported by the finding that, whilst T-lymphocytes obtained from malignant effusions proliferate vigorously in response to autologous effusion of non-T-cells, peripheral blood T-lymphocytes fail to do so, suggesting that reactive T-lymphocytes are present at sites of disease activity [3]. Taken together, these data suggest that powerful accessory cells required for a local immune response are present in the pleural space during tumour-associated inflammatory reactions.

Dendritic cells are bone marrow derived cells, which play a pivotal role in the generation of cell-mediated immune responses by virtue of their ability to function as powerful accessory cells [23]. Dendritic cells have previously been isolated from a variety of organs, including peripheral blood [24], lung interstitium [10, 15], and peritoneal cavity [25]. Moreover, they have been observed in visceral pleura [26], but never described in pleural effusions. The phenotypic characterization of human dendritic cells is difficult because of the lack of a specific marker; in addition, dendritic cells isolated from various anatomical sites do not express a unique constellation of markers [27]. We have demonstrated that a significant number of cells present in LAM and NANR fraction are HLA-DR+ cells that do not bear markers specific for B- and T-lymphocytes, and mononuclear phagocytes. Additionally, a significant number of cells express the RFD1 surface marker, which has been suggested to differentiate dendritic cells from macrophages [14]. The combination of these findings with the results of mixed leucocyte reaction experiments strongly supports the concept that dendritic cells are present in malignant pleural effusions. It remains undetermined whether "resident" dendritic cells are present in the pleura or whether dendritic cells present in exudative malignant pleural effusions reach the pleural space from the blood. In this respect, we tried to isolate dendritic cells from transudative pleural effusions, selected as the equivalent of a "pleural lavage" in the absence of a local inflammatory process. However, our attempts were unsuccessful, due to the low numbers of total cells recovered (data not shown).

The powerful accessory function exerted by NANR and LAM fractions may also be due to the interaction between dendritic cells and other cells, such as mononuclear phagocytes. In this respect, studies performed on

animal models have demonstrated that, although pulmonary interstitial dendritic cells are effective accessory cells when first isolated from their native state, they become significantly more potent immunostimulatory cells when exposed to the cytokines interleukin-1 and granulocyte/macrophage colony-stimulating factor, or when cultured in the presence of interstitial macrophages [28]. These findings strongly support the hypothesis that immune responses in lung interstitium can be regulated by modulation of the ability of antigen-presenting cells to initiate T-cell responses, and suggest that similar mechanisms might be responsible for the development of cell-mediated immune reactions within the pleural space. Further studies, aimed at isolating pure populations of dendritic cells from the pleural space, are necessary to demonstrate this hypothesis.

In summary, our results suggest that enriched cell populations from exudative malignant pleural effusions may have heterogeneous accessory activity on allogeneic T-cell proliferation, and that the potent accessory activity exerted by nonadherent nonrosetting and loosely adherent mononuclear cell fractions is related to the presence of dendritic cells in pleural effusions.

Acknowledgements: The authors thank L.W. Poulter for providing the anti-RFD1 MoAb and G. Bonsignore for helpful discussions and continuous support.

References

- Sahn SA. State of the art: the Pleura. *Am Rev Respir Dis* 1988; 138: 184–234.
- Guzman J, Bross KJ, Costabel U. Malignant pleural effusions due to small cell carcinoma of the lung: an Immunocytochemical cell surface analysis of lymphocytes and tumor cells. *Acta Cytol* 1990; 34: 497–501.
- Uchida A, Micksche M. Autologous mixed lymphocyte reaction in the peripheral blood and pleural effusions of cancer patients. *J Clin Invest* 1982; 70: 98–104.
- Nicod LP, Lipscomb MF, Weissler JC, Toews GB. Mononuclear cells from human lung parenchyma support antigen-induced T-lymphocyte proliferation. *J Leukoc Biol* 1989; 45: 336–344.
- Kuntz Crow M, Kunkel HG. Human dendritic cells: major stimulators of the autologous and allogeneic mixed leukocyte reactions. *Clin Exp Immunol* 1982; 49: 338–346.
- Freudenthal PS, Steinman RM. The distinct surface of human blood dendritic cells, as observed after an improved isolation method. *Proc Natl Acad Sci USA* 1990; 87: 7698–7702.
- Van Vooris WC, Valinsky J, Hoffman E, Luban E, Hair LS, Steinman RM. Relative efficacy of human monocytes and dendritic cells as accessory cells for T-cell replication. *J Exp Med* 1983; 158: 174–191.
- Light RW, MacGragor I, Luchsinger PC, Ball WC. Pleural effusion: the diagnostic separation of transudates and exudates. *Ann Intern Med* 1972; 77: 507–513.
- Spatafora M, Merendino A, Chiappara G, et al. Lung compartmentalization of increased tumor necrosis factor releasing ability by mononuclear phagocytes in active pulmonary sarcoidosis. *Chest* 1989; 96: 542–549.
- Nicod LP, Lipscomb MF, Toews GB, Weissler JC. Separation of potent and poorly functional human lung accessory cells based on autofluorescence. *J Leukoc Biol* 1989; 45: 458–465.
- Poulter LW, Norris A, Power C, Condez A, Schmekel B, Burke C. T-cell dominated inflammatory reactions in the bronchi of asthmatics are not reflected in matched bronchoalveolar lavage specimens. *Eur Respir J* 1992; 5: 182–189.
- Melis M, Gjomarkaj M, Pace E, Malizia G, Spatafora M. Increased expression of leukocyte function associated antigen-1 (LFA-1) and intercellular adhesion molecule-1 (ICAM-1) by alveolar macrophages of patients with pulmonary sarcoidosis. *Chest* 1991; 100: 910–916.
- Yamada M, Tamura N, Shirai T, Kira S. Flow cytometric analysis of lymphocyte subsets in bronchoalveolar lavage fluid and peripheral blood of healthy volunteers. *Scand J Immunol* 1986; 24: 559–565.
- Poulter LW, Campbell DA, Munro C, Janossy G. Discrimination of human macrophages and dendritic cells by means of monoclonal antibodies. *Scand J Immunol* 1986; 24: 351–357.
- Pollard AM, Lipscomb MF. Characterization of murine lung dendritic cells: similarities to Langerhans cells and thymic dendritic cells. *J Exp Med* 1990; 172: 159–167.
- Gjomarkaj M, Pace E, Melis M, Spatafora M, Toews GB. Mononuclear cells in exudative malignant pleural effusions: characterization of pleural phagocytic cells. *Chest* 1994; 106: 1042–1049.
- Kubicka U, Olszewski WL, Maldyk J, Wierzbicki Z, Orkiszewska A. Normal human immune peritoneal cells: phenotypic characteristics. *Immunobiol* 1989; 180: 80–92.
- Hance AJ, Douches S, Winchester RJ, Ferrans VJ, Crystal RG. Characterization of mononuclear phagocyte subpopulations in the human lung by using monoclonal antibodies: changes in alveolar macrophage phenotype associated with pulmonary sarcoidosis. *J Immunol* 1985; 134: 284–292.
- Melnicoff MJ, Horan PK, Breslin EW, Morahan PS. Maintenance of peritoneal macrophages in the steady state. *J Leukoc Biol* 1988; 44: 367–375.
- Melnicoff MJ, Horan PK, Morahan PS. Kinetics of changes in peritoneal cell populations following acute inflammation. *Cell Immunol* 1989; 118: 178–191.
- Rich EA, Tweardy DJ, Fujiwara H, Ellner JJ. Spectrum of immunoregulatory functions and properties of human alveolar macrophages. *Am Rev Respir Dis* 1987; 136: 258–265.
- Robinson E, Segal R, Vesely Z, Mekori T. Lymphocyte subpopulations in peripheral blood and malignant pleural effusions of cancer patients. *Eur J Cancer Clin Oncol* 1986; 22: 191–193.
- Steinman RM, Nussenzweig MC. Dendritic cells: features and functions. *Immunol Rev* 1980; 53: 127–147.
- Rasanen L, Lehto M, Leinikki P. Enrichment of dendritic cells from human peripheral blood. *J Leukoc Biol* 1988; 43: 343–348.
- Steinman RM, Witmer MD. Lymphoid dendritic cells are potent stimulators of the primary mixed leukocyte reaction in mice. *Proc Natl Acad Sci USA* 1978; 75: 5132–5136.
- Sertl K, Takemura T, Tschachler E, Ferrans VJ, Kaliner MA, Shevach EM. Dendritic cells with antigen-presenting capability reside in airway epithelium, lung parenchyma, and visceral pleura. *J Exp Med* 1986; 163: 436–451.
- Crowley M, Inaba K, Witmer-Pack M, Steinman RM. The cell surface of mouse dendritic cells: FACS analyses of dendritic cells from different tissues including thymus. *Cell Immunol* 1989; 118: 108–125.
- Armstrong LR, Christensen PJ, Paine RIII, et al. Regulation of the immunostimulatory activity of rat pulmonary interstitial dendritic cells by cell-cell interactions and cytokines. *Am J Respir Cell Mol Biol* 1994; 11: 682–691.