Immunology 2003 **110** 58–65

Unimpaired dendritic cell functions in *MVP/LRP* knockout mice

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SUMMARY

Dendritic cells (DCs) act as mobile sentinels of the immune system. By stimulating T lymphocytes, DCs are pivotal for the initiation of both T- and B-cell-mediated immune responses. Recently, ribonucleoprotein particles (vaults) were found to be involved in the development and/or function of human DCs. To further investigate the role of vaults in DCs, we examined the effects of disruption of the major vault protein (MVP/LRP) on the development and antigen-presenting capacity of DCs, using our *MVP/LRP* knockout mouse model. Mononuclear bone marrow cells were isolated from wild-type and knockout mice and stimulated to differentiate to DCs. Like human DCs, the wild-type murine DC cultures strongly expressed MVP/LRP. Nevertheless, the MVP/LRP-deficient DCs developed normally and showed similar expression levels of several DC surface markers. No differences were observed in *in vitro* studies on the antigen uptake and presenting capacities of the wild-type and *MVP/LRP* knockout DCs. Moreover, immunization of the *MVP/LRP*-deficient mice with several T-cell antigens led to responses similar to those observed in the wild-type mice, indicating that the *in vivo* DC migration and antigen-presentation capacities are intact. Moreover, no differences were observed in the induction of the T cell-dependent humoral responses and orally induced peripheral T-cell tolerance. In conclusion, vaults are not required for primary DC functions. Their abundance in DCs may, however, still reflect basic roles in myeloid cell proliferation and DC development.

INTRODUCTION

Dendritic cells (DCs) are a heterogeneous population of antigen-presenting cells (APC) that function as mobile sentinels of the immune system. By stimulating T lymphocytes, DCs are pivotal for the initiation of both T- and B-cell-mediated immune responses (reviewed by Cella *et al*.[1] and Banchereau *et al*.[2]). Moreover, by determining the nature of T-cell responses, DCs regulate the T helper 1/ T helper 2 (Th1/Th2) balance[3] and play an important role in peripheral T-cell tolerance. Immature DCs reside in non-lymphatic tissues and are characterized by a high capability for antigen uptake and processing. Upon maturation, the cells lose their antigen-uptake ability, whereas major histocompatibility complex (MHC) and costimulatory molecules are upregulated. The cells migrate to the lymphoid organs, an appropriate environment for the activation of antigen-specific T cells.[1] Recently, two ATP-dependent transmembrane transporter molecules, Pgp and MRP1, both known for their role in multidrug resistance (MDR), have been identified as mediators in this migration process.[4,5] Whereas the role of Pgp in this migration is as yet unclear, MRP1 regulates DC migration by transporting the leukotriene C_4 (LTC_4). More recently, Schroeijers *et al*. confirmed the upregulation of Pgp on developing DCs.[6] In this study, DCs from different origins, such as peripheral blood monocytes, $CD34^+$ peripheral blood cells and cells from patients with chronic myeloid leukemia, were investigated. Interestingly, another possible mediator of drug resistance, the intracellular vault complex, was also found to be strongly upregulated during human DC differentiation.

Received 6 January 2003; revised 2 June 2003; accepted 12 June 2003.

Abbreviations: DC, dendritic cell; DNCB, dinitro-chlorobenzene; FITC, fluorescein isothiocyanate; KLH, keyhole limpet haemocyanin; LRP, lung resistance-related protein; Mϕ, macrophage; MLR, mixed lymphocyte reaction; MRP1, multidrug resistance-associated protein; MVP, major vault protein; OVA, ovalbumin; PE, phycoerythrin; Pgp, P-glycoprotein; Th, T helper.

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Moreover, addition of major vault protein (MVP)-specific antibodies in the culture medium resulted in decreased viability of the maturated DCs. In addition, these DCs showed decreased expression levels of differentiation and maturation markers, as well as costimulatory molecules.[6] Their capacities to induce T-cell proliferation and interferon-γ (IFN-γ) release appeared to be impaired, suggesting that besides Pgp and MRP1, MVP and vaults may also play a role in the survival and functioning of DCs.

Vaults are large ribonucleoprotein particles that are found in the cytoplasm of most eukaryotic cells, as reviewed by Scheffer *et al*.[7] The complex consists of three different proteins of M_r 100 000, 193 000 and 240 000, known as MVP, VPARP and TEP1, respectively, and multiple copies of small, untranslated RNAs of 88–141 bases. The typical shape of the vault complex – a barrel with an invaginated waist and two protruding caps – as well as its constituents, appear to be conserved between species.[8–10]

As the MVP, which makes up 70% of the total mass of the complex, was identified as the lung resistance-related protein (LRP),[11] many studies on the function of this intriguing particle have focused on a role of vaults in drug resistance. Indeed, in several independent studies, an upregulation of MVP/LRP was observed in drug-resistant cell lines and tumours,[12–15] and evidence was obtained supporting the view that vaults act by transporting drugs away from their cellular targets.[16,17] However, to date a direct involvement of vaults in MDR could not be demonstrated in the *MVP/LRP* knockout mouse model that we recently generated, despite extensive studies on the sensitivity of the *MVP/LRP*-deficient animals and cells to various cytostatic drugs.[18]

Using our *MVP/LRP* knockout mouse model, we set out to study the role of MVP/LRP and vaults on the development and functioning of DCs in mice. It was hypothesized that putative DC malfunctioning might lead to an impairment of both the T-cell immune responses as well as the T-cell-dependent humoral immune responses. Hence, besides *in vitro* studies on the development of DCs, we tested the *in vivo* immune responsiveness of mice deficient for MVP/LRP. As, in humans, a high expression of MVP/LRP is observed in macrophages (Mϕ)[12] we also examined the migration and function of Mϕ derived from the *MVP/LRP*-deficient mice.

MATERIALS AND METHODS

Animals

MVP/LRP knockout mice were generated, as described previously,[18] and housed under conventional conditions at the animal facility of the Erasmus Medical Center. All animals used in this study were of a mixed background of 129/Ola and C57BL/6 strains, backcrossed for one to four generations to C57BL/6. Wild-type littermates were used as controls in all experiments.

Bone marrow-derived DC cultures

Bone marrow-derived DCs were produced essentially as described previously.[19] In short, femurs and tibias were isolated from $MVP/LRP^{+/+}$ and $MVP/LRP^{-/-}$ littermates. The bones were cleaned and crushed using a sterile mortar in DC medium [RPMI-1640 containing 10% fetal calf serum (FCS), 100 units/ml penicillin, 100 μg/ml streptomycin and 0·1 mM β-mercaptoethanol] in order to extract the cells. Subsequently, the cells were filtered through a 100-μm cell strainer (Becton-Dickinson, Franklin Lakes, NJ) and pelleted by centrifugation at 500 ***g*** for 10 min. Mononuclear cells were separated from the bone marrow cell suspension by density-gradient centrifugation over lymphoprep 1·077 (Nycomed Pharma AS, Oslo, Norway) for 20 min at 833 ***g***. The deceleration time of the centrifuge was set to 5 min. Cells were seeded at a density of $1{\cdot}25 \times 10^6$ cells in 5 ml of DC medium, complemented with 100 U/ml of granulocyte–macrophage colony-stimulating factor (GM-CSF) (PeproTech/Tebu, Le Perray en Yvelines, France) in 25-cm^2 bottles and cultured at 37° in a humidified atmosphere containing 5% CO_2. Three days after isolation of the mononuclear cells, an additional 5 ml of DC medium containing GM-CSF was added to the DC culture. Half of the DC medium was refreshed on days 6 and 8, and on this last day, lipopolysaccharide (LPS) from *Escherichia coli* (Sigma-Aldrich, St Louis, MO) was added to the medium at a final concentration of 1 μg/ml. Samples of 1×10^6 cells for Western blot analysis were taken on days 0, 3, 6, 8 and after stimulation overnight with LPS (day 9).

Phenotypic and functional analysis of DC

DC phenotype was determined by fluorescence-activated cell sorter (FACS) analysis using the following monoclonal antibodies (mAbs): anti-CD11c-conjugated fluorescein isothiocyanate (FITC), anti-MHC class II-conjugated phycoerythrin (PE), biotinylated anti-MHC class II (all purchased from PharMingen, Heidelberg, Germany); anti-CD40–PE, anti-CD80–PE, anti-CD86–PE, anti-CD11b–PE (all obtained from Beckman Coulter, Mijdrecht, the Netherlands); and the early myeloid marker-specific ER-MP58–FITC.[20] Cells incubated with biotinylated mAb were washed and stained with streptavidine-CyChrome (PharMingen). To exclude dead cells from the analysis, 40 μg/ml 7-amino-actinomycin D (7-AAD) (Molecular Probes, Leiden, the Netherlands) was added prior to analysis on a FACScan using CELLQUEST software (Becton-Dickinson, Alphen a/d Rijn, the Netherlands).

To test the antigen-uptake capacity of the DCs, 2×10^5 cells were incubated with 10 μl of a 10-mg/ml dextran–FITC solution (Sigma-Aldrich) in a total volume of 100 μl of DC medium. The cells were incubated at 37° for 1 hr. The uptake was stopped by the addition of ice-cold phosphate-buffered saline (PBS) and, after washing, the cells were incubated with anti-MHC class II–PE, as a DC marker, for an additional 30 min on ice. The background uptake of dextran–FITC was determined by incubating the cells at 4° instead of 37°.

Western blot analysis

Cell lysates were subjected to sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) and the size-fractionated proteins were transferred to nitrocellulose. The membranes were blocked in TBST [50 mM Tris–HCl (pH 7·5), 100 mM NaCl, 0·05% (vol/vol) Tween-20] containing 5% (wt/vol) non-fat dried milk (Bio-Rad, Laboratories, Hercules, CA). MVP/LRP was detected using a rabbit polyclonal antiserum raised against human MVP[21] and an anti-rabbit immunoglobulin conjugated to horseradish peroxidase (HRP) (Jackson ImmunoResearch Laboratories, Westgrove, PA) as second

antibody. Immune complexes were detected using BM chemiluminescence (Roche Molecular Biochemicals, Mannheim, Germany) and visualized on Hyperfilm ECL (Amersham-Pharmacia, Uppsala, Sweden). Quantifications were performed using IMAGEQUANT, version 3·3 (Molecular Dynamics, Sunnyvale, CA).

Allogeneic mixed lymphocyte reaction

To determine the capacity of the DCs to stimulate T-cell proliferation, splenocytes from BALB/c mice were stimulated with DCs derived from wild-type and *MVP/LRP* knockout mice with a C57BL/6-129 OLA background.[18] Spleen cells were isolated by squeezing the spleens through a wet 100-μm cell strainer (Becton-Dickinson) followed by centrifugation of the cells for 5 min at 558 ***g***. Splenocytes (10^4 per well) were mixed with DCs in ratios ranging from 20 : 1 to 1280 : 1 (spleen cells/DCs), and seeded in round-bottom 96-well plates in 200 μl of DC medium. After 3–5 days of incubation at 37°, 0·1 μCi/well [^{3}H]thymidine (Amersham, Bucks., UK) was added and the cells were incubated overnight. Cells were transferred to a glass-fibre filter using a filtermate 196 cell harvester (Packard Instruments BV, Groningen, the Netherlands) and the radioactivity retained on the filters was determined by using a topcount microplate scintillation counter (Packard Bioscience). All incubations were performed in quadruplicate.

Mϕ migration tests and uptake of dextran–FITC

To isolate Mϕs, two wild-type and two *MVP/LRP* knockout animals were injected intraperitoneally (i.p.) with 1·5 ml of paraffin oil. After 48 hr, the animals were killed and abdominal cavities flushed with 6 ml of Iscove's modified Dulbecco's medium (IMDM) (Bio Whittaker, Verviers, Belgium), containing 10% FCS and 50 IU/ml heparin, to harvest the cells. The numbers of cells, reflecting *in vivo* migration, were counted and the cells were resuspended in 200 μl of IMDM containing 10% heat-inactivated mouse serum. For the *in vitro* migration test, 0·5 μl of this cell suspension was transferred to a well of a microtitre plate and the cells were allowed to adhere for 30 min. Then, 3 × 25 μl of medium was added slowly to the wells, taking care that the cells remained attached to the plastic. Using an ocular raster, squares containing Mϕ were identified and recorded. The migration capacity of the cells was subsequently determined by counting the numbers of cells that had migrated out of these original squares after 24 hr.

To test the capacity of the Mϕ to internalize dextran–FITC, 50 μl of the Mϕ cell suspension was added to 500 μl of PBS containing 1% bovine serum albumin (BSA) and 1 mg/ml dextran–FITC. After incubation at 37° (or on ice as a negative control) for 1 hr, the cells were washed with and resuspended in 500 μl of ice-cold PBS containing 1% BSA. The amounts of intracellular dextran–FITC taken up by the cells was determined by analysis on a FACScan.

Sensitization and skin testing to different antigens

For sensitization with keyhole limpet haemocyanin (KLH) (Perimmune, Seattle, WA), five wild-type and five *MVP/LRP*-deficient mice were injected with 100 μg of KLH subcutaneously in the right-hind footpads. After 10 days, the animals were challenged with 5 and 10 μg of KLH subcutaneously in the ears. The thickness of the ears was measured before, and 4, 24, 48 and 72 h later. Following essentially the same protocol, 3 days later, all mice were again sensitized, by subcutaneous injection of 50 μg of ovalbumin (OVA) (Sigma-Aldrich) in the left-hind footpads, and skin tested 7 days later by injecting 10 μg of OVA into the right ear. A third sensitization and testing round was carried out with the widely used contact-allergen dinitro-chlorobenzene (DNCB). The animals were sensitized by application of 20 μl of a 1% DNCB solution in EtOH epi-cutaneous (e.c.) on the abdomen. After 4 days, they were challenged with 2 × 10 μl of 0·3% DNCB in EtOH e.c. on the left ear. Swelling of the ears was again measured at 4–72 hr after the challenge.

Oral tolerance test

Six wild-type mice and six *MVP/LRP* knockout mice were fed three times with 10 mg of OVA on days 1, 3 and 8. Control mice received the same volume (200 μl) of saline. Immunization was performed on day 13, with 50 μg of OVA, which was injected subcutaneously into the footpad. After 7 days, the animals were challenged intra-cutaneous (i.c.) in the ear with 10 μg of OVA. Ear swelling was measured at 4, 24, 48 and 72 hr after the challenge.

Antibody shift

Sera of other groups of six wild-type and six *MVP/LRP* knockout mice that were immunized (day 0) and challenged (day 10) with KLH, as outlined above, were tested for the production of anti-KLH by enzyme-linked immunosorbent assay (ELISA). Flat-bottom microtitre plates were coated overnight with 1 μg/well of KLH. Each of the mouse sera was diluted in a three-step dilution series from 1 : 10 to 1 : 21 870 and the anti-KLH in the serum were detected with goat anti-mouse immunoglobulin (Ig)G, IgG1 or IgG2a. As a second antibody, rabbit anti-goat-HRP was used. Between the successive steps, the plates were washed 2 × 15 second in tap water containing 0·05% Tween-20. Bound antibody was detected by a colour reaction using *o*-phenylenediamine (OPD). After 30 min the reaction was stopped by the addition of 50 μl of 2·5 M H_2SO_4 and the absorbance (*A*) at 492 nm was determined.

RESULTS

Phenotypic analysis of MVP/LRP-deficient DCs

Mononuclear cells isolated from total bone marrows from wild-type and *MVP/LRP* knockout mice were cultured for 9 days, in the presence of GM-CSF, to induce DC differentiation. On day 8, LPS was added to the culture medium as a maturating agent. Routinely, the bone marrow cultures from wild-type and MVP/LRP knockout mice yielded equal amounts of DCs. In order to investigate whether MVP/LRP deficiency might interfere with DC development, mature DCs derived from wild-type and *MVP/LRP* knockout bone marrow were harvested after overnight stimulation with LPS. By FACScan analysis, the expression of several DC surface markers and costimulatory molecules on the MVP/LRP-deficient cells was determined and compared with the levels on wild-type cells. Overlays of knockout and wild-type FACS histograms are shown in Fig. 1. The

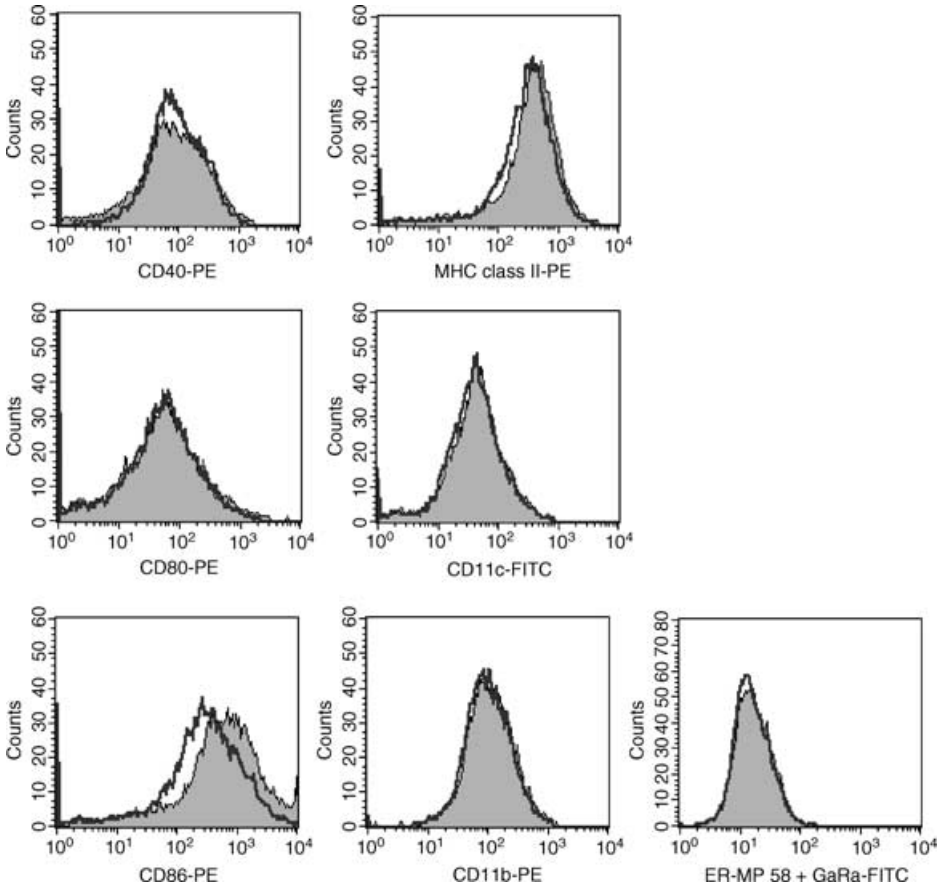

Figure 1. Phenotypic analysis of dendritic cells (DCs) derived from wild-type and major vault protein/lung resistance-related protein (*MVP*/*LRP*) knockout mouse bone marrow. Expression levels of seven different cell-surface markers on wild-type and *MVP*/*LRP*-deficient cells were determined by fluorescence-activated cell sorter (FACS) analysis. Grey plots represent marker expression in DCs derived from wild-type bone marrow, black overlays represent expression in *MVP*/*LRP*-deficient DCs. The fluorescence is shown on the *x*-axis, cell counts are shown on the *y*-axis. The fluorescence levels were clearly distinguishable from the routinely included negative controls (not shown).

cell-surface levels of the DC marker CD11c, the early myeloid marker ER-MP 58,[20] the myeloid marker CD11b, MHC class II molecules and the costimulatory molecules CD40 and CD80, were similar in the *MVP*/*LRP* knockout and wild-type DCs. One of the costimulatory markers, CD86, was expressed at a slightly lower level on the *MVP*/*LRP* knockout DCs. However, the cells were still clearly positive for this marker and the other costimulatory molecules were expressed normally.

Expression of MVP/LRP during murine DC development

Samples, corresponding to 10^6 cells, of wild-type total bone marrow, immature DCs and mature DCs, were analysed by Western blotting in order to determine the expression levels of MVP/LRP. As shown in Fig. 2, the expression of MVP/LRP in total bone marrow is relatively low. During differentiation (days 3–8) in cultures with GM-CSF, MVP/LRP became more highly expressed (fourfold compared with unstimulated mononuclear bone marrow cells). Subsequently, like in human DCs,[6] LPS stimulation (day 9), seemed to enhance the expression of MVP/LRP even further (6·5-fold compared with the unstimulated mononuclear bone marrow cells). Of note, on day 3 of the differentiation culture, when the numbers of differentiated DCs are still low,[19] MVP/LRP was already highly expressed on the cells, suggesting that MVP/LRP expression might be related to processes in early myeloid development.

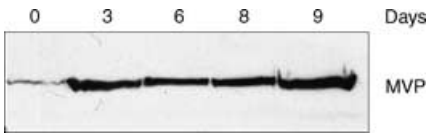

Figure 2. Major vault protein/lung resistance-related protein (MVP/LRP) expression in dendritic cells (DCs). Total bone marrow was isolated from wild-type mice. The mononuclear cells were isolated and cultured in the presence of granulocyte–macrophage colony-stimulating factor (GM-CSF). Samples were taken on days 3, 6, 8 and 9. Protein lysates corresponding to equal numbers of cells (10^6 cells) were subjected to sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) and analysed for MVP/LRP expression by Western blotting. Note that on day 8, the DCs were stimulated overnight with lipopolysaccharide (LPS).

DC antigen handling and presentation

DC function was tested by measuring the *in vitro* dextran-uptake capacity. The internalized dextran–FITC was similar in *MVP/LRP*$^{+/+}$ and *MVP/LRP*$^{-/-}$ DCs (Fig. 3a). Cells incubated with dextran–FITC at 4° (negative controls) showed only low levels of dextran–FITC uptake (Fig. 3a).

Subsequently, the ability of DCs to induce T-cell proliferation was tested by means of mixed lymphocyte reaction assays. For this, the DCs from wild-type and *MVP/LRP* knockout bone marrow were mixed with lymphocytes isolated from allogeneic spleens. The induced proliferation of the lymphocytes was measured after 4 days. As evident from Fig. 3(b), the *MVP/LRP* knockout DCs are perfectly capable of inducing T-cell proliferation. No differences were observed between the proliferation of lymphocytes incubated with wild-type and *MVP/LRP* knockout DCs.

Mϕ migration and function

To explore Mϕ migration and the capacity to internalize dextran–FITC from *MVP/LRP*-deficient mice, two wild-type and two knockout mice were injected with paraffin oil. After 2 days, Mϕ were isolated from the peritoneal cavity. As the numbers of Mϕ obtained from unstimulated peritoneal cavities never exceed more than 2–3 $\times$ 10^6 cells, we referred to the cells collected after injection of paraffin oil (≈20–30 $\times$ 10^6 cells) as cells migrating from the blood into the peritoneal cavity. Approximately 90% of the collected cells reflect inflammatory cells, of which 80–90% were Mϕ, as judged by differential counting of haematoxylin and eosin (H & E)-stained cytospins of these cells. The numbers of cells that had migrated reflect the *in vivo* migration capacity of the Mϕ. As is shown in Table 1, the numbers of Mϕ were comparable in wild-type and *MVP/LRP* knockout mice. Next, *in vitro* migration assays were performed. Percentages of migrated Mϕ were counted 24 hr after seeding and were similar in wild-type and *MVP/LRP*-deficient cells (Table 1). The capacity to take up dextran–FITC by macropinocytosis or receptor-mediated endocytosis was tested by incubating the isolated Mϕ with a dextran–FITC solution and quantification of the internalized material by FACS analysis. Percentages of cells containing dextran are shown in Table 1. The *MVP/LRP*-deficient Mϕ migrate normally and are as capable of taking up FITC-labelled dextran as the wild-type Mϕ.

In vivo immune responses

Although the *MVP/LRP*-deficient DCs have essentially similar phenotypic characteristics as the wild-type DCs and appear

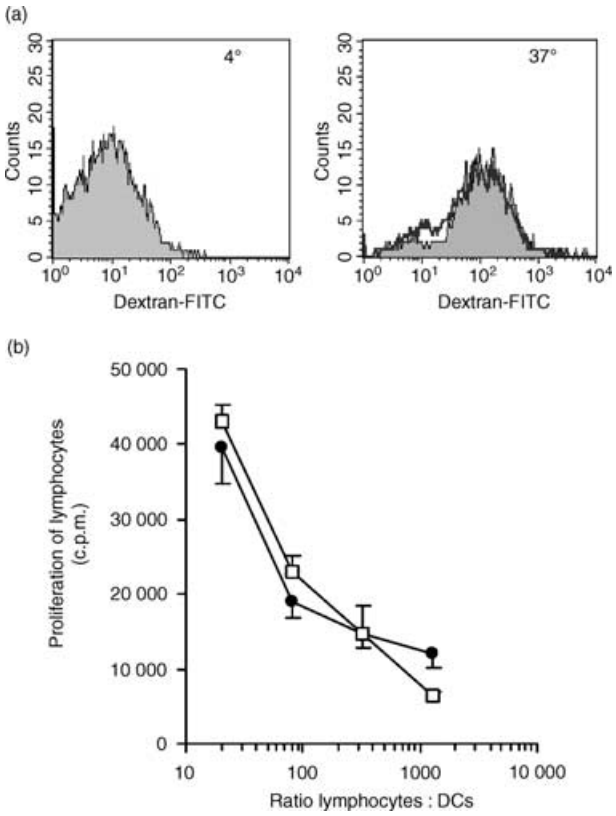

Figure 3. Functional analysis of dendritic cells (DCs) derived from wild-type and major vault protein/lung resistance-related protein (*MVP/LRP*) knockout mouse bone marrow. DCs were incubated with dextran–fluorescein isothiocyanate (FITC) either at 4° (control) or at 37°. After 1 hr the cells were washed, incubated with anti-major histocompatibility complex (MHC) class II-conjugated phycoerythrin (PE) as a DC marker, and the amount of DC-associated dextran–FITC was determined by fluorescence-activated cell sorter (FACS) analysis. (a) The left-hand panel depicts background binding of dextran–FITC at 4° to wild-type DCs. Identical results were obtained with *MVP/LRP* knockout DCs. The right-hand panel depicts the uptake of dextran–FITC at 37° by wild-type (grey plot) and *MVP/LRP*-deficient (black) DCs. (b) Mixed lymphocyte reaction (MLR). Lymphocytes were mixed with wild-type (closed circles) and *MVP/LRP*-deficient (open squares) DCs. On the *x*-axis, the ratios of lymphocytes : DCs are indicated on a logarithmic scale. The proliferation of lymphocytes is measured by the incorporation of [^{3}H]thymidine and expressed as counts per minute (c.p.m.) on the *y*-axis. Depicted are the results from a representative experiment; each data point represents a mean value with one-sided standard deviation ($n = 4$).

Table 1. Migration and capacity of macrophages (Mϕ) from wild-type and major vault protein/lung resistance-related protein (*MVP/LRP*) knockout mice to internalize dextran–fluorescein isothiocyanate (FITC)

	Cell number	Viability	Migration	Internalization of dextran–FITC
Wild-type ($n = 2$)	1·3–2·5 $\times$ 10^7	78–87%	55–64%	17·6–33·5%
Knockout ($n = 2$)	0·8–2·8 $\times$ 10^7	82–83%	64–70%	15·1–41·4%

Shown are numbers of Mϕ isolated from the peritoneal cavity (first column) and the viability of these cells (column 2). Percentage of Mϕ that migrated during the *in vitro* migration experiment are shown in column 3. The last column shows the percentages of Mϕ that have taken up dextran–FITC, after an incubation of 1 hr at 37°, as measured by fluorescence-activated cell sorter (FACScan) analysis.

functional in *in vitro* tests, their *in vivo* migration from immunization sites to the draining lymph nodes and their subsequent *in vivo* antigen-presenting capacities might still be affected. In turn, this might lead to impaired T-cell responsiveness. To test this possibility, five wild-type and five knockout animals were immunized with three different antigenic materials well established for their capacity to provoke T-cell-mediated immune responses. After challenging, immune responses were evaluated by measuring the delayed-type hypersensitivity reactions in the challenged ears. While these reactions for the protein antigens KLH and OVA are recognized as reflecting Th1 responses, the contact allergen DNCB may induce both Th1 and Th2 responses. The results of these experiments, as presented in Fig. 4(a), show that the responses to all antigens examined were similar in wild-type and *MVP/LRP* knockout mice. As expected, the PBS-injected control animals showed low responses. These data suggest that the disruption of *MVP/LRP* has no significant effects on the development of T-cell-mediated effector functions, as revealed by skin hypersensitivity reactions.

Induction of oral tolerance

The expression of MVP/LRP was found to be high in tissues of the gastrointestinal tract.[12] This might point to a role of MVP/LRP and vaults in a protective response to food allergens. To examine this option, we fed both wild-type and *MVP/LRP* knockout mice three times with 10 mg of OVA. Such repeated feeding is known to induce an incapacity of the animals to subsequently develop T-cell-mediated immune responses, reflecting immunological tolerance. When immunization with OVA was attempted, only the positive-control saline-fed animals showed proper immune responses to OVA. *MVP/LRP*-deficient animals displayed an equally decreased responsiveness to OVA as the wild-type animals (Fig. 4b), proving that induction of oral tolerance in the *MVP/LRP* knockout mice is unimpaired. Apparently, these mice do not exhibit major defects in food handling.

T-cell-mediated humoral responses

Besides the initiation of primary immune responses, DCs also play a role in the modulation of immune responses by regulation of the Th1/Th2 balance (reviewed by Shortman *et al.*[3]), as reflected by the preferential production of distinct antibody isotypes. Possibly, the disruption of *MVP/LRP* might change the preference for either a Th1 or a Th2 response and, as a consequence, the production of antibody isotypes associated with these responses, e.g. IgG1 versus IgG2a. We therefore investigated the subclasses of the antibodies produced upon immunization with KLH. Sera of KLH-immunized animals were tested by ELISA. The results are shown in Fig. 5. The titres of total IgG, IgG1 and IgG2a are similar in wild-type and knockout animals, suggesting that besides proper T-cell-mediated immunity, also the T-cell-dependent humoral antibody production to KLH is unaffected.

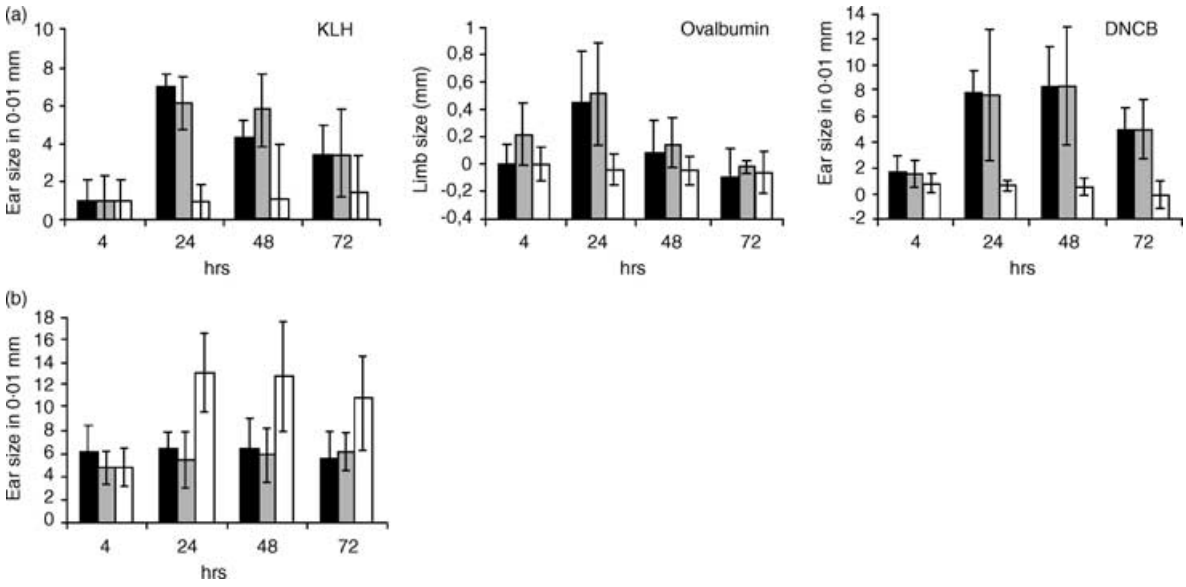

Figure 4. Immune responsiveness of wild-type and major vault protein/lung resistance-related protein (*MVP/LRP*)-deficient mice immunized with several allergens. (a) In all experiments, five wild-type and five knockout mice were immunized subcutaneously and challenged after 10 [keyhole limpet haemocyanin (KLH)], 7 [ovalbumin (OVA)] or 4 [dinitro-chlorobenzene (DNCB)] days, in the skin of the ear or limb. Control, wild-type animals were injected with equal volumes of saline. The swelling at the site of the challenge (thickness of the ear/limb minus original thickness) is a measure for the immune responsiveness and is shown on the *y*-axis. Black bars show the response of wild-type animals, grey bars show the response of MVP/LRP-deficient animals and white bars show saline-injected control animals. Error bars indicate 2 standard deviations (SD) ($n = 5$). (b) Oral tolerance test to OVA. Six wild-type and six *MVP/LRP* knockout mice, were administered 3×10 mg of OVA intragastrally. Positive controls were administered physiological salt solution. The animals were immunized in one of the limbs, and challenged in the ear. The swelling of the ear is a measure for the immune responsiveness. Black bars show the response of wild-type animals, grey bars show the response of *MVP/LRP*-deficient animals and white bars show the response of the (wild-type) positive control animals. Error bars indicate 2 SD ($n = 5$).

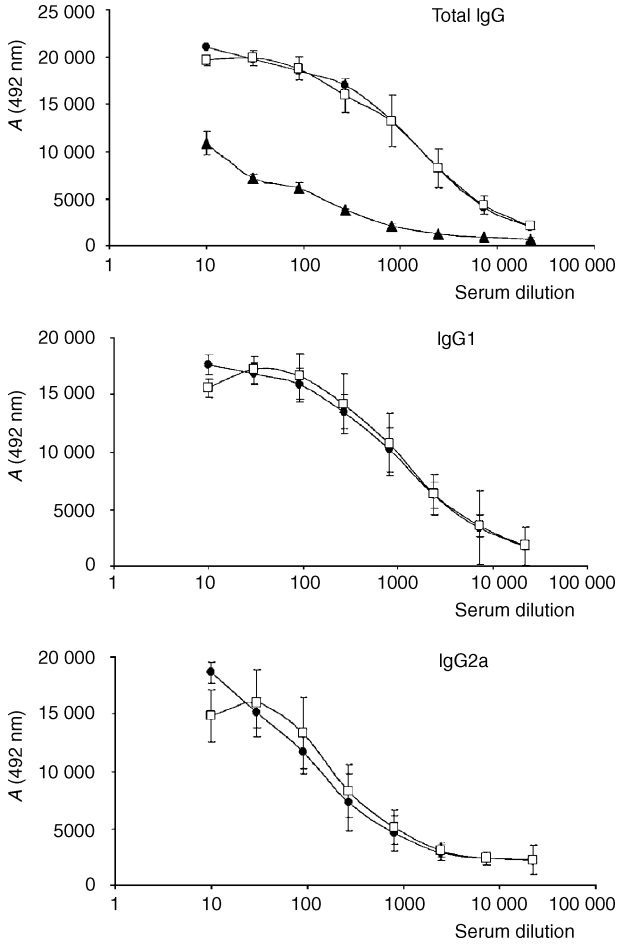

Figure 5. T-cell-dependent humoral immune responsiveness. Anti-keyhole limpet haemocyanin (anti-KLH) in the serum of KLH-immunized mice. A range of serum dilutions was analysed for total immunoglobulin (Ig)G, IgG1 and IgG2a by enzyme-linked immunosorbent assay (ELISA), as described in the Materials and methods. The results are expressed as mean ± standard deviation (SD) of six wild-type mice (closed circles), six MVP/LRP-deficient mice (open squares) or three control, saline-injected mice (closed triangles).

DISCUSSION

Recently, Schroeijers *et al*. reported that MVP/LRP, the major component of the vault complex, is upregulated during the development of human DCs.[6] Moreover, the presence of MVP/LRP-specific antibodies, presumably interfering with the function of MVP/LRP or vaults, resulted in reduced expression levels of DC markers and costimulatory molecules, and a decreased capacity to induce T-cell proliferative and IFN-γ-releasing responses. Previously, we reported the generation of an *MVP/LRP* knockout mouse model,[18] in which vault particles are absent. Using this model, we investigated the role of MVP and/or vaults in the maturation and function of DCs. Although MVP/LRP is highly expressed in mature wild-type DC cultures, the loss of this protein did not alter the *in vitro* development and function of DCs derived from mononuclear bone marrow cells. In addition, *in vivo* immunization assays showed that neither the T-cell-mediated immune response nor the T-cell-dependent humoral response are affected by the disruption of *MVP/LRP*, indicating intact antigen presenting and migration capacities of the DCs.

Apparently, the clues for an involvement of MVP/LRP and vaults in DC development and function, as reported previously for human DCs,[6] were not further substantiated using the *MVP/LRP* knockout mouse model. This could point to a fundamental difference between DCs from human and mouse. However, it should be considered that the decreased viability, lower expression of DC markers and reduced antigen-stimulatory capacity reported were observed with DCs cultured in the presence of polyclonal, or pooled monoclonal, anti-MVP/LRP. The lack of effects with individual mAbs was noted to argue against interference with functionally relevant epitopes. Rather, the blockade of DC functions could result from events secondary to the binding of anti-MVP/LRP, e.g. from the aggregation of vault particles.[6] The present results, showing that the mere absence of vault particles does not significantly affect phenotypic and functional DC markers, support the latter option. Nevertheless, a role of vaults in DC antigen-presenting functions cannot be fully excluded because the loss of MVP/LRP and vaults might still result in subtle effects beyond the scope of the presently used experimental conditions and assays. Possibly, gene products playing roles in critical cellular functions show redundancy, with other factors compensating for their eventual loss. The latter option is, however, difficult to reconcile with the unique structure of the vault complex and the lack of MVP homologues in the murine genome.[10]

In aggregate, the functional impact of highly expressed MVP/LRP in mammalian DCs remains unclear. As the high expression levels in DCs have, thus far, primarily been studied in *in vitro* cultured cells, further analyses of conditional upregulation of MVP/LRP levels in DCs *in vivo* in human and mouse lymphoid tissues are warranted. In early immuno-histopathological analyses of human lymphoid tissues we noted high levels of MVP/LRP in Mϕ-like cells, but not in DCs.[12] Given the present results, showing that a lack of vault particles also does not interfere with primary Mϕ functions (notably migration), we currently focus on their potential role in controlling cell-division processes. Such a role(s) might fit with recent data on the association of MVP/LRP with the tumour-suppressor factor PTEN,[22] as well as with their frequent overexpression in cytostatic drug-resistant tumour cells. The present data reveal that vaults are not required for primary DC functions. Their abundance in DCs may, however, still reflect basic role(s) in myeloid cell proliferation and DC development.

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