

Scavenger Receptors SR-AI/II and MARCO Limit Pulmonary Dendritic Cell Migration and Allergic Airway Inflammation¹

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The class A scavenger receptors (SR-A) MARCO and SR-AI/II are expressed on lung macrophages (MΦs) and dendritic cells (DCs) and function in innate defenses against inhaled pathogens and particles. Increased expression of SR-As in the lungs of mice in an OVA-asthma model suggested an additional role in modulating responses to an inhaled allergen. After OVA sensitization and aerosol challenge, SR-AI/II and MARCO-deficient mice exhibited greater eosinophilic airway inflammation and airway hyperresponsiveness compared with wild-type mice. A role for simple SR-A-mediated Ag clearance (“scavenging”) by lung MΦs was excluded by the observation of a comparable uptake of fluorescent OVA by wild-type and SR-A-deficient lung MΦs and DCs. In contrast, airway instillation of fluorescent Ag revealed a significantly higher traffic of labeled DCs to thoracic lymph nodes in SR-A-deficient mice than in controls. The increased migration of SR-A-deficient DCs was accompanied by the enhanced proliferation in thoracic lymph nodes of adoptively transferred OVA-specific T cells after airway OVA challenge. The data identify a novel role for SR-As expressed on lung DCs in the down-regulation of specific immune responses to aeroallergens by the reduction of DC migration from the site of Ag uptake to the draining lymph nodes. *The Journal of Immunology*, 2007, 178: 5912–5920.

The lung is constantly exposed to numerous inhaled particles and pathogens and relies on the broad ligand specificity of scavengers and other pattern recognition receptors for innate immune defense (1–3). The scavenger receptor (SR)⁵ family includes two members in the SR-A subclass that are expressed on lung macrophages (MΦs) and dendritic cells (DCs), MARCO (MΦ receptor with collagenous structure), and SR-AI/II (SR-A, types I and II) (1, 2, 4). MARCO, like SR-AI/II, binds

acetylated low-density lipoprotein and bacteria but not yeast (5–7). MARCO and SR-AI/II expressed on alveolar macrophages function to promote the uptake and clearance of inhaled particles and bacteria (7–10).

Aeroallergens constitute another common inhaled challenge to the lung's immune cells. Stimulated in part by gene expression profiling that shows increased expression of MARCO and SR-AI/II in the lungs of mice in an OVA-asthma model, we sought to determine whether SR-As contributed to defense of the lung against inhaled allergens using receptor-deficient mice and a model of allergic asthma. We found that sensitized mice lacking SR-A develop more severe airway inflammation and airway hyperresponsiveness (AHR) in response to inhaled aeroallergen. Because SR-As mediate macrophage binding and clearance of modified proteins, we initially expected that decreased clearance of Ag (OVA) by SR-A-deficient alveolar macrophages (AMs) would be a mechanism for increased allergic responses, but this postulate proved incorrect. We next evaluated the effect of SR-A-deficiency on the ability of Ag-loaded pulmonary DCs to migrate to the draining lymph nodes (LNs) and generate specific T cell responses. The data indicate that MARCO and SR-AI/II function in a novel mechanism to down-regulate migration of pulmonary DCs to thoracic LNs and thereby diminish T cell responses to specific aeroallergens.

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⁵ Abbreviations used in this paper: SR, scavenger receptor; SR-AI/II, SR class A, type I and type II; AHR, airway hyperresponsiveness; AM, alveolar macrophage; BAL, bronchoalveolar lavage; BALF, BAL fluid; Ct, threshold cycle; C3H, C3H/HeJ; DC, dendritic cell; i.t., intratracheal; KO, knockout; LN, lymph node; MARCO, macrophage receptor with collagenous structure; MΦ, macrophage; *P*_{enh}, enhanced pause; WT, wild type.

Materials and Methods

Animals

Six- to eight-week-old mice genetically deficient in MARCO (MARCO^{-/-}) or SR-AI/II (SR-AI/II^{-/-}) were used in all experiments. Age- and sex-matched wild-type (WT) (C57BL/6 and BALB/c) mice purchased from Charles River Laboratories were used as controls. MARCO^{-/-} mice were generated using targeted homologous recombination (9) and were backcrossed for at least 10 generations to the C57BL/6 background. SR-AI/II^{-/-} mice were generated by disrupting exon 4 of the SR-A gene, which is essential for the formation of functional trimeric

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receptors (11). Double knockout (KO) mice were obtained in our laboratory by intercross of single KO mice.

Both single KO mice were backcrossed in our laboratory to the BALB/c background for eight generations. DO11.10 mice, which are transgenic for the TCR recognizing OVA peptide 323–339, A/J, C3H/HeJ (C3H), and C3H/HeOJ mice were from The Jackson Laboratory. All animals were housed in sterile microisolator cages and had no evidence of spontaneous infection. Approval before all experimentation was obtained from Harvard School of Public Health institutional animal use review committee.

Mouse model of airway allergic inflammation

To compare allergic responses in SR-A and normal mice, groups of MARCO^{-/-}, SR-AI/II^{-/-}, and C57BL/6 WT control mice were sensitized i.p. with 8 μ g OVA in 1 mg of alum gel in 0.5 ml of PBS on days 0 and 7. On day 14 the sensitized mice were challenged with aerosolized 0.5% OVA or PBS for 1 h. Mice were sacrificed 72 h postchallenge, blood was collected through heart puncture, and the lungs were lavaged with PBS before they were harvested, inflated with formalin, and processed for histologic analysis.

In BALB/c mice, 10 μ g OVA in 2 mg of Alum powder were administered i.p. at days 0 and 7 followed by aerosol challenge with either PBS or 1% OVA for 30 min on days 14 and 15.

Microarray data analysis

Microarray data was acquired from the Public Expression Profiling Resource (<http://pepr.cnmcresearch.org>; see project 108). Gene expression data were calculated by using the GeneChip-Robust Multiarray Analysis algorithm (12) from the Bioconductor project (<http://www.bioconductor.org/>). The fold change in MARCO and SR-A gene expression was calculated as the ratio of the level in the OVA/control sample.

The raw *p* values were adjusted by false discovery rate correction and an adjusted *p* value <0.05 was interpreted as significant.

RT and real-time PCR

Total lung RNA was extracted from normal and OVA-sensitized and challenged BALB/c mice using a Qiagen RNeasy kit according to manufacturer's instructions. RNA purity was controlled by OD measurement. RNA concentrations were adjusted and the samples were reverse transcribed to cDNA using the novel SuperScript III first-strand cDNA synthesis kit (Invitrogen Life Technologies). cDNA samples were analyzed in duplicate in a quantitative real-time PCR using the SYBR Green Supermix (Bio-Rad) for MARCO and SR-A message with the following primer sequences (Integrated DNA Technologies): SR-A sense (5'-AGAATTTCAGCATGGC AACTG-3') and SR antisense (5'-ACGGACTCTGACATGCAGTG-3'); and MARCO sense (5'-GAAACAAAGGGGACATGGG-3') and MARCO antisense (5'-TTCACACCTGCAATCCCTG-3'). Murine β -actin was used as housekeeping control and a no-template sample was used as a negative control. Data are represented as Δ Ct (threshold cycle) values, with the lower values indicating a greater abundance of mRNA in the sample.

Measurement of airway hyperresponsiveness

AHR was measured in MARCO^{-/-}, SR-AI/II^{-/-}, and BALB/c WT mice using whole body plethysmography (Buxco; EMKA Technologies) 24 h after the last of two daily OVA or PBS aerosol challenges. The response of the airways to inhaled methacholine (Sigma-Aldrich) at concentrations ranging from 6.25 to 100 mg/ml (13) was recorded. AHR was expressed as enhanced pause (P_{enh}), a calculated value that correlates with airway resistance.

Bronchoalveolar lavage (BAL)

BAL was performed in situ with a 20-gauge catheter inserted into the proximal trachea, flushing the lower airways six times with 0.8 ml of PBS. The fluid retrieved from the first flushing was kept for ELISA. The BAL fluid cells were separated from the BAL fluid by centrifugation, resuspended in PBS, and counted and a fraction was cytospun on microscopic slides for staining with Diff-Quick (Baxter Scientific Products) for subsequent leukocyte differential counts.

OVA uptake studies

To prepare the OVA-FITC conjugate, OVA was dissolved in carbonate buffer (pH 9.2). Freshly prepared FITC in DMSO (10 mg/ml) was added at a ratio of 10 mg per 200 mg of OVA and the mixture was incubated at

room temperature in the dark for 1 h. To remove free FITC, the mixture was dialyzed for 24 h against PBS. MARCO^{-/-}, SR-AI/II^{-/-}, and C57BL/6 mice were given a 15-min aerosol treatment with a 10 mg/ml solution of OVA-FITC. BALs were performed 1 h later and cells were analyzed by flow cytometry. To test the binding of OVA to AMs in vitro, BAL fluid (BALF) cells (200 $\times$ 10³/well) from C57BL/6 WT and double KO mice were pretreated for 5 min with 5 μ M cytochalasin D and then incubated with 5 μ g/ml OVA-Alexa Fluor 488 for 40 min at 37°C and analyzed by flow cytometry.

Instillation of macromolecule solutions into the trachea

Mice were anesthetized by i.p. injection of 2.5% Avertin and received an intratracheal (i.t.) injection of 600 μ g of OVA-FITC in a volume of 60 μ l of sterile PBS. The trachea was carefully exposed via a small midline incision and the solution was inoculated. The incision was then closed with sterile silk and the mice were allowed to fully recover before being returned to the cages.

Preparation of single-cell suspensions and immunofluorescent labeling

Lung digestion medium consisted of RPMI 1640 (from Invitrogen Life Technologies) supplemented with 1 mg/ml collagenase type IV (Sigma-Aldrich) and 0.5 mg/ml DNase (deoxyribonuclease I from a bovine pancreas; Sigma-Aldrich). LN digestion medium consisted of 1 $\times$ HBSS (Cellgro; Mediatech) and 2% EDTA-treated FBS (HyClone) supplemented with 2.5 mg/ml collagenase type IV (Sigma-Aldrich). EDTA-treated FBS was prepared by adding 20 μ l of 0.5 M EDTA per ml of FBS. FACS staining buffer consisted of PBS (free of Ca²⁺ or Mg²⁺) supplemented with 5% FBS, 0.1% sodium azide, and 5 mM EDTA.

Preparation of lung and LN single-cell suspensions

Lung. Animals were euthanized by CO₂ narcosis. Following a thoracotomy, right heart catheterization was performed using a 21-gauge, 0.75-inch siliconized needle (SURFLO winged infusion set; Terumo) and the pulmonary circulation was perfused with at least 20 ml of sterile PBS to remove the intravascular pool of cells. Two milliliters of digestion medium were then injected in the trachea using a 22-gauge catheter and the trachea was quickly sealed with silk suture after the catheter was removed. The trachea and lungs were then removed and the lungs were carefully separated from the heart, thymus, and trachea and incubated at 37°C in additional 3 ml of digestion medium for 30 min. Incubation was then prolonged for an additional 30 min with vigorous pipetting of the samples at 10-min intervals with a 5-ml serological pipet. Subsequently, samples were passed through a 70- μ m nylon cell strainer, subjected to RBC lysis, incubated in calcium- and magnesium-free PBS containing 10 mM EDTA for 5 min at room temperature on a shaker, and finally resuspended in FACS staining buffer and kept on ice until immunofluorescent labeling.

Lymph nodes. For migration studies, animals were euthanized by CO₂ narcosis 24 h after an i.t. injection of OVA-FITC. For T cell proliferation studies, animals were euthanized by CO₂ narcosis 96 h after the injection of OVA. Following a thoracotomy, paratracheal and parathymic intrathoracic LNs were removed under a stereo microscope (Olympus SZ 60) and incubated at 37°C in 3 ml of LN digestion medium. After 10 min of incubation, LNs were minced with 20-gauge, 1.5-inch and 25-gauge, 0.625-inch needles (BD Biosciences) and incubation was prolonged for another 10 min. Subsequently, samples were passed through a 7- μ m nylon cell strainer, incubated in calcium- and magnesium-free PBS containing 10 mM EDTA for 5 min at room temperature on a shaker, and finally resuspended in FACS staining buffer and kept on ice until immunofluorescent labeling.

Labeling of single cell suspensions for flow cytometry

All staining procedures were performed at 4°C. Cells were preincubated for 20 min with a Fc receptor blocking Ab (anti-CD16/CD32; BD Biosciences) to reduce nonspecific binding. For lung studies, cells were subsequently stained with a PE-Cy5.5-conjugated hamster anti-mouse CD11c mAb (Caltag Laboratories) and data acquisition was performed using the FL1/FL3 template to allow assessment of the distribution of CD11c-bright cells with regard to autofluorescence. A PE-Cy 5.5-conjugated hamster IgG isotype control was used to determine background staining (Caltag Laboratories). Rat anti-F4/80 (IgG2a; clone 6F12) and rat anti Mac-3 (IgG1; clone M3/84) were from BD Biosciences. For migration studies, cells were stained with a PE-Cy5.5-conjugated hamster anti-mouse CD11c mAb (Caltag Laboratories). For T cell proliferation studies, cells were stained

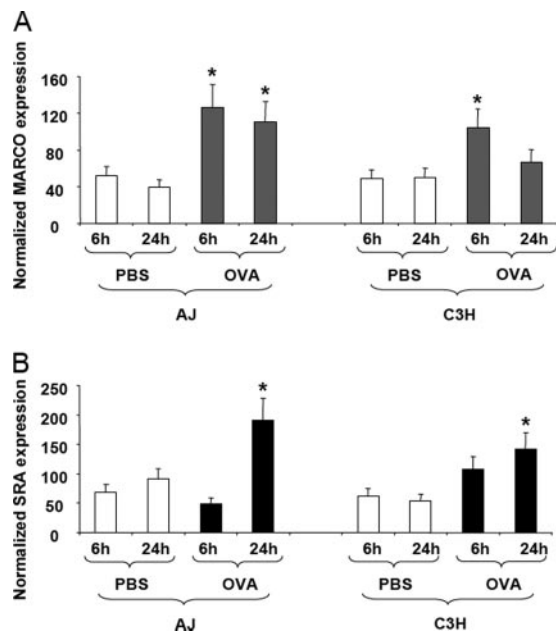


FIGURE 1. Augmented expression of MARCO and SR-AI/II in allergic asthma. Microarray analysis of RNA transcripts for MARCO (A) and SR-AI/II (SRA) (B) was performed on asthmatic and control A/J (AJ) and C3H mice at 6 h and 24 h after allergen or PBS challenge. Data are expressed as mean $\pm$ SD of normalized gene expression obtained from five mice. *, $p < 0.05$ vs same time point after PBS challenge.

with PE mouse anti-mouse DO11.10 TCR mAb (clone KJ1-26) (Caltag Laboratories). A PE-conjugated mouse IgG2a isotype control was used to determine background staining (Caltag Laboratories). Flow cytometry data acquisition was performed on a BD FACScan running CellQuest software (BD Biosciences). Flow Jo software (Tree Star) was used for data analysis. For lung and migration studies 50,000 total events were acquired for each sample. For T cell proliferation studies 500,000 total events were acquired for each sample. Dead cells were gated out based on light scatter properties.

In vivo assessment of T cell proliferation

CD4⁺ T cells were enriched from the spleens of DO11.10 mice by magnetic bead separation under sterile conditions using a mixture of biotin-conjugated mAbs against CD8a (rat IgG2a; Ly-2), CD11b (rat IgG2b; Mac-1), CD45R (rat IgG2a; B220), CD49b (rat IgM; DX5), and Ter-119 (rat IgG2b), followed by anti-biotin microbeads (colloidal superparamagnetic microbeads conjugated to a monoclonal anti-biotin Ab, mouse IgG1; clone Bio3-18E7.2) (Miltenyi Biotec). CD4⁺ DO11.10 T cells were subsequently labeled with 10 μ M CFSE (Sigma-Aldrich) at 37°C for 10 min as described by Lyons et al. (14) and then resuspended in sterile PBS. Mice received an i.v. injection of 10×10^6 CFSE-labeled DO11.10 T cells 24 h before an i.t. injection of 600 μ g of OVA in a volume of 60 μ l of PBS. Four days later T cell responses were analyzed in the draining mediastinal LNs by observing the CFSE division profiles of live KJ1-26⁺ CD4⁺ T cells. The number of transgenic T cells in each LN was calculated as percentage of KJ1-26⁺CFSE⁺ cells among the total cell number.

Statistical analysis

Student's *t* test (unpaired, two-tailed) was used to calculate significance levels for all measurements. Data are presented as mean $\pm$ SEM or SD. Differences were considered significant when $p < 0.05$.

Results

Increased MARCO and SR-AI/II gene expression in a murine model of asthma

To identify genes modulated in asthma, we analyzed public databases of microarray expression profiling in experimental murine asthma models. In a project conducted by M. Wills-

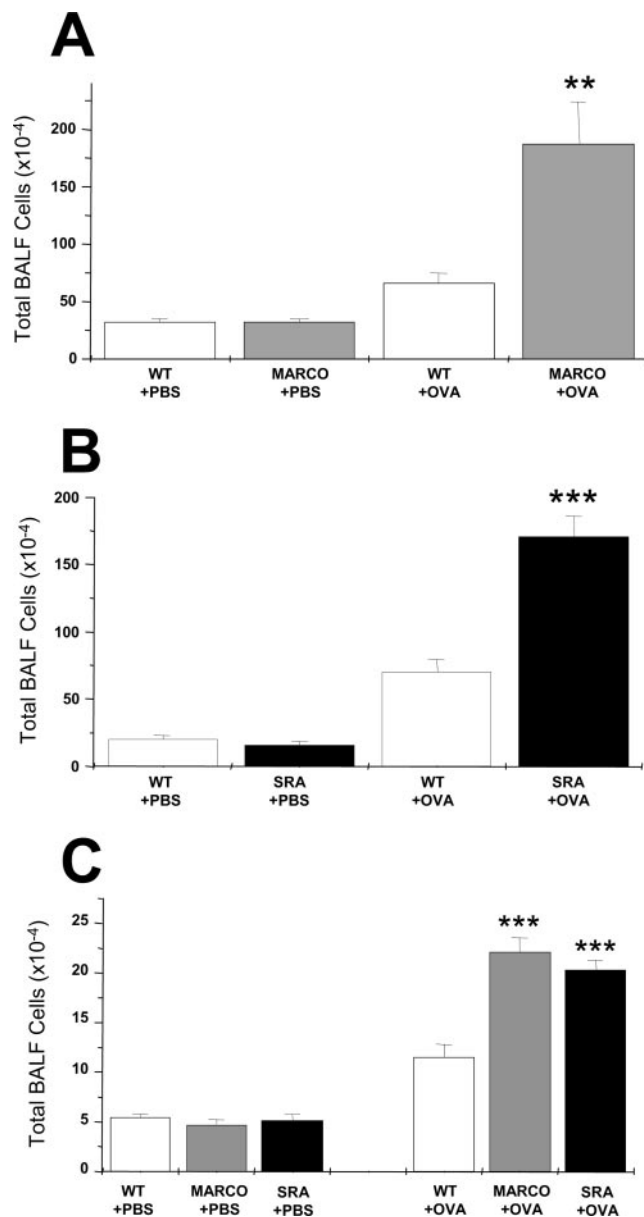


FIGURE 2. BAL cell yields after OVA-sensitization and challenge. Control (C57BL/6 or BALB/c), MARCO^{-/-} and SR-AI/II^{-/-} (SRA) mice were sensitized twice with OVA in alum and exposed once to 1% aerosolized OVA (+OVA). As a negative control, mice were immunized with OVA and challenged with PBS aerosol (+PBS). Seventy-two hours after aerosol exposure, lungs were lavaged and then fixed in formalin for H&E staining. Total leukocyte counts were determined in BALF of MARCO^{-/-}, SR-AI/II^{-/-}, and their WT counterparts in both C57BL/6 (A and B) and BALB/c (C) backgrounds. Data shown here are representative of 18 (A), 12 (B), and 6 mice (C) per group. **, $p < 0.01$; ***, $p < 0.001$; for OVA vs PBS challenge.

Karp (Cincinnati Children's Hospital Medical Center, Cincinnati, OH; data openly available online at the Public Expression Profiling Resource (<http://pepr.cnmcresearch.org/>)), the response to OVA exposure at 6 and 24 h following allergen challenge in both the A/J and C3H strains was determined by using five replicates of whole lung RNA from each experimental group. We processed the data as described in *Materials and Methods*. A comparison of allergen-challenged mice to saline-challenged mice revealed a significant up-regulation of MARCO and SR-AI/II after exposure to OVA in both strains (Fig. 1, A and B). A

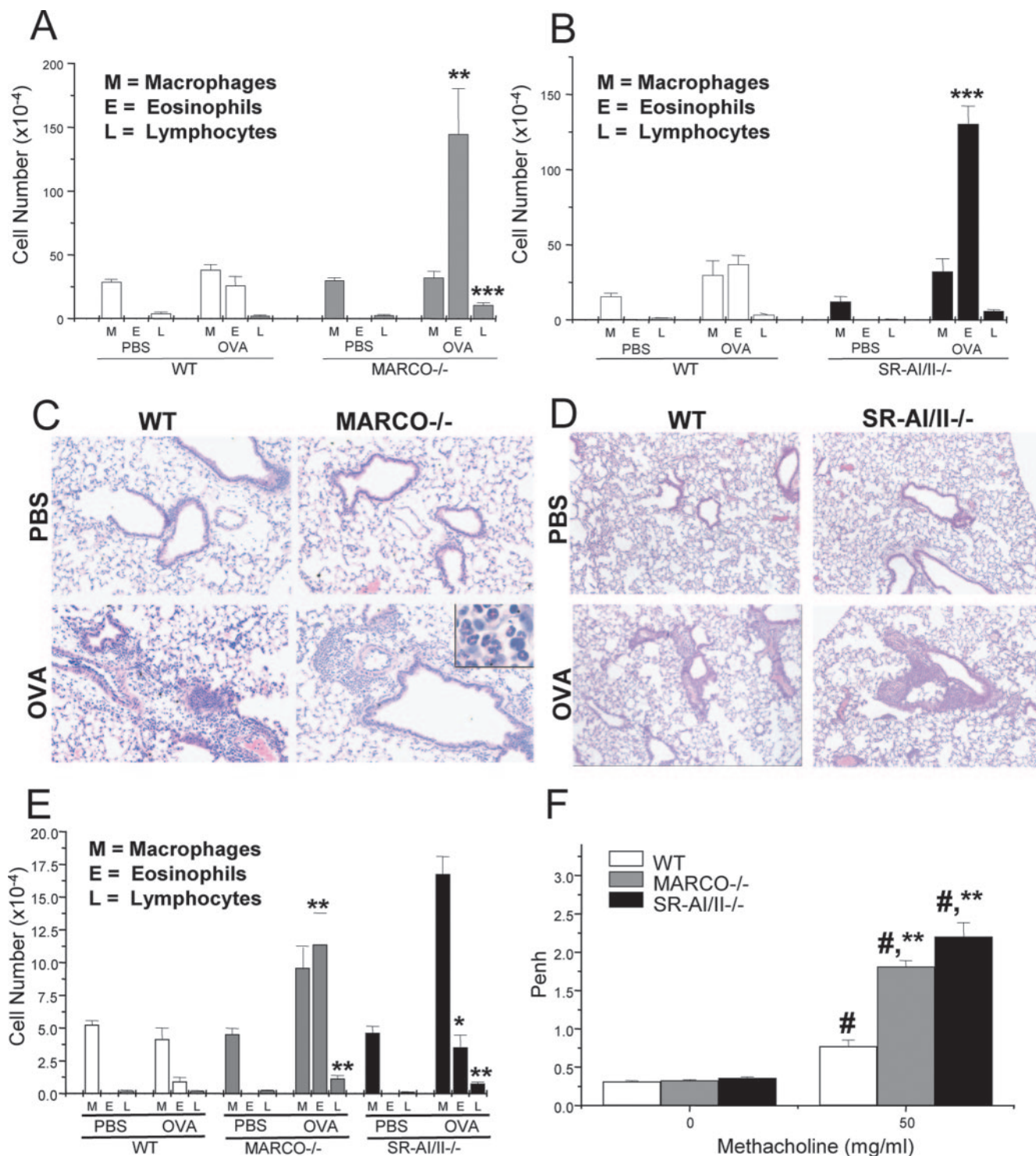


FIGURE 3. Response to OVA challenge in WT vs SR-A-deficient mice. Differential counts showing the amounts of BALF macrophages, eosinophils, and lymphocytes were determined on stained cytospin slides from MARCO^{-/-}, SR-AI/II^{-/-}, and their WT counterparts in both C57BL/6 (A and B) and BALB/c backgrounds (E). Data shown here are representative of 18 mice per group (A), 12 mice per group (B), and 6 mice per group (E). *, $p < 0.05$; **, $p < 0.01$; and ***, $p < 0.001$; for OVA vs PBS challenge. Representative photomicrographs of tissue histopathology are shown from WT (C and D), MARCO^{-/-} (C), and SR-AI/II^{-/-} (D) mice challenged with either PBS or OVA. *Inset* (C, lower right panel) shows higher magnification ($\times 600$) of eosinophils and mononuclear cells comprising the inflammatory infiltrates. Images are shown at a $\times 200$ original magnification for MARCO^{-/-} and $\times 100$ for SR-AI/II^{-/-} mice. SR-A-deficient mice also show enhanced airway hyperreactivity (F) as measured by whole body plethysmography in conscious OVA-sensitized and challenged BALB/c mice. P_{enh} , an index of airway obstruction, was recorded after aerosolization of either PBS or 50 mg/ml methacholine. Data represent mean P_{enh} values $\pm$ SEM (six mice per group for OVA/OVA and three mice per group for OVA/PBS). #, $p < 0.05$ vs PBS challenge; **, $p < 0.01$ vs WT mice.

similar trend was found in studies using the C57BL/6 strain (search for GDS348 on the Gene Expression Omnibus DataSets site: <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gds>).

We also observed increased MARCO and SR-A gene expression in RT-PCR analysis of lung samples from OVA-sensitized and exposed mice compared with controls (e.g., ΔCT values for

OVA vs controls were 5.3 ± 0.6 vs 6.6 ± 0.4 (SR-A) and 7.2 ± 1.1 vs 9.2 ± 1.5 (MARCO); lower ΔCT values indicate a greater abundance of mRNA targets).

Increased severity of airway inflammation in SR-A deficient mice during allergic asthma

We next directly analyzed the physiologic relevance of the two SR-A receptors, MARCO and SR-AI/II, in vivo in a murine model of allergic airway inflammation caused by OVA sensitization and aerosol challenge. Due to the unavailability of SR-A KO mice in the susceptible A/J and resistant C3H backgrounds used in the microarray studies, we used the C57BL/6 and BALB/c strains, both known to show pulmonary expression of SR-As (8, 9) and to be prone to OVA-induced airway inflammation (15, 16). Seventy-two hours after the aerosol challenge, sham-challenged mice (OVA/PBS groups) showed no sign of inflammation, whereas all OVA/OVA groups showed a remarkable increase in the total number of leukocytes recruited to the airways (Fig. 2, A and B). Notably, the total number of eosinophils and lymphocytes in the BAL samples from OVA/OVA groups was substantially greater in the SR-A-deficient mice relative to their control counterparts (Fig. 3, A and B). OVA/PBS mice, in contrast, did not show any recruitment of eosinophils into their airways. Consistent with the increased leukocyte numbers in lavage samples of OVA-challenged SR-A-deficient mice, histologic analysis of lungs harvested from these mice showed allergic inflammation consisting of peribronchial and perivascular cell infiltrates of eosinophils and mononuclear cells (Fig. 3, C and D).

Unlike C57BL/6 mice, allergen-sensitized BALB/c mice develop easily detectable AHR following exposure to inhaled allergen (15, 16). MARCO^{-/-} and SR-AI/II^{-/-} mice on the BALB/c background also showed a significant increase in eosinophils and macrophages (Fig. 3E) and total cell number (Fig. 2C) in the BALF following OVA challenge, compared with their WT counterparts. The basis for the increased macrophage number in the KO mice on the BALB/c background is unknown. It is also worth mentioning that the discrepancy in both the intensity and the nature of cellular inflammatory responses between C57BL/6 and BALB/c strains after exposure to inhaled OVA is an expected result of the different induction protocols we have used to achieve significant eosinophilic recruitment and the Ag dose-dependent response in these strains. Whole body plethysmography was used to evaluate pulmonary function changes after OVA challenge in WT vs MARCO^{-/-} and SR-AI/II^{-/-} mice. Following aerosolized bronchoconstrictor (methacholine) challenge, WT mice showed a slight, but significant, increase in AHR relative to the baseline ($p < 0.05$). In contrast, MARCO^{-/-} and SR-AI/II^{-/-} mice showed a much more robust response ($p < 0.01$; Fig. 3F), consistent with their greater allergic inflammatory response.

WT and SR-A-deficient lung MΦs show normal uptake of inhaled OVA allergen

AMs can efficiently bind and internalize unopsonized particles and bacteria through SR-As, leading to the clearance of inhaled matter from the airways and the reduction of the resulting inflammation (7–9) and are known to similarly bind modified proteins (17–19). To determine whether SR-As could reduce allergic inflammation by simply “scavenging” aeroallergen with a resulting decrease in allergen dose, we measured their ability to internalize inhaled allergens using FITC-OVA. WT and KO mice were exposed to inhaled fluorescent OVA, the airways were lavaged 1 h later, and the total fluorescence of AMs was evaluated by flow cytometry.

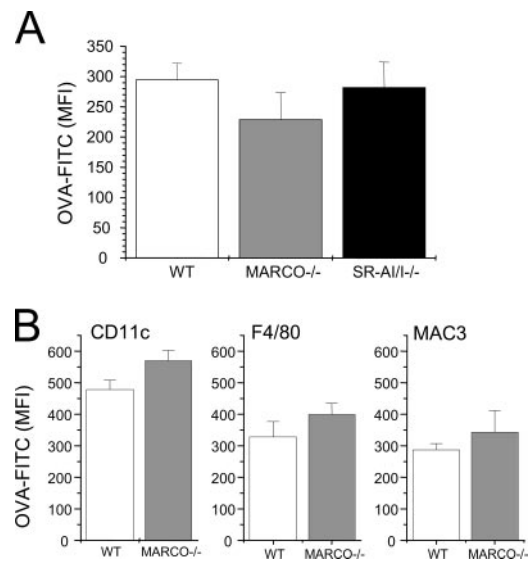


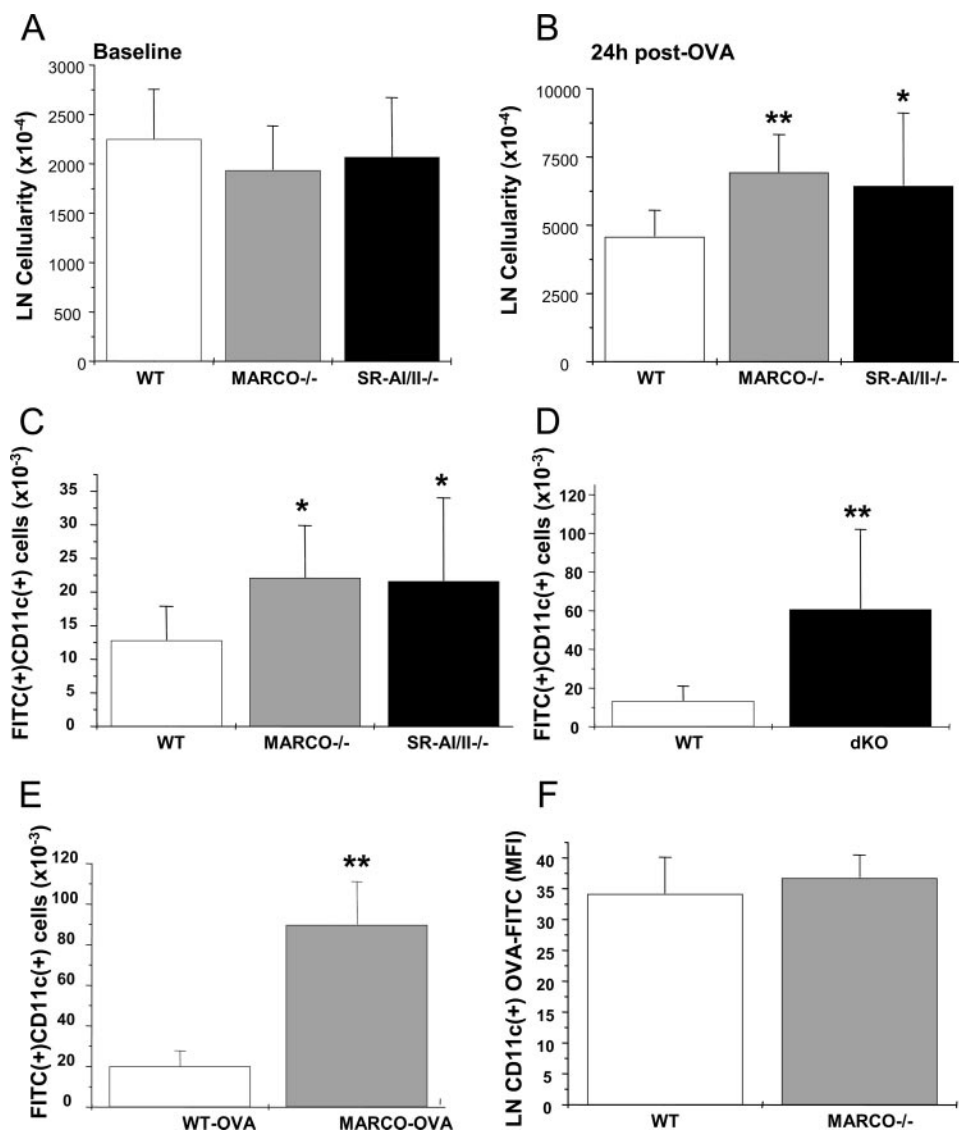
FIGURE 4. Absence of SR-As on AMs does not affect the clearance of inhaled allergen. **A**, Control (C57BL/6), MARCO^{-/-}, and SR-AI/II^{-/-} mice were challenged with aerosolized OVA-FITC (10 mg/ml) for 15 min. The lungs were lavaged 1 h later with PBS and cells were analyzed by flow cytometry to compare the uptake of fluorescent OVA. Data shown represent the mean ± SEM of four mice per group. **B**, In separate experiments, three WT and three MARCO^{-/-} mice received 600 μg of OVA-FITC i.t. The lungs were harvested 4 h later and cell suspensions prepared by homogenization were labeled with Abs to either CD11c, F4/80, or MAC3 Abs and the mean green fluorescence intensity (MFI) of the double positive cells was determined.

Similar amounts of FITC-OVA were found associated with AMs in WT, MARCO^{-/-}, and SR-AI/II^{-/-} mice ($p > 0.05$), indicating essentially identical uptake in vivo (Fig. 4A). In parallel experiments, FITC-OVA was administered i.t. to the mice and the amount of OVA associated with the MΦ population was determined on the cells isolated from whole lung homogenates. The total amount of FITC-OVA on MΦs, as discriminated by gating of the CD11c⁺, F4/80⁺, or MAC3⁺ populations, was similar in both WT and MARCO^{-/-} mice ($p > 0.05$; Fig. 4B). In vitro assays confirmed that the absence of receptors did not affect AM binding of OVA, as double-deficient AMs bound Alexa Fluor 488-OVA to nearly the same extent as did control AMs (data not shown). These findings are consistent with previous reports indicating that, unlike chemically modified albumin, native albumin binding to MΦs is not mediated through SR-As (17–19), and they also indicate that nebulization does not per se denature the allergenic proteins sufficiently to create SR-A binding domains.

Allergen-loaded SR-A-deficient DCs show increased migration from the lungs to the draining LNs

We next sought to investigate whether another SR-A-expressing cell type, the lung DC, was involved in the increased asthmatic phenotype seen in SR-A-deficient mice. Airway DCs capture Ags in the lungs and migrate to the regional LNs where they present the Ag to the specific T cells. To track DC migration from the lungs to the draining LNs, we administered OVA-FITC i.t. and analyzed cell suspensions prepared from mediastinal LNs 24 h later. DCs were labeled with anti-CD11c Ab and the number of cells expressing the CD11c and also carrying FITC was determined by flow cytometry. Although there are no significant differences in LN cellularity under basal conditions (Fig. 5A), OVA challenge of the airways resulted in an increase in LN cellularity, an increase which

FIGURE 5. Increased migration of MARCO^{-/-} and SR-AI/II^{-/-} pulmonary DCs in response to an inhaled Ag challenge. *A* and *B*, The total cell content of homogenized mediastinal LNs from C57BL/6, MARCO^{-/-}, and SR-AI/II^{-/-} mice was determined before (*A*) or 24 h after (*B*) they received 600 μ g of OVA-FITC i.t. *C–F*, LN suspensions from OVA-FITC-challenged mice were stained for CD11c and the fraction of FITC⁺ DCs was determined by flow cytometry (*C*). A similar protocol was applied to double KO (dKO) mice (*D*) and OVA-sensitized C57BL/6 and MARCO^{-/-} mice (*E*). Also, the amount of green fluorescence associated with the DCs was determined (*F*). Data represent the mean $\pm$ SD from two or more separate experiments with at least six mice per genotype, *, $p < 0.05$; **, $p < 0.01$.



is greater in the SR-A-deficient mice (Fig. 5*B*). A striking finding was that SR-A-deficient mice showed a significantly greater number of Ag-loaded DCs in the thoracic LNs (Fig. 5*C*), indicating that DC migration is more efficient in the KO mice. Double KO mice showed an even greater migration of airway DCs after OVA challenge compared with control mice and single deficient mice (Fig. 5*D*). These studies were performed in unimmunized mice. We next assessed DC migration in OVA-sensitized WT and MARCO^{-/-} mice. Notably, although the sensitized WT mice showed an elevated migration of airway DCs to the LNs after OVA exposure (note the expanded range of the y-axis), the increase was even more marked in the MARCO^{-/-} mice (Fig. 5*E*). To determine whether differences in Ag (OVA) uptake by WT or SR-A-deficient DCs could mediate the enhanced allergic responses in SR-A-deficient mice, we also evaluated the OVA-FITC content of the DCs that reach the LNs after Ag challenge (measured as green fluorescence). We observed the same amounts of Ag in the DCs reaching the LNs in both WT and MARCO^{-/-} mice (Fig. 5*F*). This indicates that SR-A-deficiency does not alter the uptake of OVA-FITC Ag by DCs, a finding similar to data obtained with macrophages (Fig. 4). To evaluate the potential of trace endotoxin in the OVA preparation to modulate DC migration, we performed OVA-FITC in-

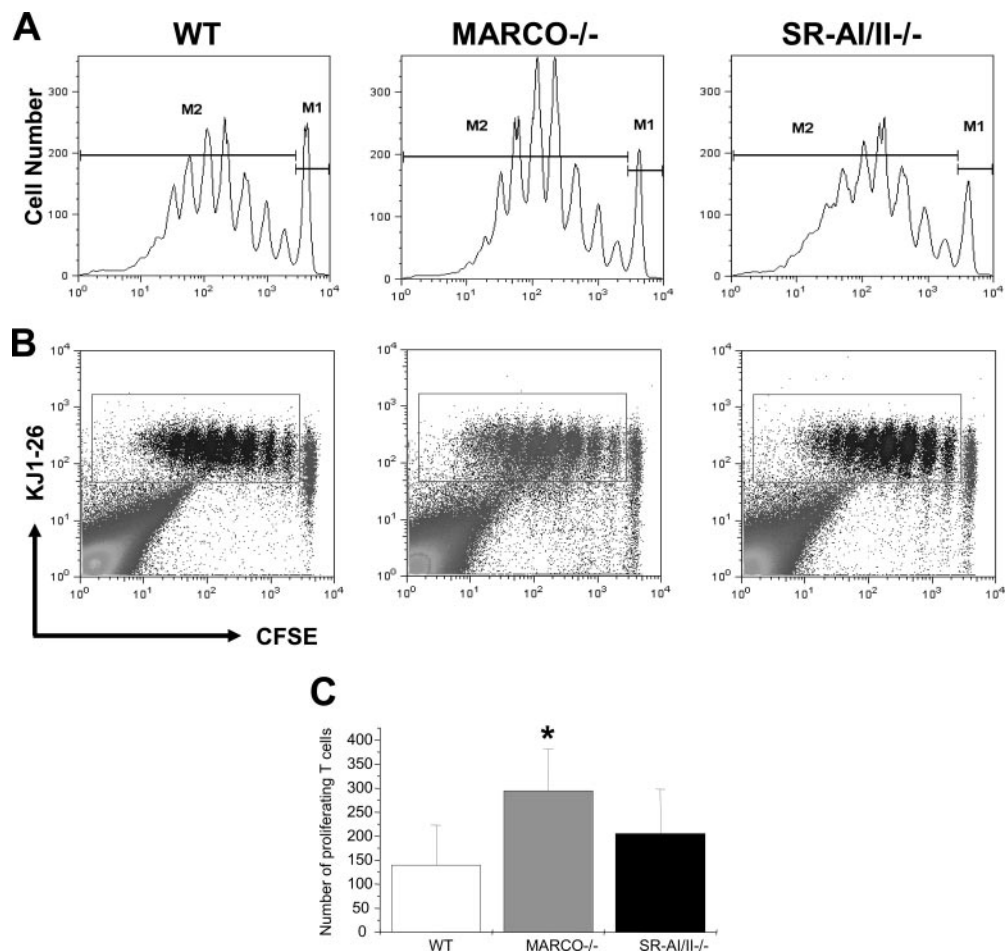
stillation into endotoxin-sensitive and resistant (C3H/OuJ and C3H/HeJ respectively). No differences were observed in the numbers of migrated FITC⁺CD11c⁺ DCs found in thoracic LNs in the two strains of mice (data not shown).

To evaluate the possibility that the enhanced DC migration in KO mice was due to a higher basal number of DCs in the lungs, we quantified the lung DC population in naive MARCO^{-/-} and control mice. Lung DCs were defined as bright CD11c⁺ cells with low autofluorescence, as described by Vermaelen and Pauwels (20). We found that the number of lung DCs was not statistically different between MARCO^{-/-} and their control WT mice (data not shown).

Allergen challenged SR-A deficient mice show enhanced T cell priming in the draining LNs

To more directly test the functional significance of augmented Ag-loaded DC migration in SR-A-deficient mice, we used an adoptive transfer model to assess T cell proliferation in the draining LNs after Ag challenge. BALB/c WT, MARCO^{-/-}, and SR-AI/II^{-/-} mice were injected i.v. with CFSE-labeled OVA-specific CD4⁺ T lymphocytes from DO11.10 transgenic mice. Recipient mice were challenged i.t. with OVA 24 h later. The mediastinal LNs were

FIGURE 6. Enhanced Ag-induced T lymphocyte proliferation in mediastinal LNs of SR-A-deficient mice. CFSE-labeled spleen CD4⁺ T cells from DO11.10 mice were transferred i.v. into BALB/c, MARCO^{-/-}, and SR-AI/II^{-/-} mice 24 h before i.t. administration of OVA. Ninety-six hours later, cell suspensions were prepared from draining LNs and stained with KJ1-26-PE Ab. Representative histograms (A; cells in the M2 zone have undergone at least one division) and dot plots (B; cells in the rectangles have undergone at least one division) are shown for control, MARCO^{-/-}, and SR-AI/II^{-/-} mouse groups. The deduced absolute number of cells that underwent at least one division is shown in C. Data represent the mean $\pm$ SD from eight (MARCO^{-/-}) and 12 mice (SR-AI/II^{-/-}). *, $p < 0.05$.



harvested 96 h after OVA challenge for an analysis of dye dilution as a function of cell division.

Comparable numbers of adoptively transferred DO11.10 T cells reached the mediastinal LNs in all three groups of mice (data not shown) and, similarly, comparable fractions underwent at least one division (percentage of cells showing decreased CFSE was 91, 93.5, and 92%, respectively, in WT, MARCO^{-/-}, and SR-AI/II^{-/-} mice). However, there was a greater proliferative response in the LNs of MARCO^{-/-} mice ($295 \pm 83 \times 10^3$; mean $\pm$ SD) compared with WT mice ($140 \pm 83 \times 10^3$), indicating that a higher absolute number of T cells had undergone a greater number of divisions in the MARCO^{-/-} mice (Fig. 6). This indicates that the larger numbers of Ag-loaded DCs that migrate to the draining LNs of the MARCO^{-/-} mice result in a greater proliferative response by Ag-specific T lymphocytes. SR-AI/II^{-/-} mice showed a similar trend that did not reach statistical significance in T cell proliferation ($206 \pm 92 \times 10^3$) compared with control mice.

Discussion

The data presented identify a novel role for SR-As expressed on lung DCs in modulating pulmonary responses to aeroallergens. The context for our findings includes the recognition of the important role of DCs in the pathogenesis of asthma (21) and as professional APCs that bridge innate and adaptive immunity (22, 23). DCs express SR-AI/II, which functions in Ag presentation and adaptive immunity (17, 19, 24–28). For example, SR-AI/II^{-/-} mice are deficient in mounting an efficient T cell response to maleylated murine serum albumin, a known SR-AI/II ligand (29). In

contrast, the role of MARCO receptors in modulating adaptive immunity has not been examined.

It has been postulated that MARCO expression is induced upon DC maturation (30, 31). Although we did not directly address the maturation state of pulmonary DCs in naive WT mice, we know the following: 1) only immature DCs can take up and process Ag (32); 2) immunohistochemical studies show the expression of MARCO only on MΦs in the normal lung (8) with an absence of MARCO labeling in normal airways that contain CD11c⁺ airway DCs; and 3) mediastinal LN DCs express MARCO after OVA challenge (data not shown). This suggests that pulmonary DCs start to express MARCO after allergen encounter, consistent with the increased MARCO gene expression observed after OVA challenge in microarray studies.

Some limitations of the study merit discussion. For some control experiments, only MARCO-deficient mice were analyzed (e.g., the migration of DCs in OVA-sensitized mice; Fig. 5E). Hence, the full extent to which SR-AI/II deficiency mirrors the findings with MARCO-deficient mice requires further characterization. One potential problem to be considered is the confounding effects of trace endotoxin in the OVA allergen. Two lines of evidence argue against this possibility. First, no differences were observed in the numbers of migrated FITC⁺CD11c⁺ DCs found in either endotoxin-sensitive or endotoxin-resistant thoracic LNs (C3H/OuJ and C3H/HeJ respectively). Second, we have previously reported similar levels of cytokine release (TNF- α and MIP-2) by AMs from WT and KO mice in response to LPS in vitro (9), arguing against differential responses on this basis.

In peripheral tissues such as the lungs, DCs exist normally in an immature state and provide a sentinel function for foreign Ags (32). Upon Ag encounter, DCs undergo a process of maturation that triggers their migration to draining LNs and enhances their Ag-presenting capacity (23). The migration of Ag-loaded DCs from peripheral tissues to the LNs is a critical step in generating an optimal immune response (33, 34) and, hence, a potential regulatory point.

SR-As may inhibit DC migration through a number of mechanisms. SR-As have been shown to promote adhesion to matrix molecules (35, 36) and to other cells, e.g., marginal zone macrophages to B cells (37), and either of these interactions could potentially reduce cell migration. Pikkarainen et al. (38) have previously shown that fibroblastic cell lines transfected with MARCO undergo significant morphologic changes through the induction of dendritic plasma membrane processes. These processes include the appearance of large lamellipodia-like structures and long plasma membrane extensions. Moreover, a clear correlation exists between MARCO expression and the rearranged actin cytoskeleton of mature DCs (30), although in this study MARCO expression upon maturation was associated with a decrease in filopodia and a round phenotype. The morphologic changes induced by simple MARCO expression do not require its interaction with any given ligand. The rearrangements induced by MARCO in fibroblastic cell lines were shown to be partially dependent on Rac1 (38). Rac, together with Rho and Cdc42, represent a group of small GTPases involved in the formation of filopodia and podosomes in immature DCs (39), structural changes that could increase adhesion and reduce migration. To test the speculation that adhesion to a matrix might mediate some of the observed effects, studies of the kinetics of induction of MARCO expression on airway and LN DCs after allergen challenge are warranted, as well as a comparison of the migration capacity of MARCO-deficient and WT DCs. Additional mechanisms are suggested by data indicating the ability of scavenger receptors, when present, to skew the cytokine milieu and immune response toward Th1-type immunity (40). Indeed, one of the mechanisms leading to increased AHR in allergic asthma is the enhanced recruitment of eosinophils into the allergen-challenged airways as we observed in the lungs of SR-A deficient mice. This may be a consequence of the increased recruitment of Th lymphocytes and an altered cytokine milieu.

Innate immune responses are increasingly recognized as critical modifiers of adaptive immunity (41, 42). In the example presented here, the innate pattern recognition receptors, the SR-As, mediate reduced amounts of total Ag delivery to LNs (through decreased numbers of DCs carrying similar amounts of Ag per cell). SR-A-mediated down-regulation of lung immune responses likely contributes to the reduction of unwanted immune responses to commonly encountered environmental aeroallergens.

Disclosures

The authors have no financial conflict of interest.

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