Dendritic cells, but not macrophages or B cells, activate major histocompatibility complex class II-restricted CD4$^+$ T cells upon immune-complex uptake *in vivo*

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Summary

Professional antigen-presenting cells (APC) are able to process and present exogenous antigen leading to the activation of T cells. Antigen–immunoglobulin (Ig)G complexes (IC) are much more efficiently processed and presented than soluble antigen. Dendritic cells (DC) are known for their ability to take up and process immune complex (IC) via FcγR, and they have been shown to play a crucial role in IC-processing onto major histocompatibility complex (MHC) class I as they contain a specialized cross-presenting transport system required for MHC class I antigen-processing. However, the MHC class II-antigen-processing pathway is distinct. Therefore various other professional APC, like macrophages and B cells, all displaying FcγR, are thought to present IC-delivered antigen in MHC class II. Nonetheless, the relative contribution of these APC in IC-facilitated antigen-presentation for MHC class II *in vivo* is not known. Here we show that, in mice, both macrophages and DC, but not B cells, efficiently capture IC. However, only DC, but not macrophages, efficiently activate antigen-specific MHC class II restricted CD4$^+$ T cells. These results indicate that mainly DC and not other professional APC, despite expressing FcγR and MHC class II, contribute significantly to IC-facilitated T cell activation *in vivo* under steady-state conditions.

Keywords: dendritic cells; macrophages; antigen presentation/processing; Fc receptors

Introduction

CD4$^+$ T cells can control many activities of the immune system. In order to exert their action, the CD4$^+$ T cells need to recognize their specific antigen in the context of major histocompatibility complex (MHC) class II molecules. In contrast to MHC class I molecules, MHC class II molecules have a more restricted distribution pattern, and they are predominantly expressed, under normal conditions, by professional antigen-presenting cells (APC), like B cells, macrophages and dendritic cells. These professional APC also express a variety of receptors that are involved in the uptake of potentially antigenic materials. For example, it has been shown that DC and macrophages can capture antigens through the uptake of, for example, apoptotic cells, antigen-heat shock protein-complexes and antigen–immunoglobulin (Ig)G complexes (IC).[1] For the uptake of these antigens, the APC display several receptors like mannose-receptors, CD91, CD36, complement receptors, and FcγR. Although these receptors are crucial for the clearance of pathogens or apoptotic cells, they are at the same time also involved in the more efficient presentation of the exogenously acquired antigen to the cellular arm of the immune system.[2,3] For example, several *in vitro* studies have shown that APC present IgG-complexed antigen much more efficiently to MHC class I-restricted CD8$^+$ T cells than soluble antigen.[4,5] This efficient cross-presentation is dependent on the expression of FcγR, and is

Abbreviations: APC, antigen-presenting cell; DC, dendritic cell; DTR, diphtheria toxin receptor; FcγR, Fc receptor for IgG; GFP, green fluorescent protein; IC, immune complex; Ig, immunoglobulin; MACS, magnetic activated cell sorting; OVA, ovalbumin; pMF, peritoneal macrophages.

thought to be an unique property of DC as these cells possess a specialized cross-presentation transport system for MHC class I antigen-presentation.[6–10]

Although DC can efficiently present antigen from IC in a FcγR-dependent fashion in the context of both MHC class I and class II molecules[11,12] the rules governing antigen-presentation for MHC class I and II molecules are rather distinct. MHC class I-presentation of internalized antigen taken up by DC results from antigen-shuttling from endocytic compartments into the cytoplasm for further processing.[13] In contrast, internalized antigen can be loaded directly onto MHC class II-molecules in MHC class II-loading compartments for subsequent presentation to $CD4^+$ T cells.[1] Thus, unlike the requirements for MHC class I processing and presentation, the requirements to allow presentation of exogenously acquired antigen for MHC class II seem to be generally distributed among professional APC.

Because MHC class II expression in conjunction with FcγR expression is not confined to DC, it has been postulated that also other professional APC, like macrophages and B cells, can equally well cross-present IgG-complexed antigen in the context of MHC class II.[5] Indeed, several studies performed *in vitro* indicated that FcγR can mediate capture and presentation of IC by both B cells and macrophages.[14–18] However, the relative contribution of these professional APC to IC-facilitated antigen-presentation in the context of MHC class II molecules *in vivo* has not been studied in detail.

Here, we set out to determine the cross-presentation of IC for MHC class II presentation by professional APC (i.e. B cells, macrophages, and DC) *in vivo* in order to define the relative importance of these cell types for IC-dependent antigen-presentation *in vivo*. Our studies is the first that showed that DC, and not macrophages or B cells, despite MHC class II expression and the apparent presence of the required MHC class II-processing machinery, efficiently activate MHC class II-restricted $CD4^+$ T cells *in vivo* after injection of IC.

Materials and methods

Mice

BALB/c mice ($H\text{-}2^d$) were obtained from Charles River Nederland (Maastricht, the Netherlands). DO11.10 transgenic mice, which have a transgenic T-cell receptor (TCR) specific for the ovalbumin $(OVA)_{323-339}$ epitope in the context of $I\text{-}A^d$, were bred in our own specific pathogen-free animal facility. CD11c-DTR (diphtheria toxin receptor) transgenic mice ($H\text{-}2^d$), which carry a transgene encoding a simian DTR-GFP (green fluorescent protein) fusion protein under control of the murine CD11c promoter[10,19] were also bred in our own facility.

Antibodies

The following antibodies were purchased from PharMingen (San Diego, CA): phycoerythrin (PE)-coupled anti-CD11c antibody (HL3), fluoroscein isothiocyanate (FITC)-coupled anti-CD11b antibody (M1/70), FITC-coupled goat anti-mouse (GAM) antibody, allophycocyanin (APC)-coupled GAM antibody and APC-coupled anti-CD4 antibody (L3T4). The PE labelled mouse monoclonal antibody to the mouse DO11.10 TCR (KJI-26) was purchased from Caltag Laboratories (Burlingame, CA). Stainings were performed at 4° for 20 min. After washing, the stained cells were analysed using a FACSCalibur® flow cytometer equipped with CellQuest software (Becton Dickinson, San Jose, CA).

Generation of OVA immune complexes

Immune complexes (IC) were generated by incubating soluble OVA (Sigma-Aldrich, Zwijndrecht, the Netherlands) with polyclonal OVA-specific rabbit IgG (rIgGαOVA) (MP Biomedicals, Aurora, OH), at a ratio of 1 μg OVA to 25 μg rIgGαOVA in phosphate-buffered saline (PBS), for 30 min at 37°. As a control, soluble OVA was preincubated with 25 μg control rIgG (Sigma-Aldrich). IC were then injected intravenously (i.v.) into BALB/c or CD11c-DTR transgenic mice.

^{99m}Tc-labelling of OVA

To follow association of soluble OVA and IC to different APC *in vivo*, OVA was directly labelled with ^{99m}Tc before generation of IC. Briefly, 10 μl of an OVA solution (10 mg/ml) was added to 4 μl of a mixture of 950 mg/l $SnCl_2.2H_2O$ and 2 g/l sodium pyrophosphate.$10H_2O$ in PBS. Immediately thereafter, 2 μl of a solution containing 10 mg/ml of KBH_4 in 0·1 M NaOH was added into this mixture.[20] After addition of 0·1 ml of ^{99m}Tc-sodium pertechnetate solution (^{99m}Tc, approximately 200–500 MBq/ml, Technekow, Mallinckrodt Medical BV, Petten, the Netherlands) the mixture was gently stirred at room temperature for 1 hr. More than 95% of the OVA was radioactively labelled with ^{99m}Tc. This labelled compound will further be referred to as ^{99m}Tc-OVA. The radioactive labelled OVA was further used for the generation of IC as described above. ^{99m}Tc-labelled OVA and IC were then i.v. injected into BALB/c mice.

Isolation of antigen-presenting cells

At several time points after i.v. injection of soluble OVA or IC in BALB/c mice, spleen cells from these mice were isolated and sorted for DC, macrophages and B cells. Spleen cells were first sorted in a $CD11c^+$ (the DC in mice) and a $CD11c^-$ fraction, using CD11c-specific

microbeads and the magnetic-activated cell sorting (MACS) system (LS$^+$ columns) (Miltenyi, CLB, Amsterdam, the Netherlands) according to manufacturer's instructions. The CD11c$^-$ cells were further used to isolate macrophages and B cells. Therefore, these cells were stained for FITC-coupled anti-CD11b antibody (for macrophages) or FITC-coupled GAM antibody (for B cells) in PBS/0·5% bovine serum albumin (BSA) for 15 min at 4°. Then the CD11c$^-$ CD11b$^+$ and CD11c$^-$ GAM$^+$ cells were purified using anti-FITC microbeads and the MACS system (LS$^+$ columns). After sorting, the purified CD11c$^+$ cells (DC), the CD11c$^-$ CD11b$^+$ cells (macrophages), and the CD11c$^-$ GAM$^+$ cells (B cells) were washed and resuspended in Iscove's modified Dulbecco's medium (IMDM) (BioWhittaker, Verviers, Belgium) supplemented with 8% heat-inactivated fetal calf serum (FCS; Bodinco, Alkmaar, the Netherlands), 100 IU/ml penicillin/streptavidin (BioWhittaker), 2 mM L-glutamine (Invitrogen, Breda, the Netherlands) and 20 μM 2-mercaptoethanol (2-ME; Merck, Hohenbrunn, Germany). This yielded cell populations that contained 92·2 ± 3·0% CD11c$^+$ cells (DC-fraction), 85·3 ± 2·6% CD11b$^+$ cells (macrophage-fraction), or 98·9 ± 0·3% surface Ig$^+$ cells (B cell-fraction). Similar techniques were applied as well in experiments analysing the distribution of IC using ^{99m}Tc-labelled OVA or IC. The radioactivity to each cell fraction was determined in a dose-calibrator (VDC.101, Veenstra Instruments, Joure, the Netherlands) at each interval/step of cell purification.

Peritoneal macrophages were isolated from the peritoneal cavity 24 hr following injection of 2 ml thioglycolate medium. The peritoneal cavity was flushed with 5 ml of ice-cold PBS. After lysis of the erythrocytes and washing of the obtained cells, the macrophages were allowed to adhere to tissue-coated culture plates for 1 hr. The non-adherent cells were removed thoroughly and the macrophages were incubated with OVA-IC for 2 hr. Subsequently, the IC was washed away and the macrophages were collected using a cell scraper and transferred to a 96-flat-bottomed plate to be used in a T-cell stimulation assay. For these purposes, we took advantage of DO11.10-hybridoma cells that secrete IL-2 after activation.

T-cell proliferation assay

To detect OVA presentation, the sorted DC, macrophages and B cells were used as stimulators for OVA-specific DO11.10 cells in a [^{3}H]-thymidine incorporation assay. Irradiated APC (1×10^5, $1{\cdot}5 \times 10^5$ or 2×10^5) were incubated with $0{\cdot}5 \times 10^5$ DO11.10 cells in round bottom plates in 150 μl IMDM. As a positive control, 0·01 μg/ml, 0·1 μg/ml or 1 μg/ml $OVA_{323–339}$ peptide was added to APC derived from untreated mice. After 72 hr, the plates were pulsed for 18 hr with 0·5 μCi/well of [^{3}H]-thymidine and harvested.

Antigen-presentation in vivo

To follow T-cell proliferation *in vivo*, DO11.10 cells were labelled with the intracellular fluorescent dye 5(6)-carboxyfluorescein diacetate succinimidyl estes (CFSE) (Molecular Probes, Leiden, the Netherlands). Briefly, spleen and lymph node cells were isolated from DO11.10 mice and CD11c$^+$ cells were removed using CD11c-specific microbeads and the MACS system. Cells were washed with PBS containing 0·1% BSA (Sigma-Aldrich) and resuspended at 10×10^6 cells/ml in PBA/0·1% BSA. Next, 5 μM CFSE was added and cells were incubated for 10 min at 37°. Then 10% FCS was added and cells were washed twice with IMDM and resuspended in PBS/1%BSA. DO11.10 cells (3×10^6) were injected i.v. into BALB/c or CD11c-DTR transgenic mice.

To deplete CD11c$^+$ cells *in vivo*, CD11c-DTR transgenic mice were injected intraperitoneally (i.p.) with 4 ng diphtheria toxin (DT; Sigma)/4 g body weight in 200 μl PBS. In these mice the DTR is inserted under the CD11c-promoter.[19] Eighteen hr after DT injection, BALB/c and CD11c-DTR transgenic mice were i.v. injected with IC. Three days thereafter, cells from the spleen and inguinal lymph nodes were analysed for proliferation of the DO11.10 cells using a FACSCalibur® flow cytometer.

Results

IC are efficient in inducing CD4$^+$ T-cell proliferation *in vivo*

Professional APC are able to capture, process and present exogenous antigen leading to activation of T cells. Several *in vitro* studies showed that antigen complexed with IgG are much more efficiently presented than soluble antigen in a FcγR-mediated fashion.[4,5] To determine whether this enhancement also occurs *in vivo* in the context of MHC class II, wild type mice were injected with CFSE-labelled OVA-specific CD4$^+$ T cells derived from DO11.10 mice and treated with different concentrations of soluble OVA or OVA bound to IgGαOVA. Three days after injection of the antigen, T-cell proliferation in the spleen and lymph nodes was analysed.

Soluble OVA induces T-cell proliferation when injected at a concentration of 100 μg/mouse, but no proliferation was detectable any more when OVA was administrated at a concentration of 10 μg or lower. In contrast, injection of OVA incubated with anti-OVA IgG still resulted in significant proliferation of DO11.10 cells when injected at a dose of 0·1 μg OVA/mouse (Fig. 1). Injection of OVA incubated with control IgG did not induce proliferation at a concentration of 10 μg or lower (data not shown). These results indicate that IC are at least 100 times more efficient in inducing CD4$^+$ T-cell proliferation than soluble OVA.

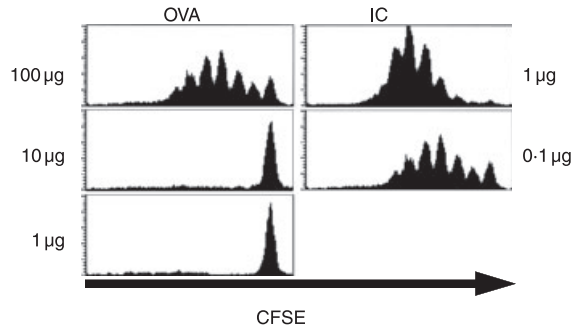

Figure 1. OVA-IC are at least 100 times more efficient then soluble OVA in inducing T-cell proliferation. CFSE labelled DO11·10 cells were transferred into mice. These mice were subsequently injected i.v. with different concentrations of soluble OVA or OVA complexed to anti-OVA IgG ($n = 2$). Three days after injection, proliferation of the DO11·10 cells ($CD4^+/KJI26^+$) was analysed in the spleen and lymph nodes (not shown). One representative experiment out of two performed is presented.

DC and/or macrophages are the dominant APC that present IgG-complexed antigen *in vivo*

DC are known to take up and process IC via FcγR. Also other professional APC, like macrophages and B cells, display FcγR and are thought to present antigen that have been acquired through FcγR.[4,5,14,18,21]

To test the importance of the different APC in IC-mediated antigen-presentation to $CD4^+$ T cells directly *in vivo*, wild-type and CD11c-DTR transgenic mice were injected with CFSE-labelled OVA-specific $CD4^+$ T cells and immunized with IC. $CD11c^+$ DC in the latter mouse strain can be depleted *in vivo* by injection of DT.[10] As shown in Fig. 2, injection of IC induces T-cell proliferation in both mouse strains. Injection of DT into wild-type mice had no effect on T-cell proliferation (Fig. 2a), showing that DT does not affect antigen-specific T-cell proliferation of DO11.10 cells. However, when DC were depleted in the CD11c-DTR transgenic mice by injection of DT, proliferation of the T cells was almost completely abolished (Fig. 2b). Even when a 10 times higher concentration of IC was injected, no proliferation was induced in mice depleted for $CD11c^+$ cells (data not shown). Until recently, these data indicate that the DC, but not the macrophages and B cells, are the predominant cell type responsible for IC-mediated, MHC class II-restricted $CD4^+$ T cell activation *in vivo*. However, a recent publication elegantly showed that DT injection into these CD11c-DTR transgenic mice also depletes marginal zone and metallophilic macrophages.[22] Therefore, these experiments can not make a distinction between the contributions of macrophages versus DC in the presentation of immune-complexed antigen to $CD4^+$ T cells *in vivo*, but do show that B cells are not involved in this process.

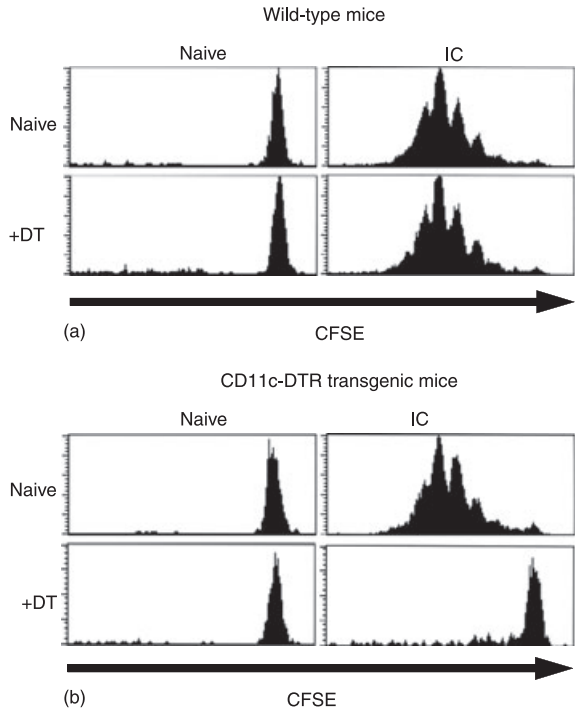

Figure 2. DC and/or macrophages are the predominant cells for presentation of IgG-complexed OVA to OVA-specific $CD4^+$ T cells *in vivo*. CFSE-labelled DO11.10 cells were transferred into wild-type (a) or CD11c-DTR transgenic mice (b). These mice were or were not treated with DT, as indicated, and injected i.v. with 1 µg IC ($n = 2$). Three days after IC injection, proliferation of the DO11.10 cells ($CD4^+/KJI26^+$) was analysed. One representative experiment out of two performed is presented.

Macrophages do capture IC efficiently

As macrophages and B cells readily express FcγR, they are thought to be able to capture IC. To determine whether macrophages and B cells capture IgG-complexed OVA, 5 µg radioactively labelled (^{99m}Tc) OVA or ^{99m}Tc-OVA complexed to anti-OVA IgG was injected into mice. One hr after injection, spleens were taken and sorted for the different types of APC (see Materials and methods and Fig. 3). The time point of 1 hr was chosen as previous studies have shown that 100% of IC are cleared from the bloodstream within this period.[23] Uptake of IC by the different APC was analysed by measuring the radioactivity present in the DC-, macrophage- and B-cell fractions. To this end, cells were counted and the amount of ^{99m}Tc present per 10^6 cells was determined. As shown in Fig. 4(a), a high level of radioactive label was present in the macrophage-fraction. This was even higher than the radioactivity measured in the DC-fraction (on a per cell basis). Although B cells were also able to acquire ^{99m}Tc-IC, the amount of radioactivity measured in the B-cell fraction was relatively low. ^{99m}Tc-OVA, not complexed with anti-OVA IgG, was hardly detectable in any cell fraction at this concentration.

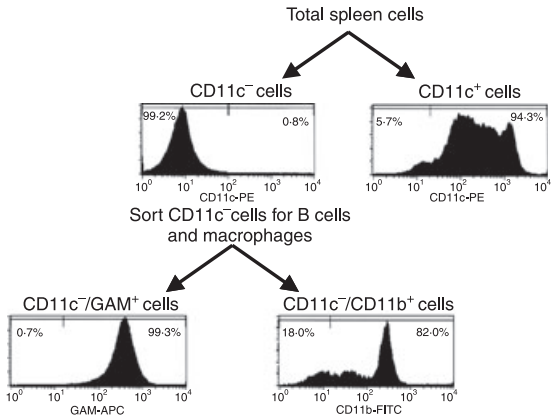

Figure 3. Purity of APC populations. Total spleen cells were first sorted in a $CD11c^+$ (the DC) and a $CD11c^-$ fraction (see Materials and methods). The $CD11c^-$ cells were further divided into a $CD11b^+$-fraction (macrophages) and a GAM^+-fraction (B cells). The purified cell populations contained 92·2 ± 3·0% $CD11c^+$ cells (DC), 98·9 ± 0·3% $CD11c^-$ GAM^+ cells (B cells), and 85·3 ± 2·6% $CD11c^-$ $CD11b^+$ cells (macrophages).

To demonstrate that the ^{99m}Tc-labelled IC follows the same pattern of antigen-presentation as unlabelled IC, wild-type mice, injected with CFSE-labelled OVA-specific $CD4^+$ T cells, were infused with labelled- or unlabelled IC and $CD4^+$ T-cell proliferation was analysed. As shown in Fig. 4(b), ^{99m}Tc-labelling had no apparent effect on the ability to present immune-complexed antigen. Together, these results indicate that both macrophages and DC, but not B cells, are able to capture IC efficiently and further emphasize the inability of B cells to present immune-complexed antigen to $CD4^+$ T cells.

Macrophages do not present IC to $CD4^+$ T cells

To gain more insight into the ability of the different types of APC to capture and present IC to $CD4^+$ T cells, we infused 5 μg IC i.v. into naive mice. One hr after IC injection, spleen cells were taken and separated into the different APC fractions. These APC populations (i.e. DC, macrophages and B cells) were then analysed for their ability to induce proliferation of OVA-specific $CD4^+$ T cells.

Figure 5(a) shows that only the $CD11c^+$ cell fraction, but not macrophages or B cells, are able to induce proliferation of DO11.10 cells *in vitro* after administration of 5 μg IC *in vivo*. Also at relatively high stimulator to responder cell ratio's no significant proliferation of T cells stimulated by macrophages was observed (Fig. 5b). When OVA-peptide was added to the different APC populations, all fractions were able to activate DO11.10 cells (Fig. 5c), indicating that lack of proliferation in the B-cell and macrophage cultures was not a consequence of an hampered ability of these cells to present antigen and

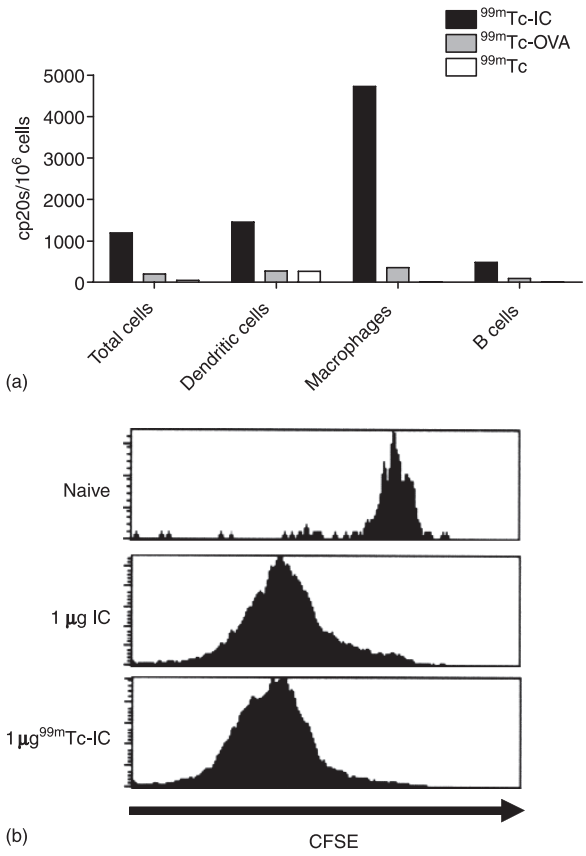

Figure 4. DC and macrophages are able to capture IC. (a) Wild-type mice were injected i.v. with 5 μg ^{99m}Tc-labelled soluble OVA, OVA-IC or free ^{99m}Tc as control. One hr after injection, spleen cells were isolated and sorted for DC, macrophages and B cells. The amount of ^{99m}Tc acquired by the cells was subsequently determined. Data show the counts/20 s/10^6 cells. One representative experiment out of two performed is presented. (b) Mice, previously injected with CFSE-labelled DO11.10 cells, were injected i.v. with the unlabelled or the ^{99m}Tc-labelled IC. Three days after IC injection, proliferation of the DO11·10 cells ($CD4^+$/$KJI26^+$) was analysed in the spleen.

activate DO11.10 cells. When soluble OVA was injected in the same concentration as IC, none of the different types of APC were able to induce T-cell proliferation (data not shown). Likewise, peritoneal macrophages (pMF) loaded *in vitro* with 1 μg OVA-IC were not able to activate DO11.10 hybridoma cells unless loaded exogenously with free OVA-peptide. In contrast, DC loaded with the same amount of OVA-IC efficiently activated DO11.10 cell (Fig. 5d). Together, these results indicate that the DC, and not the macrophages and B cells, are the most prominent APC in IC-mediated, MHC class II-restricted, antigen-presentation.

Discussion

In this study we investigated the ability of DC, macrophages and B cells to acquire and present OVA-IC to

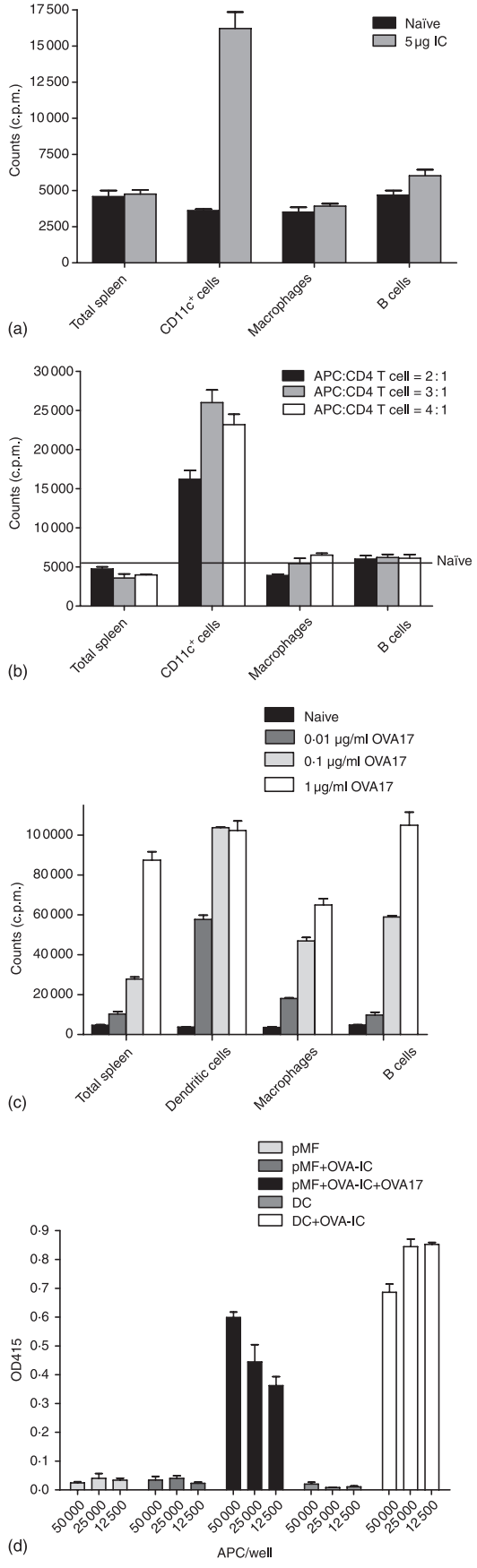

Figure 5. DC take up and present IgG-complexed OVA to OVA-specific $CD4^+$ T cells. Wild-type mice were injected i.v. with 5 μg OVA complexed to anti-OVA IgG or were left untreated (a). One hr after injection, spleen cells were isolated and sorted for DC ($CD11c^+$ cells), macrophages and B cells. These cells were subsequently added to DO11·10 cells in a ratio of 2 : 1 (see Materials and methods), to analyse their ability to stimulate T cells. Proliferation of the DO11·10 cells was determined by [^{3}H]-thymidine incorporation. (b) The same experiment as described above was performed with S : R ratios of 3 : 1 and 4 : 1. (c) To control whether the different isolated APC fractions are able to stimulate T cells, the APC were incubated with 0·01 μg/ml, 0·1 μg/ml or 1 μg/ml OVA peptide (see Materials and methods). Proliferation of the DO11.10 cells was determined as described in (a). Error bars indicate SEM of triplicate wells. One representative experiment out of four performed is presented. (d) Macrophages (pMF; 50 000; 25 000 and 12 500 cells/well) isolated from the peritoneal cavity were incubated with 1 μg OVA-IC and subsequently used for stimulation of 50 000 DO11.10-hybridoma cells. Only pMF loaded with OVA-peptide were able to activate DO11.10 cells. In contrast, DC were able to activate DO11.10 cells efficiently after incubation with IC. Activation of DO11.10 cells was detected by the production of IL-2 in the supernatant using enzyme-linked immunosorbent assay. The OD at 450 nm is depicted. Error bars indicate SEM of triplicate wells. One representative experiment out of two performed is presented.

OVA-specific $CD4^+$ T cells *in vivo*. Our results revealed that DC, but not macrophages or B cells are able to activate $CD4^+$ T cells efficiently following FcγR-facilitated antigen capture, despite the fact that macrophages readily capture IC *in vivo* and express abundant amounts of FcγR, costimulatory molecules and MHC class II molecules (data not shown).[21] These results are important as they show that the predominant cell type able to present antigen derived from IC to $CD4^+$ T cells is the DC.

Previously, it has been shown that DC efficiently facilitate IC-mediated antigen-presentation in the context of MHC class I molecules.[9] However, the pathways leading to antigen-presentation via MHC class I or class II molecules are distinct.[1] MHC class I molecules primarily present peptides derived from endogenously synthesized proteins of either self or pathogen origin. In contrast, MHC class II molecules generally present peptides derived from exogenous proteins that enter the cell through the endocytic route. Nevertheless, exogenous antigen can also be presented in the context of MHC class I molecules by DC, as these cells express specialized compartments that are thought to be involved in antigen-shuttling from endocytic compartments into the cytoplasm and further processing for MHC class I.[7,13,24] In contrast, internalized antigen present in the endocytic compartment can be loaded directly onto MHC class II molecules.[1] Earlier studies have shown that all three types of APC (i.e. DC, macrophages and B cells) are able to take up and present soluble antigen *in vivo*.[25]

As macrophages and B cells also express FcγR, next to MHC class II and MHC-loading compartments, they are thought to be able to take up and present IgG-complexed antigen to CD4$^+$ T cells.[5] Although the macrophage fraction was able to induce some T-cell proliferation (stimulation index: 2·9 versus 12·3 by DC-fraction) when a very high dose of IC was injected (i.e. 40 μg, a higher dose is lethal; unpublished observations), our results indicate that the ability to present exogenously acquired IC-derived antigen in the context of MHC class II molecules for activation of CD4$^+$ T cells is a characteristic that is predominantly confined to DC.

Our findings contrast other reports that show that macrophages present exogenously loaded IgG-complexed antigen to MHC class II-restricted T cells *in vitro*. It has been reported that targeting of antigen to FcγR on activated macrophages *in vitro* can bias a T helper 1 (Th1) response to a Th2-like response.[14] In addition, several *in vitro* studies demonstrated that also B cells can capture and present IC via FcγR[16,18] although others indicated that FcγRII, expressed by B cells is incompetent for endocytosis.[26] In all cases, direct evidence that macrophages and B cells present IgG-complexed antigen *in vivo* is lacking. Our results in the experiments with radioactive labelled IC show that macrophages, but not B cells, readily capture IC but hardly activate CD4$^+$ T cells. The reasons underlying these observations are intriguing, and are conceivably related to unique features displayed by DC. It has been shown that DC, but not B cells, can accumulate antigen for later presentation.[27] After exposure to inflammatory signals or other activating signals, like FcγR-cross-linking, newly formed immunogenic MHC class II-peptide complexes are transported to the cell surface through the formation of tubules from lysosomal compartments that go on to fuse directly with the plasma membrane.[28,29] Although it is not clear whether this tubule-mediated pathway is unique for DC, tubular extensions in macrophages and B cells formed in response to certain activators have not been reported to mediate transport to the cell surface but instead are thought to facilitate phagosome–lysosome fusion.[30,31] Macrophages thus seem to function more as scavenger cells, which quickly remove the IgG-bound antigen from the body, then as APC for activation of T cells.

Professional APC, particularly macrophages, are playing a crucial role in the defence against micro-organisms like bacteria. In several model systems, it has been shown that macrophages can present bacterial antigen to MHC class II-restricted T cells. However, in most instances, these pathogens, like *Mycobacterium tuberculosis* or *Listeria monocytogenes*, are residing inside the macrophages. Thus, in these cases, rather than that they have been obtained from exogenous sources, the antigen are from sources constitutively present inside macrophages, possibly allowing proper antigen-processing and presentation. Although, several studies indicated the ability of DC to cross-present antigen for MHC class II, in general, and IC in particular, it might not be surprising that macrophages are not able to cross-present IC-derived antigen. An important function of CD4$^+$ T cells resides in their ability to enhance macrophage activity to endow the macrophages with the capacity to kill intracellular pathogens. In case, however, the antigen is derived from exogenous sources, no such enhancement is needed, and therefore, in this case, the macrophage does not need to communicate with CD4$^+$ T cells.

Macrophages and B cells are also playing a pivotal role in various autoimmune diseases. Next to their ability to produce pro-inflammatory cytokines after FcR-triggering, it is proposed that they are also able to present antigen to CD4$^+$ T cells.[32–35] Although our study is performed under steady-state conditions, rather than under inflammatory conditions as found in ongoing autoimmune diseases, our findings suggest that these professional APC are not contributing to the induction and perpetuation of autoimmune diseases through presentation of antigen to (pathogenic) CD4$^+$ T cells. Therefore, we consider it more likely that the IC-related contribution of these professional APC to autoimmune diseases is associated to release of pro-inflammatory cytokines and molecules after FcγR-cross-linking, the inefficient clearance of IC, or the destruction of autoantibody opsonized cells. The ability to orchestrate and steer the T-cell response is the responsibility of DC, further emphasizing the crucial role of DC in controlling (ongoing) T-cell responses.

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