The Journal of Immunology

A Novel Role of Complement Factor C1q in Augmenting the Presentation of Antigen Captured in Immune Complexes to $CD8^+$ T Lymphocytes

Nadine van Montfoort, Judith M. H. de Jong, Danita H. Schuurhuis, Ellen I. H. van der Voort, Marcel G. M. Camps, Tom W. J. Huizinga, Cees van Kooten, Mohamed R. Daha, J. Sjef Verbeek, Ferry Ossendorp and René E. M. Toes

J Immunol 2007; 178:7581-7586; ;
doi: 10.4049/jimmunol.178.12.7581
http://www.jimmunol.org/content/178/12/7581

The Journal of Immunology is published twice each month by
The American Association of Immunologists, Inc.,
1451 Rockville Pike, Suite 650, Rockville, MD 20852

Print ISSN: 0022-1767 Online ISSN: 1550-6606.

A Novel Role of Complement Factor C1q in Augmenting the Presentation of Antigen Captured in Immune Complexes to CD8$^+$ T Lymphocytes[1]

Nadine van Montfoort,[2]* Judith M. H. de Jong,[2†] Danita H. Schuurhuis,* Ellen I. H. van der Voort,[†] Marcel G. M. Camps,* Tom W. J. Huizinga,[†] Cees van Kooten,[‡] Mohamed R. Daha,[‡] J. Sjef Verbeek,[3§] Ferry Ossendorp,[3,4]* and René E. M. Toes[3†]

Ag-IgG immune complexes (IC) are efficiently taken up, and Ag-derived peptides are subsequently processed and presented by APC. In vitro experiments indicate that IgG Fc Receptors (FcγR) facilitate the efficient uptake of IC by dendritic cells. Previous experiments showed that the cross-presentation of Ag-derived peptides after s.c. administration of IC is FcγR-dependent. To study the role of different FcγR and complement in MHC class I Ag presentation after i.v. administration, we used mice deficient for FcγRs and complement components. These mice were injected with CFSE-labeled OVA-specific CD8$^+$ T cells followed by administration of IC composed of OVA and rabbit anti-OVA IgG i.v. to measure MHC class I presentation of OVA-derived peptides. The Ag presentation was partly reduced in FcRγ-chain-deficient mice, but not affected in FcγRI/II/III-deficient mice, complement factor C3-deficient mice, or FcγRI/II/III × C3-deficient mice. Importantly, CD8$^+$ T cell proliferation was significantly reduced in mice deficient for C1q. This proliferation could be restored when IC were incubated with purified human C1q before injection. Likewise, purified C1q could strongly enhance the uptake and presentation of IC by dendritic cells in vitro. Heat inactivation abrogated the C1q-mediated uptake of IC. In addition, in vivo uptake of OVA-IC in the spleen was significantly reduced in C1q-deficient mice compared with wild-type mice. Together, these results indicate a novel function of C1q, which is present in high levels in the bloodstream, by directly enhancing the uptake and MHC class I presentation of Ag captured in IC by APC to CD8$^+$ T cells. ***The Journal of Immunology,*** **2007, 178: 7581–7586.**

Professional APC, such as dendritic cells (DC),[5] are able to process and present exogenous Ag in MHC class I, leading to the activation of naive CD8$^+$ T cells, in a process called cross-presentation (1). DC may acquire exogenous Ag by receptor-mediated uptake, pinocytosis or phagocytosis. Receptor-mediated uptake is generally very efficient. Ag may enter DC via a variety of receptors, such as mannose receptors or IgG Fc receptors (FcγR) (2–5).

We have previously demonstrated that the FcγR-mediated uptake of the model Ag OVA enabled cross-presentation to CD8$^+$ T cells in vitro (5, 6) and that OVA-anti-OVA IgG immune complexes (OVA-IC) are 100-1000-fold more efficiently presented compared with free OVA protein.

FcR are receptors for the Fc part of Ig, providing a link between the cellular and the humoral part of the immune response. FcR are membrane glycoproteins, which belong to the Ig supergene family. In mice, four types of FcγR are described, that is, three activating receptors, FcγRI (CD64), FcγRIII (CD16), and FcγRIV, and one inhibitory receptor, FcγRII (CD32) (7–9). Activating FcγR are expressed by many cell types of the immune system, particularly DC, monocytes, macrophages, NK cells, mast cells, eosinophils, and neutrophils. The inhibitory receptor FcγRII is expressed on all hemopoietic cells, except T and NK cells. When FcγRs are cross-linked by immune complexes (IC), the balance between activation and inhibition signals determines the cellular response (10).

Next to FcγR, also the complement system is known to interact with IC. Three pathways of complement activation have been described: the classical pathway, the alternative pathway, and the lectin (i.e., mannan-binding lectin and ficolins) pathway (11, 12). Each pathway has its own activation and recognition mechanism, resulting in the formation of C3-convertases that cleave the central complement component C3 into the fragments C3a and C3b. Binding of C3b enables clearance of pathogens and IC as well as the generation of the lytic membrane attack complex.

C1q is one of the activator compounds of the classical complement pathway and plays an important role in the activation of the classical complement pathway and the clearance of IC from the circulation (13, 14). It is present in high levels in the bloodstream (15). C1q contains a collagen-like tail region to which the serine proteases C1r and C1s are bound, connected to a globular head region responsible for ligand binding. The heads recognize defined regions within the C_H2 domain of Ag-complexed IgG molecules,

*Department of Immunohematology and Blood Transfusion; †Department of Rheumatology; ‡Department of Nephrology; and §Department of Human and Clinical Genetics, Leiden University Medical Center, Leiden, The Netherlands

Received for publication November 23, 2006. Accepted for publication April 12, 2007.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This work was supported by the following grants: Dutch Cancer Society UL 2004-3008 (to N.v.M.), The Netherlands Organisation for Scientific Research (NOW) 901-07-249 (to J.M.H.d.J.), and VIDI (to R.E.M.T.).

[2] N.v.M. and J.M.H.d.J. equally contributed to this work.

[3] J.S.V., F.O., and R.E.M.T. equally contributed to this work.

[4] Address correspondence and reprint requests to Dr. Ferry Ossendorp, Leiden University, Albinusdreef 2, Leiden, The Netherlands. E-mail address: F.A.Ossendorp@lumc.nl

[5] Abbreviations used in this paper: DC, dendritic cells; FcγR, IgG Fc receptors; OVA-IC, OVA-anti-OVA IgG immune complexes; IC, immune complexes; B6, C57BL/6K^h; WT, wild-type; KO, knockout; rIgGαOVA, OVA-specific rabbit IgG; hC1q, human-derived C1q; OVA-Alexa488, Alexa Fluor488-conjugated OVA.

thereby opsonizing ICs. Upon binding to its ligand, C1q changes conformation, which leads to the activation of its associated serine proteases, C1r and C1s.

In vitro, uptake of OVA-IC and cross-presentation of OVA-derived peptides is FcγR-dependent (6). In vivo, s.c. injected IC are preferably taken up via FcγR without the apparent involvement of the complement system (16). In this study, we analyzed the contribution of FcγR and the complement system in the cross-presentation of peptides derived from i.v. administered OVA-IC. Our results identify an important role for C1q in IC uptake and Ag presentation, as mice deficient for C1q displayed a substantially reduced ability to present immune-complexed Ag to CD8$^+$ T cells.

Materials and Methods

Mice

C57BL/6K^h (B6; H-2^b) and OT-I/CD45.1 mice, which have a transgenic Vα2Vβ6 TCR specific for the OVA$_{257–264}$ epitope in the context of H2-K^b, were bred in the specific pathogen-free animal facility of the Leiden University Medical Center. FcRγ-chain-deficient mice were provided by Dr. T. Saito, (Laboratory for Cell Signaling RIKEN Research Center for Allergy and Immunology, Yokohama, Japan). C3-deficient mice were provided by Dr. M. C. Carroll (CBR Institute for Biomedical Research, Harvard Medical School, Boston, MA). C1q-deficient mice were kindly provided by M. Botto (Rheumatology Section, Faculty of Medicine, Hammersmith College, London, U.K.) (17). FcγRI/II/III-deficient mice and FcγRI/II/IIIxC3-deficient mice were generated in our laboratory. All mice were backcrossed on a B6 background for at least six generations. The animal experiments have been reviewed and approved by the animal experimental committee of Leiden University.

Adoptive transfer of CFSE-labeled OT-1 cells

To follow T cell proliferation in vivo, OT-I/CD45.1$^+$ cells labeled with the intracellular fluorescent dye CFSE (Molecular Probes) were used. Single-cell suspension from spleen and lymph node cells from OT-I/CD45.1$^+$ mice were depleted for DC using CD11c-specific microbeads and the MACS system (LS$^+$ columns; Miltenyi Biotec) according to the manufacturer's instructions. Cells were incubated with 5 μM CFSE in PBS/0.1% BSA (Sigma-Aldrich) for 10 min at 37°C. FACS analysis was performed to assess CFSE labeling efficiency and the percentage of CD8$^+$Vα2$^+$ cells in the OT-1 donor-derived population. Wild-type (WT) or knockout (KO) recipients were injected with OT-1 cells containing a standardized number of 3×10^6 CD8$^+$Vα2$^+$ cells in 200 μl PBS/0.1% BSA i.v.

Generation of OVA IC for i.v. injection

IC were generated by incubating OVA (Worthington Biochemical) with polyclonal OVA-specific rabbit IgG (rIgGαOVA; Valeant Pharmaceuticals), at a ratio of 1 μg OVA to 50 μg rIgGαOVA in PBS, for 30 min at 37°C. IC containing 1 μg OVA were injected i.v. into the WT or the KO mice, 1 day after infusion of the OT-1 cells.

For the experiments described later (see Fig. 5*B*), IC were incubated with purified human-derived C1q (25 μg hC1q/1 μg IC) for 1 h at room temperature before i.v. administration. C1q was obtained as described (18).

Analysis of CD8$^+$ T cell proliferation in vivo

To analyze proliferation of the CD8$^+$ T cells derived from OT-I/CD45.1$^+$ mice in vivo, spleens were collected from the recipient mice 3 days after the injection of OVA-IC. Spleen cells were incubated with allophycocyanin-coupled anti-CD8α Ab and PE-coupled anti-CD45.1 Ab (BD Pharmingen) and analyzed using a FACSCalibur flow cytometer equipped with CellQuest software (BD Biosciences). Live CD8$^+$/CD45.1$^+$ T cells were gated and the CFSE division profile was analyzed. The contribution of the different FcγR and complement was assessed by comparing CD8$^+$ T cell proliferation between the KO mouse strains and the WT controls included in each experiment.

Uptake of Alexa Fluor488-conjugated OVA (OVA-Alexa488) by DC

D1 cell line DC were cultured as described (19). OVA-Alexa488-IC were generated by incubating 1 μg/ml OVA-Alexa488 (Molecular Probes) with 50 μg/ml polyclonal rIgGαOVA (Valeant Pharmaceuticals) for 30 min at 37°C. OVA Alexa488 in IC were incubated with different concentrations of hC1q for 1 h at room temperature in a flat-bottom 96-well plate. In the experiments using heat-inactivated C1q, the C1q molecules were, before adding to the IC, incubated for 60 min at 56°C. After 1 hour, 100,000 D1 cells were added to each well to incubate with the IC and C1q for 1 h at 37°C. After 1 hour, cells were washed intensively to remove IC and C1q. Cells were then analyzed by FACS.

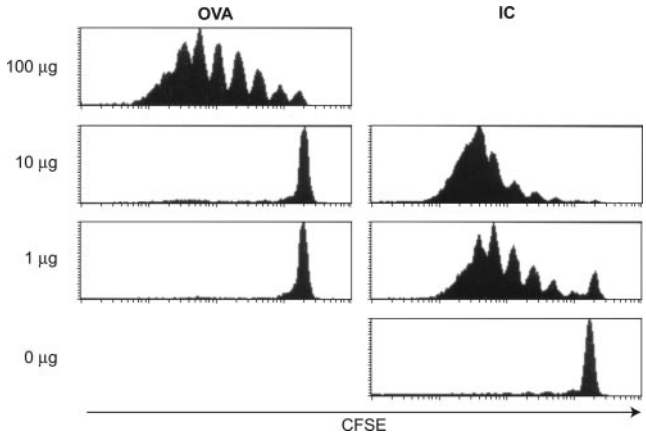

FIGURE 1. Efficient presentation of Ag captured in IC to CD8$^+$ T cells. CFSE-labeled OVA-specific OT-1/CD45.1$^+$ cells were transferred into B6 mice. The following day, these mice were injected i.v. with different amounts of free OVA or OVA complexed with rabbit anti-OVA IgG (OVA-IC) ($n = 2$). Three days after injection, proliferation of the CD8$^+$/CD45.1$^+$ T cells was analyzed in the spleen. One experiment of three providing similar results is presented.

Cross-presentation of OVA-IC by DC in vitro

IC were generated by incubating OVA (Worthington Biochemical) with polyclonal rIgGαOVA (Valeant Pharmaceuticals), at a ratio of 1 μg OVA to 50 μg rIgGαOVA in PBS, for 30 min at 37°C. OVA-IC were incubated with different concentrations of hC1q for 1 h at room temperature in a flat-bottom 96-well plate. In the experiments using heat-inactivated C1q, the C1q molecules were, before adding to the IC, incubated for 60 min at 56°C. After 1 hour, 50,000 D1 cells were added to each well to incubate with the IC and C1q for 1 h at 37°C. After 1 hour, cells were washed intensively to remove IC and C1q and subsequently incubated with 5×10^4 B3Z T cells overnight at 37°C. B3Z hybridoma cells recognize the OVA CTL epitope (SIINFEKL) in H-2K^b and express the lacZ reporter gene behind the IL-2 promoter (6, 20). Ag presentation is quantified in a colorometric assay at an optimal density of 590 nm by adding the substrate chlorophenol red-β-D-galactopyranoside to B3Z cell lysates.

Uptake of OVA-Alexa488 by DC in vivo

WT or C1q-deficient mice were injected i.v. with 1 μg of OVA-Alexa488 in IC. After 1 h, the mice were sacrificed and the spleens were isolated.

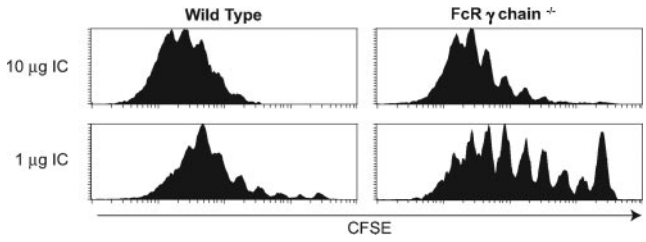

FIGURE 2. Activating FcγR are partly, but not crucially, involved in the presentation of OVA-IC to CD8$^+$ T cells after i.v. injection. CFSE-labeled OVA-specific OT-1/CD45.1$^+$ cells were transferred into recipient mice. These mice were injected i.v. with 1 μg or 10 μg OVA-IC. Three days after injection, proliferation of the CD8$^+$/CD45.1$^+$ T cells was analyzed in the spleen. FcRγ-chain-deficient mice ($n = 5$) as recipients were compared with WT mice ($n = 5$). One experiment of two providing similar results is presented.

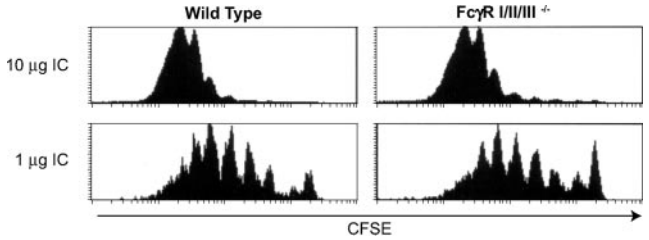

FIGURE 3. FcγRI/II/III are not crucially involved in the presentation of OVA-IC to $CD8^+$ T cells after i.v. injection. CFSE labeled OVA-specific OT-1/$CD45.1^+$ cells were transferred into recipient mice. FcγRI/II/$III^{-/-}$ mice ($n = 4$) as recipients were compared with wild-type mice ($n = 4$). These mice were injected i.v. with 1 μg or 10 μg OVA-IC. Three days after injection, proliferation of the $CD8^+$/$CD45.1^+$ T cells was analyzed in the spleen.

Splenocytes were incubated with anti-CD11c-allophycocyanin and analyzed by FACS. $CD11c^+$ cells were gated and the percentage of OVA-Alexa488-positive cells was calculated.

Results

Efficient presentation of Ag captured in IC to $CD8^+$ T cells

We and others have previously demonstrated that the uptake of IC composed of the model Ag OVA bound to OVA-specific IgG enabled cross-presentation of OVA-derived peptides to $CD8^+$ T cells (6, 21). In vitro, peptides derived from OVA-IC are much more efficiently presented than peptides from noncomplexed Ag in a FcγR-mediated fashion. In vivo, we have shown that FcγR are involved in IC-mediated Ag-presentation when the IC are administered s.c. (16). Likewise, it has been shown that FcγR are crucially involved in IgG-mediated enhancement of Ab responses after i.v. immunization with IgG/Ag complexes (22). In this study, we analyzed IC-mediated Ag-presentation to $CD8^+$ T cells after i.v. administration.

MHC class I Ag presentation of OVA-derived peptides in vivo was analyzed by using $CD8^+$ T cells derived from OT-I/CD45.1 transgenic mice, which have a transgenic TCR specific for the $OVA_{257–264}$ epitope as presented by H2-K^b.

WT B6 mice were infused i.v. with CFSE-labeled OVA-specific T cells, and after 24 h were injected i.v. with different amounts of free OVA or OVA bound to rIgGαOVA. Three days after injection of the Ag, T cell proliferation in the spleen was analyzed.

T cell proliferation could be induced when free OVA was administered at a dose of 100 μg/mouse (Fig. 1). No proliferation was detected when noncomplexed OVA was administrated at doses of 10 μg/mouse or lower. In contrast, i.v. injection of OVA incubated with anti-OVA IgG resulted in significant proliferation of OT-I cells at 1 μg OVA/mouse. These results indicate that OVA complexed with anti-OVA IgG in IC administered i.v. is 10 to 100 times more efficient in inducing $CD8^+$ T cell proliferation than noncomplexed OVA.

Reduced Ag presentation in the FcRγ-chain-deficient mice, but not in FcγRI/II/III-deficient mice

FcR γ-chain-deficient mice lack the expression of activating FcγR (23). We analyzed the involvement of the activating FcγR in the presentation of immune-complexed OVA after i.v. injection. As shown in Fig. 2, $CD8^+$ T cell proliferation was partly reduced in the FcRγ-chain-deficient mice compared with $CD8^+$ T cell proliferation in WT mice after injection of 1 μg IC, while no differences were observed at 10 μg IC. To obtain a better appreciation of the FcγR responsible for the diminished Ag presentation in the absence of activating FcR, we analyzed the ability of FcγRI/II/III-deficient mice to facilitate Ag presentation to $CD8^+$ T cells. In FcγRI/II/III-deficient mice, only the newly described FcγR IV (7) is present. As shown in Fig. 3, no apparent differences in Ag presentation were observed between the FcγRI/II/III-deficient mice and WT mice after i.v. injection of 1 μg IC. These results together indicate that, after i.v. injection of IC, activating FcγR are involved in presentation of the Ag to $CD8^+$ T cells, however other receptors or mediators are likely to play a role as well.

The central complement component C3 is not crucial for presentation of Ag from IC to $CD8^+$ T cells

Because circulating IC interact with complement components, and professional APC express receptors for several complement factors, we studied the involvement of complement in the presentation of OVA-IC to T cells. C3 has a central role in the activation of the complement system, because all three pathways are dependent on the presence of C3.

As shown in Fig. 4, no difference in $CD8^+$ T cell proliferation was found between WT and C3-deficient mice when injected with IC i.v. These results, therefore, indicate that C3, or those components that are activated directly or indirectly by the action of C3, do not play a crucial role in IC-mediated Ag-presentation to $CD8^+$ T cells.

As it is conceivable that the system is redundant, we also analyzed the $CD8^+$ T cell proliferation in mice deficient for both the FcγR and C3 (FcγRI/II/$III^{-/-}$ × $C3^{-/-}$) after i.v. injection of IC. Again, no changes in T cell proliferation were found (Fig. 4), indicating that neither the FcγRI, FcγRII, FcγRIII, nor C3, or the molecules downstream from C3, are involved in IC-mediated Ag-presentation after i.v. administration of the IC.

C1q is crucially involved in IC-mediated Ag presentation in vivo

IC activate the complement system via interaction with C1, the first component of the classical complement pathway. C1q is present in high levels in the bloodstream. One of the major roles for C1q is the clearance of IC from the circulation. To examine the

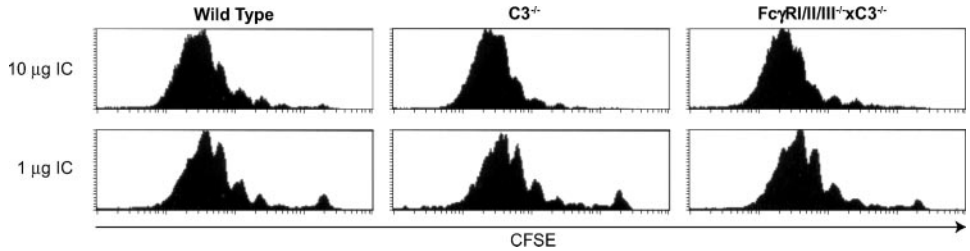

FIGURE 4. C3 is not required for the presentation of OVA-IC to $CD8^+$ T cells after i.v. injection. CFSE-labeled OVA-specific OT-1/$CD45.1^+$ cells were transferred into WT mice, $C3^{-/-}$ mice, or FcγRI/II/$III^{-/-}$ × $C3^{-/-}$ mice. These mice were injected i.v. with 1 μg or 10 μg OVA-IC ($n = 4$). Three days after injection, proliferation of the $CD8^+$/$CD45.1^+$ T cells was analyzed in the spleen.

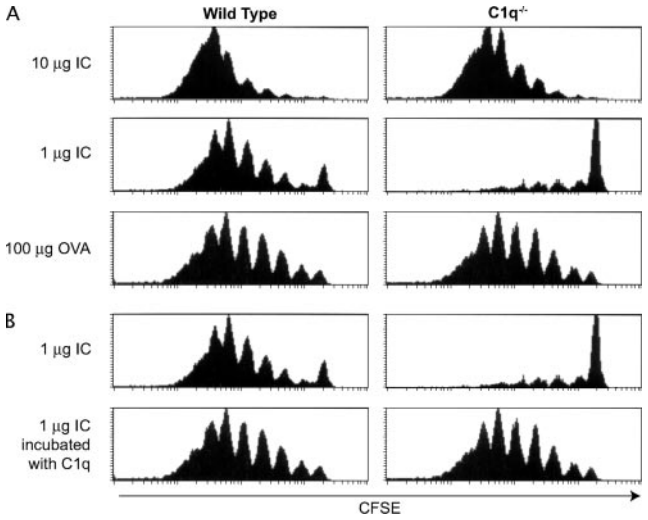

FIGURE 5. C1q is crucially involved in the presentation of OVA-IC to CD8$^+$ T cells after i.v. injection. CFSE-labeled OVA-specific OT-1/CD45.1$^+$ cells were transferred into WT mice ($n = 3$) or C1q$^{-/-}$ mice ($n = 3$). These mice were injected i.v. with 1 μg or 10 μg OVA-IC or 100 μg of free OVA ($n = 3$) (*A*) or 1 μg OVA-IC $\pm$ 25 μg/ml purified human-derived C1q (*B*). Three days after injection, proliferation of the CD8$^+$/CD45.1$^+$ T cells was analyzed in the spleen. One experiment of three (*A*) or two (*B*) performed, providing similar results, is presented.

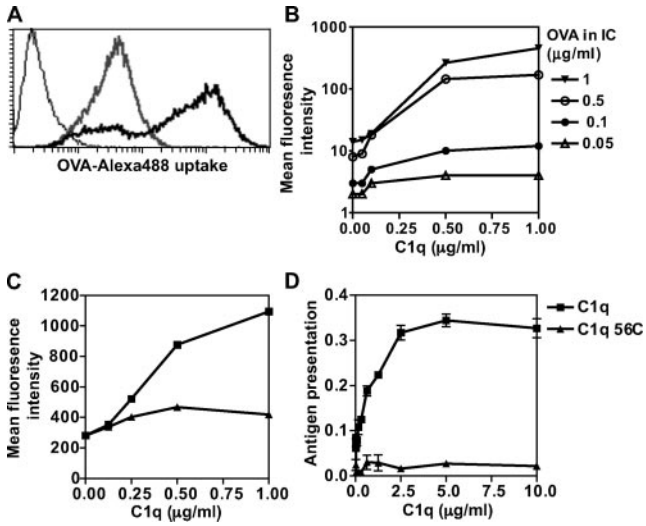

FIGURE 6. C1q enhances uptake and cross-presentation of immune-complexed Ag by DC in vitro. *A–C*, DC were incubated for 1 hour with (*A*) medium (thin line), 1 μg/ml OVA-Alexa488 in IC (thick gray line), or 1 μg/ml OVA-IC that were also incubated with 0.5 μg/ml purified human C1q before the addition to DC (thick black line); (*B*) different concentrations of OVA-Alexa488 in IC preincubated with increasing concentrations of purified human C1q (*x*-axis); (*C*) 1 μg/ml OVA-Alexa488 in IC preincubated with increasing concentrations of purified human C1q (■) or purified human C1q that was heat-inactivated at 56°C (▲). After 1 hour, DC were harvested and analyzed by FACS for Alexa488 fluorescence (*y*-axis). *D*, DC were incubated for 1 hour with 1 μg/ml OVA-IC preincubated with increasing concentrations of human C1q (■) or human C1q that was heat-inactivated at 56°C (▲). After 24 h, B3Z OVA-specific CD8$^+$ T cells were added overnight, and Ag presentation was measured in a colorimetric assay at optimal density 590 nm (see *Materials and Methods*).

possibility that C1q is also involved in the presentation of the OVA-derived antigenic peptide after i.v. injection of OVA-IC, CD8$^+$ T cell proliferation was analyzed in mice deficient for C1q.

As shown in Fig. 5, proliferation of the OVA-specific CD8$^+$ T cells was strongly reduced in C1q-deficient mice compared with WT mice after injection of 1 μg IC i.v. No changes in proliferation were found when high amounts (100 μg) of OVA protein were administrated i.v. in soluble form, indicating that DC from C1q-deficient mice are not generally deficient in endocytosis, processing, and presentation of Ag. In addition, no intrinsic abnormalities in the uptake of IC by primary DC cultures derived from C1q KO mice were observed (data not shown).

When the IC were first incubated with purified hC1q before injection into the C1q-deficient mice, proliferation of the CD8$^+$ T cells was restored (Fig. 5*B*). These results indicate that C1q plays a prominent role in IC-mediated Ag presentation.

C1q enhances the uptake and Ag presentation of IC by DC in vitro

To determine the efficiency of OVA uptake by DC, we developed a system using fluorescent OVA-Alexa488 protein to measure uptake by FACS. In this way, we show that C1q can strongly enhance the uptake of IC by DC (Fig. 6*A*). Alexa488-labeled IC were incubated with different concentrations of human C1q and added to DC in vitro. After 1 hour, the uptake of IC by the DC was measured. The uptake of IC at an OVA concentration of 0.5 μg/ml or higher is greatly enhanced when increasing amounts of C1q were added (Fig. 6*B*). The C1q binding activity is mediated via the globular heads of the C1q molecule, which can interact with the constant domain of the IgG molecule. The binding function of these globular heads is classically heat sensitive. As shown in Fig. 6*C*, the enhancement of IC uptake by DC in combination with C1q is abrogated when C1q is heat-inactivated at 56°C. C1q-mediated enhancement of IC uptake by DC resulted in improved MHC class I presentation of the OVA-derived SIINFEKL epitope (Fig. 6*D*). This enhancement of MHC class I presentation was also abrogated by heat inactivation of C1q. These results together indicate that C1q is actively involved in the MHC class I presentation of peptides derived from circulating immune-complexed Ag.

Decreased uptake of OVA-IC in splenic DC of C1q-deficient mice after i.v. injection

To analyze whether C1q also enhances the uptake of IC by DC in vivo, we injected C1q-deficient and WT mice with OVA-Alexa488-IC. In the C1q-deficient mice, significant lower numbers of CD11c$^+$ cells have taken up OVA-IC (Fig. 7). These results correlate with our findings obtained in vitro, indicating a direct effect of C1q in the enhancement of uptake of circulating IC. These results also indicate that the reduced Ag presentation in vivo in the absence of C1q is related to decreased uptake of circulating IC by

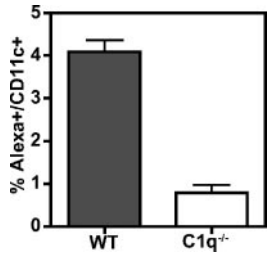

FIGURE 7. Decreased uptake of OVA-IC in splenic DC of C1q-deficient mice after i.v. injection. WT or C1q$^{-/-}$ mice ($n = 2$) were injected i.v. with 1 μg of OVA-Alexa488 in IC. After 1 h, the mice were sacrificed and the spleens were isolated. CD11c$^+$ spleen cells were analyzed on OVA-Alexa488 uptake by FACS analysis. Student *t* test was performed to assess the difference, $p = 0.01$. The experiment was performed three times with similar results.

splenic DC. Together, these findings indicate that C1q facilitates the presentation of peptides derived from immune-complexed Ags to $CD8^+$ T cells by enhancing the DC-mediated uptake of IC.

Discussion

In this study, we investigated the contribution of the different murine FcγR and complement to the activation of OVA-specific $CD8^+$ T cells in vivo following i.v. vaccination with OVA-IC.

Previously, it has been shown that the protein Ag contained within the IC is much more efficiently processed and presented in MHC molecules than free Ag by DC in vitro (6, 21). In this study, we also show that i.v. injected immune complexed-Ag is presented more efficiently than noncomplexed Ag in vivo. The presentation of IC after i.v. administration is partly dependent on activating FcγR, however it is not crucially dependent. This is in agreement with an earlier publication, where it was shown that the ability of $CD8^+$ DC, in contrast to $CD8^-$ DC, to induce $CD8^+$ T cell proliferation was not affected in FcRγ-chain$^{-/-}$ mice after i.v. injection with IC (24).

Our results indicate a pivotal contribution of C1q in the presentation of Ag derived from i.v. administered IC to $CD8^+$ T cells in vivo, indicating that C1q can accommodate the presentation of Ag complexed in IC. The enhancing effect, mediated by C1q in the presentation of immune complexed Ag in vivo, might be a consequence of an altered clearance of IC from the blood in C1q-deficient mice, as it has been shown that the splenic uptake of IC is significantly reduced in mice deficient for C1q (14). Although an altered clearance or deposition of IC could certainly contribute to the effects observed in vivo, our results obtained in vitro indicate that C1q-binding to IC also directly enhances the uptake and presentation of Ag from IC by DC. Because immature DC are a source of C1q (18), the direct enhancement may be mediated at the local environment in the tissue.

Although we cannot formally conclude that C1q plays a similar role in vivo under unmanipulated conditions involving all murine ingredients, we consider it unlikely that the findings are a consequence of using rabbit IgG and human C1q: complement components are highly conserved and can bind and be activated by rabbit IgG, as shown, for example, by the replaceability of human C1q and murine C1q in hemolytic assays (25) and the ability of murine C3 to be deposited on rabbit IgG following complement activation (26). Likewise, murine FcR can readily interact with rabbit Ig IC, as shown by their crucial role in the cross-presentation of immune-complexed Ag in vitro (5) and after s.c. administration of IC (16). Moreover, we have shown in vitro that the uptake of IC by DC is similar when mouse Ig is used instead of rabbit Ig (data not shown). Adding hC1q to the IC composed of mouse IgG gave a similar enhanced uptake (data not shown) as seen with IC composed of rabbit IgG (Fig. 6, *B* and *C*).

As the enhanced uptake of Ag in IC was abolished after inactivating C1q at 56°C, which results in the melting of the globular heads of the molecule, it is likely that intact globular heads are crucial for the enhancement of uptake. The globular heads of C1q are shown to bind to the C_H2 domain in the Fc-tail of the Ab (13), indicating that C1q directly influences the stability, composition, or size of the IC, thereby enhancing the binding and presentation of IC by DC.

In line with our findings, C1q has been shown to enhance the phagocytosis of apoptotic cells, pathogens, and opsonized erythrocytes in a dose-dependent way (27–31). The enhancement of the uptake of opsonized erythrocytes could be blocked by anti-C1q Abs, and was only observed when the collagenous part of C1q was present, indicating that an interaction of C1q with a C1q receptor was involved in the enhancement. To date, several types of putative C1q-binding cell surface-expressed receptors have been described (13, 32–37). Although the receptors are shown to interact with C1q, so far they are not described to mediate the enhanced endocytosis of IC. If the C1q-dependent enhancement of uptake requires an interaction of C1q with one of the candidate receptors, it is most likely that the collagen-like region of C1q binds the receptor(s) (38). However, the globular heads cannot be ruled out as a ligand, because the binding of C1q to the Fc region involves only two of the six globular heads, leaving room for unoccupied heads to bind to other molecules, including receptors. Therefore, based on our in vitro results and the literature discussed, a direct effect of C1q on the conformation of the IC, the clearance of IC in vivo, and/or the involvement of candidate C1q receptors that interact with C1q and/or synergize with FcγR may be involved in the enhanced C1q-dependent presentation of peptides derived from immune complexed Ag.

In conclusion, we have shown that C1q, which is present in high levels in the bloodstream, plays a pivotal role in the cross-presentation of IC-derived peptides to $CD8^+$ cells in vivo after i.v. administration, thereby bridging innate and adaptive immune response. This novel function of C1q may be of relevance for the design of improved vaccination strategies against infectious diseases and cancer.

Disclosures

The authors have no financial conflict of interest.

References

1. Heath, W. R., G. T. Belz, G. M. Behrens, C. M. Smith, S. P. Forehan, I. A. Parish, G. M. Davey, N. S. Wilson, F. R. Carbone, and J. A. Villadangos. 2004. Cross-presentation, dendritic cell subsets, and the generation of immunity to cellular antigens. *Immunol. Rev.* 199: 9–26.
2. Bonifaz, L. C., D. P. Bonnyay, A. Charalambous, D. I. Darguste, S. Fujii, H. Soares, M. K. Brimnes, B. Moltedo, T. M. Moran, and R. M. Steinman. 2004. In vivo targeting of antigens to maturing dendritic cells via the DEC-205 receptor improves T cell vaccination. *J. Exp. Med.* 199: 815–824.
3. Burgdorf, S., V. Lukacs-Kornek, and C. Kurts. 2006. The mannose receptor mediates uptake of soluble but not of cell-associated antigen for cross-presentation. *J. Immunol.* 176: 6770–6776.
4. Regnault, A., D. Lankar, V. Lacabanne, A. Rodriguez, C. Thery, M. Rescigno, T. Saito, S. Verbeek, C. Bonnerot, P. Ricciardi-Castagnoli, and S. Amigorena. 1999. Fcγ receptor-mediated induction of dendritic cell maturation and major histocompatibility complex class I-restricted antigen presentation after immune complex internalization. *J. Exp. Med.* 189: 371–380.
5. Schuurhuis, D. H., N. van Montfoort, A. Ioan-Facsinay, R. Jiawan, M. Camps, J. Nouta, C. J. Melief, J. S. Verbeek, and F. Ossendorp. 2006. Immune complex-loaded dendritic cells are superior to soluble immune complexes as antitumor vaccine. *J. Immunol.* 176: 4573–4580.
6. Schuurhuis, D. H., A. Ioan-Facsinay, B. Nagelkerken, J. J. van Schip, C. Sedlik, C. J. Melief, J. S. Verbeek, and F. Ossendorp. 2002. Antigen-antibody immune complexes empower dendritic cells to efficiently prime specific $CD8^+$ CTL responses in vivo. *J. Immunol.* 168: 2240–2246.
7. Nimmerjahn, F., P. Bruhns, K. Horiuchi, and J. V. Ravetch. 2005. FcγRIV: a novel FcR with distinct IgG subclass specificity. *Immunity* 23: 41–51.
8. Ravetch, J. V., and J. P. Kinet. 1991. Fc receptors. *Annu. Rev. Immunol.* 9: 457–492.
9. Ravetch, J. V., and S. Bolland. 2001. IgG Fc receptors. *Annu. Rev. Immunol.* 19: 275–290.
10. Amigorena, S., and C. Bonnerot. 1999. Fc receptor signaling and trafficking: a connection for antigen processing. *Immunol. Rev.* 172: 279–284.
11. Walport, M. J. 2001. Complement: first of two parts. *N. Engl. J. Med.* 344: 1058–1066.
12. Walport, M. J. 2001. Complement: second of two parts. *N. Engl. J. Med.* 344: 1140–1144.
13. Sontheimer, R. D., E. Racila, and D. M. Racila. 2005. C1q: its functions within the innate and adaptive immune responses and its role in lupus autoimmunity. *J. Invest. Dermatol.* 125: 14–23.
14. Nash, J. T., P. R. Taylor, M. Botto, P. J. Norsworthy, K. A. Davies, and M. J. Walport. 2001. Immune complex processing in C1q-deficient mice. *Clin. Exp. Immunol.* 123: 196–202.
15. Trouw, L. A., M. A. Seelen, J. M. Duijs, H. Benediktsson, C. van Kooten, and M. R. Daha. 2003. Glomerular deposition of C1q and anti-C1q antibodies in mice following injection of antimouse C1q antibodies. *Clin. Exp. Immunol.* 132: 32–39.
16. de Jong, J. M., D. H. Schuurhuis, A. Ioan-Facsinay, E. I. van der Voort, T. W. Huizinga, F. Ossendorp, R. E. Toes, and J. S. Verbeek. 2006. Murine Fc

receptors for IgG are redundant in facilitating presentation of immune complex derived antigen to CD8$^+$ T cells in vivo. *Mol. Immunol.* 43: 2045–2050.
17. Botto, M., C. Dell'Agnola, A. E. Bygrave, E. M. Thompson, H. T. Cook, F. Petry, M. Loos, P. P. Pandolfi, and M. J. Walport. 1998. Homozygous C1q deficiency causes glomerulonephritis associated with multiple apoptotic bodies. *Nat. Genet.* 19: 56–59.
18. Castellano, G., A. M. Woltman, A. J. Nauta, A. Roos, L. A. Trouw, M. A. Seelen, F. P. Schena, M. R. Daha, and C. van Kooten. 2004. Maturation of dendritic cells abrogates C1q production in vivo and in vitro. *Blood* 103: 3813–3820.
19. Winzler, C., P. Rovere, M. Rescigno, F. Granucci, G. Penna, L. Adorini, V. S. Zimmermann, J. Davoust, and P. Ricciardi-Castagnoli. 1997. Maturation stages of mouse dendritic cells in growth factor-dependent long-term cultures. *J. Exp. Med.* 185: 317–328.
20. Sanderson, S., and N. Shastri. 1994. LacZ inducible, antigen/MHC-specific T cell hybrids. *Int. Immunol.* 6: 369–376.
21. Amigorena, S., and C. Bonnerot. 1999. Fc receptors for IgG and antigen presentation on MHC class I and class II molecules. *Semin. Immunol.* 11: 385–390.
22. Wernersson, S., M. C. Karlsson, J. Dahlstrom, R. Mattsson, J. S. Verbeek, and B. Heyman. 1999. IgG-mediated enhancement of antibody responses is low in Fc receptor γ chain-deficient mice and increased in Fc γ RII-deficient mice. *J. Immunol.* 163: 618–622.
23. Takai, T. 1996. Multiple loss of effector cell functions in FcR γ-deficient mice. *Int. Rev. Immunol.* 13: 369–381.
24. den Haan, J. M., and M. J. Bevan. 2002. Constitutive versus activation-dependent cross-presentation of immune complexes by CD8$^+$ and CD8$^-$ dendritic cells in vivo. *J. Exp. Med.* 196: 817–827.
25. Roos, A., A. J. Nauta, D. Broers, M. C. Faber-Krol, L. A. Trouw, J. W. Drijfhout, and M. R. Daha. 2001. Specific inhibition of the classical complement pathway by C1q-binding peptides. *J. Immunol.* 167: 7052–7059.
26. Mantovani, B., M. Rabinovitch, and V. Nussenzweig. 1972. Phagocytosis of immune complexes by macrophages: different roles of the macrophage receptor sites for complement (C3) and for immunoglobulin (IgG). *J. Exp. Med.* 135: 780–792.
27. Bobak, D. A., T. A. Gaither, M. M. Frank, and A. J. Tenner. 1987. Modulation of FcR function by complement: subcomponent C1q enhances the phagocytosis of IgG-opsonized targets by human monocytes and culture-derived macrophages. *J. Immunol.* 138: 1150–1156.
28. Tenner, A. J., S. L. Robinson, J. Borchelt, and J. R. Wright. 1989. Human pulmonary surfactant protein (SP-A), a protein structurally homologous to C1q, can enhance FcR- and CR1-mediated phagocytosis. *J. Biol. Chem.* 264: 13923–13928.
29. Guan, E., S. L. Robinson, E. B. Goodman, and A. J. Tenner. 1994. Cell surface protein identified on phagocytic cells modulates the C1q-mediated enhancement of phagocytosis. *J. Immunol.* 152: 4005–4016.
30. Nauta, A. J., G. Castellano, W. Xu, A. M. Woltman, M. C. Borrias, M. R. Daha, C. van Kooten, and A. Roos. 2004. Opsonization with C1q and mannose-binding lectin targets apoptotic cells to dendritic cells. *J. Immunol.* 173: 3044–3050.
31. Alvarez-Dominguez, C., E. Carrasco-Marin, and F. Leyva-Cobian. 1993. Role of complement component C1q in phagocytosis of *Listeria monocytogenes* by murine macrophage-like cell lines. *Infect. Immun.* 61: 3664–3672.
32. Eggleton, P., A. J. Tenner, and K. B. Reid. 2000. C1q receptors. *Clin. Exp. Immunol.* 120: 406–412.
33. Ghiran, I., S. R. Tyagi, L. B. Klickstein, and A. Nicholson-Weller. 2002. Expression and function of C1q receptors and C1q binding proteins at the cell surface. *Immunobiology* 205: 407–420.
34. Kishore, U., M. S. Kojouharova, and K. B. Reid. 2002. Recent progress in the understanding of the structure-function relationships of the globular head regions of C1q. *Immunobiology* 205: 355–364.
35. McGreal, E., and P. Gasque. 2002. Structure-function studies of the receptors for complement C1q. *Biochem. Soc. Trans.* 30: 1010–1014.
36. Nicholson-Weller, A., and L. B. Klickstein. 1999. C1q-binding proteins and C1q receptors. *Curr. Opin. Immunol.* 11: 42–46.
37. Kim, T. S., M. Park, R. R. Nepomuceno, G. Palmarini, S. Winokur, C. A. Cotman, U. Bengtsson, and A. J. Tenner. 2000. Characterization of the murine homolog of C1qR(P): identical cellular expression pattern, chromosomal location and functional activity of the human and murine C1qR(P). *Mol. Immunol.* 37: 377–389.
38. Eggleton, P., K. B. Reid, and A. J. Tenner. 1998. C1q: how many functions? How many receptors? *Trends Cell Biol.* 8: 428–431.