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*J Immunol* 1999; 162:3125-3130; ;
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Modulation of the Humoral Immune Response by Antibody-Mediated Antigen Targeting to Complement Receptors and Fc Receptors

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During an ongoing immune response, immune complexes, composed of Ag, complement factors, and Igs, are formed that can interact with complement receptors (CRs) and IgG Fc receptors (FcγR). The role of CR1/2 and FcγR in the regulation of the immune response was investigated using OVA that was chemically conjugated to whole IgG of the rat anti-mouse CR1/2 mAb 7G6. FACS analysis using the murine B cell lymphoma IIA1.6 confirmed that the 7G6-OVA conjugate recognized CR1/2. Incubating IIA1.6 cells with 7G6-OVA triggered tyrosine phosphorylation and Ag presentation to OVA-specific T cells in vitro. Immunizing mice with 7G6-OVA at a minimal dose of 1 μg i.p. per mouse markedly enhanced the anti-OVA Ig response, which was primarily of the IgG1 isotype subclass. The 7G6-OVA did not enhance the anti-OVA response in CR1/2-deficient mice. OVA coupled to an isotype control Ab induced a considerably lower anti-OVA response compared with that induced by OVA alone, suggesting inhibition by interaction between the Fc part of the Ab and the inhibitory FcγRIib on B cells. This finding was supported by the observation that IIA1.6 cells which were incubated with 7G6-OVA lost the ability to present Ag upon transfection with FcγRIib. In sum, 7G6-conjugated OVA, resembling a natural immune complex, induces an enhanced anti-OVA immune response that involves at least CR1/2-mediated stimulation and that may be partially suppressed by FcγRIib. The Journal of Immunology, 1999, 162: 3125–3130.

The generation of an immune response is controlled by various humoral and cellular components of the innate and acquired immune system. Immediately upon entering the body, foreign material can activate complement leading to the deposition of C3 products, which in turn allows interaction with complement receptors (CRs), initiating cellular responses. CRs type 1 and 2 (CR1 and CR2), which are expressed on B cells and follicular dendritic cells in the mouse (1) as alternatively spliced products of the single Cr2 locus (2, 3), recognize activated C3 and C4 products (4, 5). Various in vitro and in vivo studies support the important role of these surface receptors in the regulation of the humoral immune response (reviewed in Ref. 6). Co-cross-linking CR1/2 and the Ag receptor reduces the threshold for activation of B cells in vitro (7, 8). The humoral response to T cell-dependent or -independent Ags can be strongly reduced by treating mice with the anti-CR1/2 mAb 7G6 (9, 10) or with soluble CR2 (11) before immunization. Mice that are deficient in CR1 and CR2, which were generated by targeted disruption of the Cr2 gene, mounted impaired humoral responses to T cell-dependent Ags (12, 13). Evidence for the potent immunoregulatory activity of complement has been provided using a recombinant fusion protein composed of C3d and a model Ag, which was highly immunogenic in mice, indirectly suggesting a role for CR1/2 in this process (14). Consistent with that finding are results obtained using mice deficient in C3 and C4, which exhibited defective Ab responses against T cell-dependent Ags (15).

After the first contact with the innate immune system and the initiation of the production of Ag-specific Igs, foreign material can also be recognized by the acquired immune system and thus interact with receptors for the Fc part of IgG (FcγR). B cells express FcγRIib, which contains an immunoreceptor tyrosine-based inhibitor motif that is involved in the down-regulation of B cell functions (reviewed in Ref. 16). FcγRIib transfected into an FcγR-negative B cell line potently down-regulates B cell activation upon co-cross-linking with surface IgG (17). Mice deficient in FcγRIib exhibit enhanced Ig production against T cell-dependent and -independent Ags (18).

In the present study, we directly targeted Ag to CR1/2 using a complex consisting of OVA chemically conjugated to anti-CR1/2 Abs. The conjugate was generated using intact IgG of the rat anti-CR1/2 mAb 7G6, allowing direct interaction not only with CR1/2 but also with FcγR, thus resembling a natural immune complex consisting of Ag coated with both complement and IgG. To address the relative contributions of CR1/2 and FcγR in the immune response, an isotype-matched IgG control, FcγR-transfected IIA1.6 cells (an FcγR-negative murine B lymphoma cell line) and CR1/2-deficient mice were used for in vitro and in vivo studies. We found that conjugation of 7G6 to OVA strongly enhanced the anti-OVA Ab response, and that this enhancement was dependent.
upon interaction of the conjugate with CR1/2. We also found evidence that interaction of the complex with FcyRIIB could partially down-regulate this response.

Materials and Methods

Cell cultures and Abs

The murine B cell lymphoma line A20 (19) and its FcγR-negative variant IIA1.6 (both expressing CR1 and CR2) as well as the Th OVA-specific hybridoma 3DO-54.8 (20) were cultured in RPMI 1640 medium supplemented with 10% heat-inactivated FCS, 100 U/ml penicillin, 100 U/ml streptomycin, 2 mM glutamine, and 1 mM sodium pyruvate. Unless stated otherwise, cells were suspended in the same medium during the in vitro assays. FcγR transfectants of the IIA1.6 cell line were maintained in the same medium supplemented with Geneticin (G418, 0.8 mg/ml; Life Technologies, Paisley, U.K.) or with Geneticin and methotrexate (10 μM; Pharmacia, Haarlem, The Netherlands). CTL-L16 cells were cultured in RPMI 1640 medium supplemented with 100 U/ml rIL-2. Rat hybridoma 7G6 (21) (kindly provided by Dr. T. Kinoshita, Osaka University, Osaka, Japan) secreting IgG2b directed to murine CR1 and CR2 was cultured on a large scale in serum free Iscove’s modified Eagle’s medium (Life Technologies). SHL45.6 (22) purified rat IgG2b mAb specific for human CD3 was used as an isotype control (kindly provided by Dr. M. Clark, Cambridge University, Cambridge, U.K.).

Preparation of IgG-OVA conjugates

Ab-OVA conjugates were prepared using N-succinimidyl S-acetylthioaceta-
ete (SATA) (purchased from Pierce, Rockford, IL) as a chemical cross-linker (reviewed in Ref. 23). Briefly, IgG at a concentration of ≥5 mg/ml suspended in a 50 mM sodium phosphate buffer containing 1 mM EDTA (pH 7.5) was incubated with a 15-fold molar excess of SATA and subsequently incubated with a 10-fold smaller volume of 0.5 M hydroxylamine HCl (Pierce) in 50 mM sodium phosphate and 25 mM EDTA (pH 7.5) for 2 h at room temperature. Next, the solution was diluted four times in 0.1 M sodium phosphate, 0.15 M NaCl, and 0.1 M EDTA (pH 7.2) containing a molar IgG-OVA ratio of 1:1.5. The coupling reaction was allowed to proceed for 90 min at room temperature and was stopped by the addition of 2-ME at a final concentration of 10 mM. Conjugates were subsequently purified from unconjugated proteins by fast protein liquid chromatography using a HiLoad Superdex 200 HR column (Pharmacia LKB Biotech, Uppsala, Sweden) equilibrated with PBS. Fractions containing molecules with an estimated molecular mass ranging from 200 to 573 kDa (based on the retention time of molecular mass markers) were pooled and used for the Ag presentation and immunization experiments. The concentration of the pooled fractions was estimated by measuring the absorbance at 280 nm, and small aliquots were immediately frozen for long-term storage. Conjugates were analyzed by 6% SDS-PAGE under nonreducing conditions.

Flow cytometry

The murine B lymphoma cell line IIA1.6 was used to analyze the capacity of 7G6-OVA conjugates to bind CR1/2 by flow cytometry. IIA1.6 cells were washed twice and suspended at a concentration of 2 × 10^5 cells/ml of PBS containing 2.5% FCS and 0.05% sodium azide. The cells were then incubated with nonconjugated 7G6 or with 7G6-OVA conjugates at different concentrations for 30 min at 4°C, followed by two washes. Next, the cells were incubated with FITC-conjugated mouse anti-rat IgG (Jackson ImmunoResearch Laboratories, West Grove, PA) or with polyclonal rabbit anti-OVA IgG (Cappel, Durham, NC) followed by incubation with FITC-conjugated goat anti-rabbit IgG; each incubation was performed for 30 min at 4°C. The cells were then washed twice and analyzed by flow cytometry.

Tyrosine phosphorylation assay

IIA1.6 cells, at a concentration of 2 × 10^5 cells/ml, were incubated with 10 μg/ml 7G6-OVA or with the same concentration of SHL45.6-OVA for 20 min at 4°C. Next, the cells were washed twice with cold serum-free RPMI 1640 medium and resuspended in aliquots of 20 μl containing 5 × 10^4 cells. The cells were then incubated in a waterbath at 37°C, and the reaction was stopped at various time periods by adding reducing sample buffer (v/v) containing 8% sodium lauryl sulfate, 20% 2-ME, and 1 mM sodium orthovanadate. After denaturing the samples by boiling, lysed cells were loaded onto a 10% SDS-polyacrylamide gel (2.5 × 10^4 cells/lane), and the proteins were subsequently electrotransferred to polyvinylidene difluoride Immobion-P membranes (Millipore, Bedford, MA). The membranes were washed in PBS containing 0.1% Tween 20 (PBS-Tween), blocked with 1% BSA in PBS-Tween, and probed with 0.8 μg/ml of the anti-phosphotyrosine mAb 4G10 (Upstate Biotechnology, Lake Placid, NY) for 1.5 h at room temperature. After washing three times with PBS-Tween, membranes were incubated with 0.5 μg/ml peroxidase-labeled rabbit anti-mouse Ig (Dako A/S, Glostrup, Denmark); bound Abs were detected using the enhanced chemiluminescence system (Amersham, Buckinghamshire, U.K.).

Ag presentation in vitro

The effect of the interaction of Ab-Ag complexes with CR1/2 or FcγR on the ability of IIA1.6 and A20 cells to present OVA Ag to OVA-specific Th cells was studied using 7G6-conjugated OVA and OVA conjugated to the isotype control SHL45.6. The IIA1.6 or A20 cells, at a concentration of 2 × 10^5 cells/ml, were incubated with OVA-specific Th cell hybridoma (2 × 10^4 cells/ml) for 24 h at 37°C in the presence of various concentrations of Ag ranging from 1 to 300 ng/ml. As a positive control for T cell reactivity, high concentrations of free OVA (≥30 μg/ml) were used. As negative controls, all experiments were also performed in the absence of Th cells, IIA1.6 cells, or Ag. The release of IL-2 by the Th cells in the culture supernatants, as a measure of efficient Ag presentation, was determined using a CTLL proliferation assay (17). CTL-L16 cells are T cells that are derived from C57BL/6 mice and that proliferate in an IL-2-dependent fashion. High concentrations of free OVA (≥30 μg/ml) were used. As negative controls, all experiments were also performed in the absence of Th cells, IIA1.6 cells, or Ag. The release of IL-2 by the Th cells in the culture supernatants, as a measure of efficient Ag presentation, was determined using a CTLL proliferation assay (17). CTL-L16 cells are T cells that are derived from C57BL/6 mice and that proliferate in an IL-2-dependent fashion.

Immunization protocol

For immunization experiments, female BALB/c of ~8 wk of age were used. In some experiments, female C57BL/6-deficient mice (12A. A.B. B.) were used; mice with a mixed genetic background of C57BL/6×129Sv were used as wild-type controls. Two i.p. injections with similar doses were administered in five mice per group over 26-day intervals using various doses of Ag per mouse as indicated. In some cases, mice were injected with a single mixture of OVA and an equal volume of CFA (Difco Laboratories, Detroit, MI), followed by a booster injection of the same concentration of OVA in IFA (Difco). The mice were bled at 3 wk after the first immunization and at 1 wk and 3 wk after the second immunization for analysis of the serum anti-OVA response.

ELISA for serum anti-OVA Ab

OVA was coated onto the surfaces of 96-well Maxisorp immunoplates (Nunc, Roskilde, Denmark) by overnight incubation with 10 μg OVA per ml PBS at 4°C. The plates were washed and blocked with PBS containing 1% BSA for 1 h at room temperature, followed by two washes. Next, the plates were incubated with serial dilutions of sera of immunized mice or of preimmune serum as a control. After two washes, the plates were incubated with alkaline phosphatase-conjugated goat anti-mouse IgG (H+L) (Southern Biotechnology Associates, Birmingham, AL) to determine total Ig concentrations or with alkaline phosphatase-conjugated goat Ab specific for mouse IgG1, IgG2a, or IgG2b (Southern Biotechnology Associates) to determine the isotype subclass of the responses. The plates were washed twice and developed using p-nitrophenyl phosphate substrate (Kirkegaard and Perry Laboratories, Gaithersburg, MD). Titers were determined by calculating the dilution of each serum giving an absorbance at 405 nm that was twice that of preimmune serum.

Results

Binding of 7G6-OVA to CR1/2 in vitro

The ability of the 7G6-OVA conjugate to bind to CR1/2 was determined by flow cytometry using the CR1/2-expressing murine B lymphoma cell line IIA1.6. After incubation of these cells with 10 μg/ml 7G6-OVA and subsequently with an FITC-conjugated mouse anti-rat Ab, a positive fluorescence was observed (Fig. 1b); this fluorescence was within the same range when unconjugated 7G6 instead of the whole conjugate was used (Fig. 1a). In addition, at a lower concentration of 1 μg/ml 7G6-OVA, a significant fluorescence was detected that was comparable with that seen when using nonconjugated 7G6 (data not shown). Cell-bound 7G6-OVA could be detected using a polyclonal rabbit anti-OVA Ab (Fig. 1d),...
Tyrosine phosphorylation

Next, we questioned whether bound conjugates can initialize an intracellular response in murine B cells. The pattern of activation following the binding of 7G6-OVA to CRs on IIA1.6 cells was investigated by the detection of protein phosphorylation on tyrosine residues. Strong phosphorylation of two protein bands of an estimated molecular mass of 110 kDa and 74 kDa was detectable after 10 min of incubation at 37°C of cells treated with 7G6-OVA, showing induction of an intracellular signaling response (Fig. 2).

This phosphorylation pattern is not visible after the incubation of cells with an isotype-matched control conjugated to OVA (Fig. 2), attesting that the binding of 7G6-conjugated OVA to CRs is responsible for the phosphorylation of the proteins mentioned. Control experiments with purified Ab 7G6 alone showed a similar pattern of phosphorylation of 110 kDa and 74 kDa protein bands (data not shown), indicating that conjugation of 7G6 with OVA is basically not modifying the signaling pattern of the targeted receptors.

Ag presentation in vitro

The ability of CRs to modulate the efficiency of Ag presentation in vitro was studied using 7G6-conjugated OVA, IIA1.6 cells, and an OVA-specific T cell clone. When IIA1.6 cells were incubated with 7G6-OVA, efficient Ag presentation to OVA-specific T cells was observed (Fig. 3a). Free OVA induced detectable Ag presentation by IIA1.6 cells only when using concentrations that were 50- to 100-fold higher (≥15 μg/ml) (Fig. 3a). OVA conjugated with the isotype control Ab SHL45.6 could not be presented by IIA1.6 cells (Fig. 3a), indicating that the observed effect of 7G6-OVA was specific for CR1/2. As a positive control, IIA1.6 cells transfected with human FcγRI were incubated with SHL45.6-OVA, which resulted in efficient Ag presentation (Fig. 3b), confirming the ability of this conjugate to interact with FcγRI leading to the presentation of OVA epitopes. The Ag presentation by nontransfected IIA1.6 cells that was induced by 7G6-OVA could be blocked completely by the addition of free 7G6 but not by CR1-specific mAb 8C12 (data not shown), suggesting that this process involved at least CR2. These results indicate that OVA can be presented efficiently by B cells when conjugated to 7G6, and that this process involves at least CR2 expressed on these cells.

Next, murine FcγRIIb-transfected IIA1.6 cells were used to investigate whether an additional interaction of the 7G6-OVA conjugate with FcγRIIb on B cells would interfere with Ag presentation. Ag presentation by IIA1.6 cells, when incubated with 7G6-OVA, was completely inhibited upon transfection with FcγRIIb (Fig. 3d). This finding was supported by the inability of the natively
FcγRIIb-expressing B cell line A20 to present Ag after incubation with 7G6-OVA (Fig. 3c). These results imply that interaction of an Ag-Ab complex with FcγRIIb on B cells inhibits CR-mediated enhancement of Ag presentation by these cells.

**Immunization with 7G6-OVA conjugate**

Next, the effect of targeting OVA to CR1/2 and FcγR2 on the in vivo response was investigated. When normal BALB/c mice were immunized with 5 μg 7G6-OVA conjugate, the anti-OVA Ab response was markedly enhanced compared with the response after immunization with free OVA (Fig. 4). The enhancing effect on the secondary response was most pronounced (Fig. 4). The effect of the 7G6-OVA conjugate on the Ab response was obvious when using doses of ≥1 μg per mouse (Fig. 5). The anti-OVA Ig response induced by 7G6-OVA was primarily of the IgG1 isotype subclass (Fig. 6). No significant enhancement was observed after immunization with maleimide-activated OVA (data not shown). When mice were immunized with OVA in CFA as a positive control, the primary and secondary anti-OVA responses were strongly enhanced (Fig. 4).

Immunization with the 7G6-OVA conjugate had no effect in CR1/2-deficient mice (Fig. 7); however, this conjugate did enhance the anti-OVA response in wild-type controls of a similar genetic background (data not shown). As a positive control, CR1/2-deficient mice responded strongly to OVA in CFA (Fig. 7); the response was within the same range as that seen for wild-type control mice (data not shown).

When mice were immunized with SHL45.6-OVA conjugate as an isotype control, the anti-OVA response was considerably lower compared with immunization with free OVA (Fig. 5), raising the possibility that the response was down-regulated by interaction of this conjugate with the inhibitory FcγRIIb on B cells. This observation was supported by in vitro experiments showing that transfection of IIA1.6 cells with FcγRIIb inhibited the presentation of OVA epitopes to OVA-specific T cells after incubation with 7G6-

**Discussion**

The present results demonstrate that the Ab-mediated targeting of Ag to CR1/2 using OVA complexed to anti-CR1/2 mAb (7G6)
conclusions. Several lines of evidence support the above.

FIGURE 7. Anti-OVA response in CR2-deficient mice induced by 7G6-OVA. CR2-deficient (CR2-KO) mice were injected i.p. twice (at 26-day intervals) with 5 μg of 7G6-OVA ( ), nonconjugated OVA ( ), nonconjugated OVA in CFA ( ), or with saline ( ). Results are expressed as titers of anti-OVA Ig in the serum at 3 wk after the first immunization and at 1 and 3 wk after the second immunization. Mean values of five mice are shown. Arrows indicate days of injection.

enhances the humoral immune response against this Ag. Interaction of these Ag-Ab complexes with FcγRIIB may partially suppress this response. Several lines of evidence support the above conclusions.

First, incubation of the FcγR-negative B lymphoma cell line IIA1.6 with 7G6-conjugated OVA resulted in the efficient presen-
tation of OVA epitopes to an OVA-specific T cell line in vitro. This process was dependent upon interaction of the complex with CR1/2, because SHL45.6-OVA as an isotype-matched control had no effect. Second, the anti-OVA Ab response was markedly en-
hanced when mice were immunized with 7G6-OVA but not after immunization with SHL45.6-OVA. The enhanced anti-OVA re-
sponse was dependent upon interaction of the 7G6-OVA complex with CR1/2, because such a response was not observed in CR1/2-
deficient mice. These mice did respond to OVA in CFA, indicating they are able to mount an anti-OVA Ab response depending upon the method used to stimulate the immune system. The 7G6-OVA-
mediated enhancement was most pronounced during the secondary response, suggesting the involvement of memory B cells or Th cells. The observation that the enhanced response induced by 7G6-
OVA was primarily of the IgG1 subclass suggests that targeting to CR1/2 preferentially triggers Th2 responses, following the same isotype specificity compared with OVA alone. The enhanced anti-
OVA response in mice immunized with 7G6-OVA was accompa-
nied by a strong IgG response against rat IgG (data not shown), suggesting efficient presentation of Ags in both parts of the complex. Mice immunized with the isotype control SHL45.6-OVA, which hardly raised an anti-OVA response, also mounted only a weak specific anti-rat IgG response. Treating mice with 7G6-OVA did not increase the total Ig concentration in the serum (W.L.W.H., unpublished observations), indicating that the enhancement was not associated with polyclonal B cell activation. The present re-
results, based on the direct targeting of Ag to CR1/2, are in agree-
ment with the observation reported previously that coupling of a model Ag to C3d, a ligand for CR1/2, enhances the immunoge-
nicity of this Ag (14).

Our results showed that the targeting of Ag to CR1/2 on IIA1.6 cells mediates efficient Ag presentation to specific Th clones with-
out the requirement of OVA-specific membrane Ig (mlg). This correlated with the ability of IIA1.6 cells to generate and transduce intracellular responses consisting of the phosphorylation of cellular proteins when triggered with 7G6-conjugated OVA. However, treating IIA1.6 cells with 7G6-OVA did not induce an increase in [Ca^{2+}], modifications in cell cycle (proliferation, apoptosis), secretion of IL-4, or expression of MHC class II (D.C.B. and W.L.W.H., unpublished observations). In contrast, it has been re-
ported that the co-cross-linking of CR1/2 with mlg on B cells does result in an increase in [Ca^{2+}], (7). Thus, CR1/2-mediated Ag presentation can occur independently of mlg via an as yet un-
known mechanism, which at least does not lead to a general pattern of B cell activation. One possibility may be provided by a recent report showing that the cross-linking of CR1/2 on murine splenic B cells enhances the expression of B7-1 and B7-2, which can provide costimulatory signals to T cells, thereby possibly contrib-
ting to enhanced Ag presentation (24). The significance of this restricted stimulatory effect could be related to lowering the thresh-
old of required help from Ag-specific Th cells in early stages of immune response.

The precise mechanism by which 7G6-mediated targeting of Ag to CR1/2 leads to an enhanced Ab response is currently unknown. The following possibilities can be envisaged. First, binding of the 7G6-OVA complex to CR1/2 may increase the local Ag concen-
tration at the surface of Ag-specific B cells, facilitating the interac-
tion between Ag and its receptor. Second, 7G6-OVA may induce cross-linking between CR1/2 and the Ag receptor on OVA-
specific B cells, resulting in enhanced intracellular signaling and/or Ag internalization. Previously reported data indicated that human CR2 is an amplifier of low intracellular signaling through the B cell receptor, by showing synergistic increases in [Ca^{2+}], follow-
ing cross-linking CR2 to mlgM at suboptimal activation doses for the triggering of mlgM on B cells (7). Theoretically, it is possible that the binding of 7G6-OVA to CR1/2 provides an additional stimulus by locally activating complement. Third, interaction be-
 tween the 7G6-OVA and CR1/2 expressed on Ag-nonspecific B cells may also lead to Ag presentation, thereby contributing to Ab production. This possibility is supported by our observation that 7G6-OVA induces tyrosine phosphorylation and Ag presentation by IIA1.6 cells, which do not recognize OVA via their surface mlg. Finally, CR2 that is expressed on the surface of follicular dendritic cells and is involved in the retention of complement-
coated Ag and the maintenance of long-term B cell memory (25) may contribute to the effect of 7G6-mediated targeting.

In separate experiments, we have injected mice with nonconjugated 7G6 Abs 24 h before immunization with free OVA. In these mice, the primary anti-OVA Ab response was inhibited by 80% and the secondary response by 30% compared with control mice injected with buffer before immunization with OVA (D.C.B. and W.L.W.H., unpublished observations). These results are in agree-
ment with previous reports (9, 10) showing that the down-modu-
lation of CR1/2 by pretreatment of mice with 7G6 before immu-
nization with keyhole limpet hemocyanin or FITC-haptenated bacteria strongly inhibits Ab production. These findings support the crucial role of CR1/2 during a normal immune response against a protein Ag.

Remarkably, when mice were immunized with OVA conjugated to SHL45.6, an irrelevant isotype control, the anti-OVA response was impaired compared with immunization with nonconjugated OVA, suggesting inhibition of the response. The possibility that this inhibition was caused by interaction of the conjugate with the
REGULATION OF THE Ab RESPONSE BY COMPLEMENT RECEPTORS AND Fc RECEPTORS

down-regulatory FcγRIIb on B cells was supported by the observation that transfection with FcγRIIb rendered IIA1.6 cells, incubated with 7G6-OVA, unable to present OVA epitopes in vitro. This result was confirmed by the inability of A20 cells, the original cell line from which IIA1.6 is derived and which naturally expresses FcγRIIb, to present Ag after treatment with 7G6-OVA. Thus, it is conceivable that after immunization with 7G6-OVA, which allows interaction with CR1/2 and FcγR, the immune response is the result of stimulation via CR1/2 and suppression via FcγRIIb.

In addition to the down-regulatory effects of FcγRIIb, interaction of an Ag with FcγR can also have stimulatory effects on the immune response. For example, in vitro experiments using FcγR-transfected cells have shown that the IgG-mediated interaction of Ag with either murine FcγRI or FcγRIII can induce presentation to Ag-specific T lymphocytes (26). In addition, using mice expressing transgenic human FcγRI, it has been demonstrated that Ag targeting to this receptor strongly enhances the Ab response (27). This observation is consistent with our present finding that transfecting IIA1.6 cells with human FcγRI enhanced 7G6-OVA-induced Ag presentation. In contrast to nontransfected IIA1.6 cells, these cells were able to present Ag after treatment with the isotype control SHL45.6-OVA. However, two lines of evidence indicated that interaction with FcγR was not responsible for the enhanced in vivo Ab production induced by 7G6-OVA. First, conjugation of OVA to the isotype control Ab SHL45.6 resulted in suppression rather than enhancement of the anti-OVA response. Second, the finding that immunizing CR1/2-deficient mice with 7G6-OVA did not result in an enhanced response indicates that FcγR alone are not sufficient to mediate the enhancement induced by 7G6-OVA.

The results of the present study provide a better insight into the mechanism of the generation of the Ab response, which is under control of both the innate and the acquired immune system during a second contact of the organism with an Ag. The intensity and duration of the Ab response induced by a complement- and IgG-containing immune complex may be controlled by a balance between stimulation via CR1/2 and down-regulation via FcγRIIb. Most likely, stimulation of an immune reaction by CR1/2, which depends upon the presence of activated complement factors in the Ag complex, has an important function during the early phase of the response. At a later stage, dampening of the response would be required to prevent uncontrolled cellular reactivity. This shift in the balance toward down-regulation may be mediated by an increasing concentration of IgG which is induced after the onset of the response; this IgG is able to interact with FcγRIIb. The present data are relevant for the rational development of new vaccine strategies. Targeting Ag to CR1/2 may provide the adjuvant effect necessary for the generation of an efficient immune response against various Ags.

Acknowledgments

We thank Dr. M. Glennie (University of Southampton, Southampton, U.K.) for essential suggestions concerning the Ab-OVA coupling procedure; Dr. T. Kinoshita (Osaka University, Osaka, Japan) for providing the 7G6 hybridoma; Dr. M. Clark (Cambridge University, Cambridge, U.K.) for providing purified SHL45.6 Abs; and Dr. C. Bonneterre (Institute Curie, Paris, France) and Dr. I. van Herik-Oudijk and M. van Vugt (University Hospital Utrecht, Utrecht, The Netherlands) for providing the FcγR-transfected cell lines. We also thank P. van Kooten for help with mAb production, P. Aerts for consultation on fast protein liquid chromatography analysis, and A. van der Sar for animal care.

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