T Cell–dependent Immune Response in C1q-deficient Mice: Defective Interferon γ Production by Antigen-specific T Cells

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Summary

The role of the classical complement pathway in humoral immune responses was investigated in gene-targeted C1q-deficient mice ($C1qA^{-/-}$). Production of antigen-specific immunoglobulin (Ig)G2a and IgG3 in primary and secondary responses to T cell–dependent antigen was significantly reduced, whereas IgM, IgG1, and IgG2b responses were similar in control and $C1qA^{-/-}$ mice. Despite abnormal humoral responses, B cells from $C1qA^{-/-}$ mice proliferated normally to a number of stimuli in vitro. Immune complex localization to follicular dendritic cells within splenic follicles was lacking in $C1qA^{-/-}$ mice. The precursor frequency of antigen-specific T cells was similar in $C1qA^{-/-}$ and wild-type mice. However, analysis of cytokine production by primed T cells in response to keyhole limpet hemocyanin revealed a significant reduction in interferon-γ production in $C1qA^{-/-}$ mice compared with control mice, whereas interleukin 4 secretion was equivalent. These data suggest that the classical pathway of complement may influence the cytokine profile of antigen-specific T lymphocytes and the subsequent immune response.

Key words: complement • deficiency • immune response • interferon γ • gene targeting

A large body of evidence supports a role for the complement system in the induction of the humoral arm of the immune response. Mice transiently depleted of complement by cobra venom factor administration before immunization with T cell–dependent (TD)[1] antigen had markedly reduced IgG antibody production (1, 2) and deficient development of B cell memory (3). Observations made in animals with congenital deficiencies in individual complement components confirmed the findings from transiently decomplemented mice. Guinea pigs (4), dogs (5), and humans (6, 7) deficient in complement components C2, C3, or C4 exhibited significantly reduced primary and secondary total IgG production in response to TD antigens. Recently, engineered C3 and C4 knockout mice showed a similar phenotype (8). In contrast, C5-deficient mice developed normal antibody responses (9). However, the requirement for complement in the induction of antibody responses was found not to be absolute. Reduced antibody responses could be overcome in C3-deficient animals (10) and C4-deficient guinea pigs (11) by introducing antigen with adjuvant or by priming with large doses of antigen.

Two nonmutually exclusive models have been proposed to explain the influence of complement on the induction of humoral immune responses. The first suggests that complement is crucial for the correct delivery, presentation, and retention of antigen in lymphoid organs. Murine CD21 expression is primarily limited to B cells and follicular dendritic cells (FDCs) (12). FDCs retain unprocessed antigen in the form of immune complexes (13) and are thought to be involved in the generation of antibody responses within the germinal center and the maintenance of immunological memory (14, 15). Immune complex localization to germinal centers (16) and splenic lymphoid follicles (3) was abolished in mice decomplemented with cobra venom factor and in rats treated with anti-C3 mAb (17). Furthermore, enhanced uptake and presentation of antigen by B cells via CD21 was observed in vitro with Ag coupled to C3dg (18).

The second model suggests that complement, in combination with antigen, lowers the threshold required for B cell activation. This model follows from observations that activation of B cells is enhanced through interaction of C3

[1]*Abbreviations used in this paper:* FDC, follicular dendritic cell; GCDC, dendritic cell identified within murine germinal center; HAGG, heat-aggregated human γ-globulin; IDC, interdigitating dendritic cell; PNA, peanut agglutinin; TD, T cell–dependent.

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with CD21 (19). CD21 can be associated with CD19, a B cell membrane protein essential for the regulation of B cell responses, differentiation, and development (20). It has been shown in vitro that B cells can bind C3 activation products (21). Subsequent studies demonstrated that the threshold for specific B cell activation in vitro was lowered when antigen was coupled with C3d (19). Competitive inhibition or blockade of CD21–C3d interaction by soluble CR2–Ig fusion protein (22), or mAb with specificity for CR2 (23–25), abolished antibody production in vivo. Similar data were obtained from *Cr2*$^{-/-}$ mice, where reduced humoral immune responses to TD antigens were observed (26, 27). Strongest support for this model was provided by chimeric mice with CR2 expression absent exclusively on B cells. Antibody responses were comparable to those observed in *Cr2*$^{-/-}$ mice (28).

At present, little is known about the role of complement in the regulation of antibody isotype production in response to TD antigen. We report here studies on the role of the classical pathway of complement on the induction of antibody production and class switching in response to TD antigen in C1q-deficient mice (29).

Materials and Methods

Mice. *C1qA*$^{-/-}$ mice used were 129/Sv or on a (129/Sv × C57BL/6) F_2 mixed genetic background as specified in each experiment, and were generated as previously described (29). Age-, strain-, and sex-matched mice were used as controls. Animals were maintained in specific pathogen-free conditions.

Immunization Protocols for Antibody Responses. *C1qA*$^{-/-}$ and control wild-type mice were immunized intraperitoneally with (*a*) a 10% SRBC suspension in PBS and challenged with an equal dose 21 d after the initial immunization and (*b*) 6×10^6 SRBCs coupled with DNP-KLH (Calbiochem-Novabiochem Corp., La Jolla, CA). SRBCs were coated with DNP-KLH using the chromic chloride method (30) or (*c*) 10 μg DNP-KLH precipitated in alum. Mice primed intraperitoneally with 6×10^6 SRBCs coupled with DNP-KLH were challenged intravenously on day 43 with 10 μg soluble DNP-KLH. Blood samples were taken before immunization and at further time points specified for each experiment.

Measurement of Anti-SRBC Antibodies. Anti-SRBC total Ig or IgG was measured by hemagglutination assay as previously described (31). In brief, heat-inactivated serum from immunized mice was serially diluted on v-bottomed microtiter plates and mixed with a 2% SRBC suspension in the presence or absence of 2-mercaptoethanol. Plates were read "blind" by a different person than the one who had set up the assay to assess agglutination titer after a 4-h incubation at 4°C.

Measurement of DNP-specific Ig Isotypes. ELISA plates (Dynatech Labs Ltd., Billinghurst, UK) were coated with 50 μl DNP-BSA (Calbiochem-Novabiochem Corp.) or goat anti–mouse Ig (H + L; 5 μg/ml; Southern Biotechnology Assoc., Birmingham, AL) diluted in carbonate/bicarbonate buffer, pH 9.6, and incubated at 4°C overnight. Plates were blocked with 1% BSA in PBS. Test sera were diluted in PBS–Tween 0.05% and 50 μl of diluted sera was incubated for 1 h at room temperature in duplicate. Each assay included affinity-purified mouse IgM, IgG1, IgG2a, IgG2b, or IgG3 (0.5 μg/ml; Sigma Chemical Co., Poole, UK), which were titrated to generate standard curves. Plates were washed and further incubated with alkaline phosphatase–conjugated goat anti–mouse isotype-specific antibodies (0.2 μg/ml in PBS-Tween 0.05%; Southern Biotechnology Assoc.) for 1 h at room temperature. The plates were developed using the substrate *p*-nitrophenyl phosphate (Sigma Chemical Co.). The OD of the reaction mixture at 405-nm wavelength was measured using an ELISA reader (Titertek Labsystems, Basingstoke, UK). The relative concentration of specific Ig isotypes in individual samples was calculated by comparing the mean OD obtained for duplicate wells minus nonspecific binding to the titrated mouse Ig isotype standard curve.

Limiting Dilution Analysis. Wild-type and *C1qA*$^{-/-}$ mice were immunized intraperitoneally with 10 μg DNP-KLH in alum. The frequency of antigen-specific T cells in the spleen was determined by IL-2 limiting dilution analysis 15–16 d after priming, as previously described (32). In brief, splenocytes taken from unimmunized mice were depleted of T cells and irradiated (25 Gy). 4×10^4 of these antigen-presenting cells were added to limiting numbers of splenocytes, with and without 50 μg/ml KLH in 96-well plates. 24 identical wells were set up at each concentration of responder cells. 2 d later, plates were irradiated (25 Gy), and 10^4 cells of the IL-2–dependent cell line, CTLL, were added to each well. After 8 h of culture, proliferation was measured by uptake of [^{3}H]thymidine over 16 h. Positive wells exhibited a proliferation of greater than the mean plus three SD of control wells with no responder cells. The frequency of antigen-specific cells was calculated by regression analysis of the number of positive wells at each dilution of responder cells.

Measurement of Cytokines. *C1qA*$^{-/-}$ mice and controls were immunized intraperitoneally with 10 μg DNP-KLH in alum. Spleens were removed at day 14 after priming and a splenic mononuclear cell suspension was generated after lysis of red blood cells with Gey's medium. Cells were plated at 5×10^5 cells/well in 200 μl medium in round-bottomed 96-well plates. The splenic cell suspension was pulsed with 10 μg/ml soluble KLH and supernatants were taken on days 4 and 7 of culture. Supernatants were tested for IFN-γ activity by a sandwich ELISA as previously described (33). In brief, antibodies to mouse IFN-γ were used for coating of ELISA plates, followed by addition of supernatants to be tested for the presence of IFN-γ, capture by a second biotinylated IFN-γ–specific antibody, and detection with streptavidin conjugated with alkaline phosphatase. Supernatants were also tested for IL-4 activity using the IL-4–dependent cell line CT.4S (34). Proliferation was assessed by the uptake of [^{3}H]thymidine (1 μCi/well).

B Cell Proliferation. A splenic mononuclear cell suspension was generated after lysis of red blood cells with Gey's medium. B cells were purified by incubating the cell suspension with mAbs specific for CD4, CD8, and Thy-1. Stained cells were subsequently lysed with rabbit complement. Purified splenic B cells from two *C1qA*$^{-/-}$ or wild type controls were plated in triplicate at 2×10^5/ml in 96-well plates in IMDM (GIBCO, Paisley, UK) supplemented with 5% heat-inactivated FCS, 5×10^{-5} M 2-mercaptoethanol, 100 U/ml penicillin, and 100 μg/ml streptomycin (Sigma Chemical Co.). B cells were stimulated with 1 μg LPS (*Salmonella typhosa* 0901; Difco, Detroit, MI), anti-CD40, FGK 45 (35), or 10 μg monoclonal anti–mouse κ chain (187.1; American Type Culture Collection, Rockville, MD) plus IL-4 (36). B cell proliferation was assessed at 48 h by the uptake of [^{3}H]thymidine.

Localization of Immune Complexes. Human γ-globulins, Cohn's fractions II and III (Sigma Chemical Co.), were heat aggregated according to Brown et al. (37). The heat-aggregated human γ-globu-

lins (HAGG) were labeled with the fluorochrome FITC. *C1qA*$^{-/-}$ and control mice were injected intravenously with 500 μg FITC-HAGG. Spleens were removed at 24 h and frozen in Tissue-Tek OCT compound (Miles Inc., Elkhart, IN) and stored at −70°C. 6-μm sections cut on a cryostat microtome were thaw mounted onto Vectabond-treated slides (Vector Labs., Peterborough, UK), fixed in acetone for 5 min at room temperature, and stored at −70°C. Splenic sections were rehydrated in PBS before staining with peanut agglutinin (PNA)-biotin (Vector Labs.) and mAb FDC-M1-biotin. PNA and FDC-M1 staining was revealed by streptavidin–Texas red (Southern Biotechnology Assoc.) and sections were mounted using Vectashield (Vector Labs.).

Statistics. Data are shown as mean values ± SEM or SD where specified. The Mann-Whitney U nonparametric test was used to analyze the difference between *C1qA*$^{-/-}$ and wild-type immune responses. Differences were considered significant when $P < 0.05$.

Results

Antibody Responses to T Cell–dependent Antigen in C1q-deficient Mice. Primary and secondary total Ig and IgG titers were analyzed in C1q-deficient and wild-type mice immunized with SRBCs. Anti-SRBC total Ig titers, measured in hemagglutination assay, were similar in *C1qA*$^{-/-}$ and wild-type mice in the primary response. In contrast, peak anti-SRBC Ig titers were significantly reduced in *C1qA*$^{-/-}$ (939 ± 85 SEM) compared with wild-type mice (2,731 ± 629) ($P < 0.02$) in the secondary response (Fig. 1 *A*). In addition, anti-SRBC IgG titers were significantly reduced in *C1qA*$^{-/-}$ (53.3 ± 7) versus wild-type (154 ± 26) mice ($P < 0.02$) in the primary response at day 8 after immunization. Similar findings were obtained in the secondary anti-SRBC IgG response (*C1qA*$^{-/-}$ mice: 277.7 ± 51.35 versus controls: 768 ± 114.4) ($P < 0.02$) (Fig. 1 *B*).

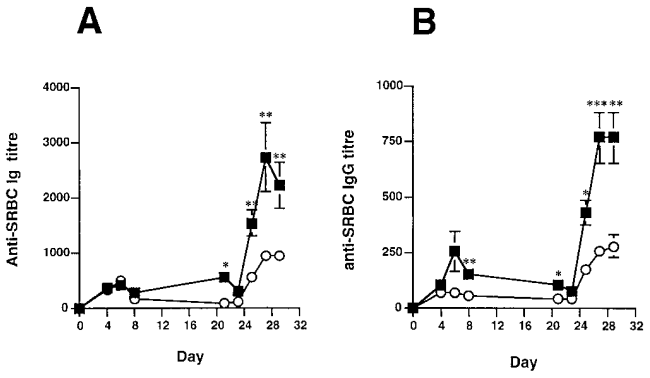

Figure 1. Anti-SRBC Ig and IgG responses in *C1qA*$^{-/-}$ mice. Wild-type (129/Sv × C57BL/6) (■; $n = 6$) and strain-matched *C1qA*$^{-/-}$ (○; $n = 6$) mice were immunized intraperitoneally with a 10% SRBC suspension and boosted on day 21 with a similar dose. Blood samples were first taken on days 0, 4, 6, and 8 and subsequently on days 21, 23, 25, 27, and 29 after immunization. Anti-SRBC total Ig (*A*) and IgG (*B*) antibody responses were determined by hemagglutination assay as detailed in the experimental procedures section. All results are represented as means ± SEM. Significance was determined by the Mann-Whitney U test. * $P < 0.05$, ** $P < 0.02$, *** $P < 0.002$.

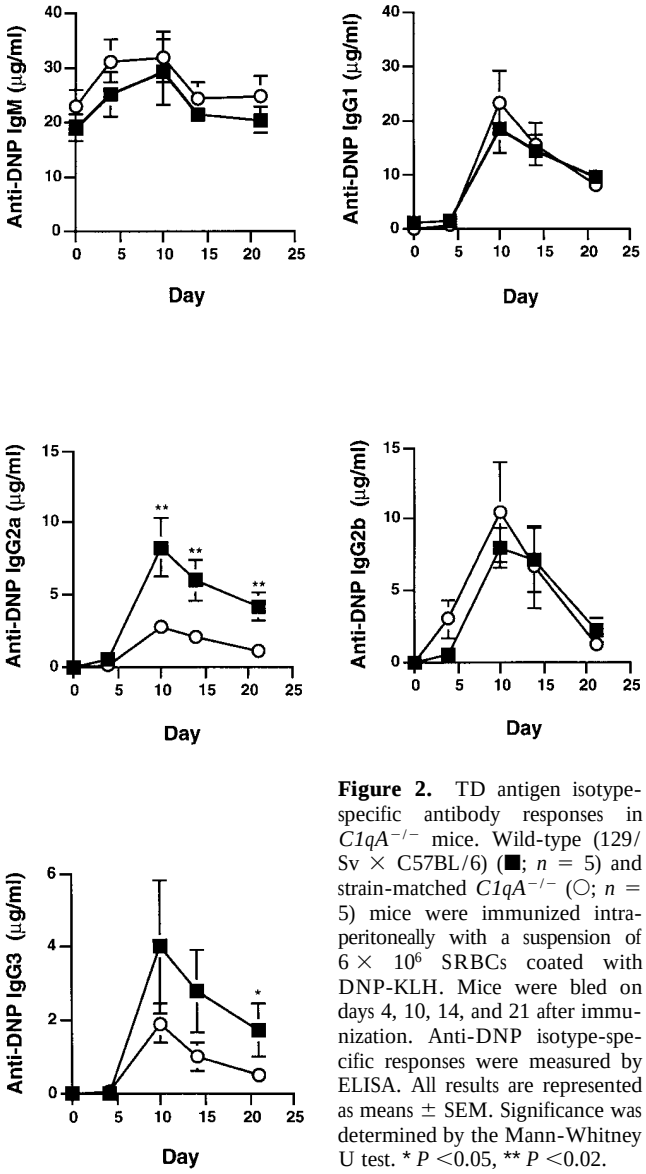

Figure 2. TD antigen isotype-specific antibody responses in *C1qA*$^{-/-}$ mice. Wild-type (129/Sv × C57BL/6) (■; $n = 5$) and strain-matched *C1qA*$^{-/-}$ (○; $n = 5$) mice were immunized intraperitoneally with a suspension of 6×10^6 SRBCs coated with DNP-KLH. Mice were bled on days 4, 10, 14, and 21 after immunization. Anti-DNP isotype-specific responses were measured by ELISA. All results are represented as means ± SEM. Significance was determined by the Mann-Whitney U test. * $P < 0.05$, ** $P < 0.02$.

Antibody responses to TD antigen were then investigated in greater detail in *C1qA*$^{-/-}$ mice. Wild-type (129/Sv × C57BL/6) F_2 and *C1qA*$^{-/-}$ mice were immunized with SRBCs coated with DNP-KLH. This model permitted the analysis of isotype-specific antibody responses to soluble hapten-protein TD antigen in the absence of adjuvant. The primary antibody response to DNP revealed a novel and unexpected pattern of isotype-specific antibody class switching. Anti-DNP–specific IgM, IgG1, and IgG2b isotype production was similar in *C1qA*$^{-/-}$ and control mice (Fig. 2). However, mean anti-DNP IgG2a antibody production on day 10 after priming was significantly reduced in C1q-deficient mice compared to wild-type animals (2.8 μg/ml ± 0.8 versus 8.3 μg/ml ± 2, respectively) ($P < 0.02$) (Fig. 2). Furthermore, anti-DNP IgG3 antibody production in *C1qA*$^{-/-}$ mice (0.5 μg/ml ± 0.25) was reduced ($P < 0.05$) when compared to the response of wild-

Table 1. *Reduced TD Antigen-specific IgG2a and IgG3 Isotype Production by C1qA$^{-/-}$ Mice*

Anti-DNP Ig isotype	129/Sv	*C1qA*$^{-/-}$
IgM	30.6 ± 17.9	16.99 ± 6.5
IgG1	18.54 ± 2.06	21.33 ± 3.60
IgG2a	16.44 ± 2.63	8.46 ± 1.92**
IgG2b	5.34 ± 1.61	4.67 ± 1.29
IgG3	0.333 ± 0.059	0.191 ± 0.0465*

Pure line 129/Sv (n = 6) and strain-matched *C1qA*$^{-/-}$ (129/Sv) mice (n = 7) were immunized intraperitoneally with 10 μg DNP-KLH in alum. Anti-DNP isotype production was measured on day 14 after priming. The data represents antigen-specific isotype production in micrograms per milliliter of serum as means ± SEM. Significance was determined by the Mann-Whitney U test. *P <0.05, ** P <0.02.

type controls (1.7 μg/ml ± 0.7) on day 21 after priming (Fig. 2). A similar pattern of isotype production was observed in response to SRBCs in the same mice (data not shown).

Anti-DNP isotype-specific responses were also measured in *C1qA*$^{-/-}$ and control mice on a pure genetic background (129/Sv). Control and gene-targeted mice were immunized with 10 μg DNP-KLH precipitated in alum. Again, significantly reduced anti-DNP IgG2a and IgG3 production was observed in *C1qA*$^{-/-}$ mice compared with controls. IgM, IgG1, and IgG2b anti-DNP production was similar in wild-type and knockout mice (Table 1).

Mice primed with SRBC–DNP-KLH were challenged with 10 μg soluble DNP-KLH at day 43 after immunization and isotype-specific anti-DNP antibody responses were analyzed. The secondary anti-DNP IgM response was similar in *C1qA*$^{-/-}$ and wild-type mice, as was a poor IgG2b antigen-specific response (Fig. 3). Anti-DNP IgG1 production was marginally reduced in *C1qA*$^{-/-}$ mice in the secondary response compared to the response in control mice (34 μg/ml ± 10.4 versus 62.9 μg/ml ± 10.3, respectively) on day 10 after challenge, though this result was not statistically significant (Fig. 3). However, anti-DNP IgG2a production was again significantly reduced in *C1qA*$^{-/-}$ mice (3 μg/ml ± 1.525) compared to wild-type controls (52.1 μg/ml ± 17) (P <0.05) at day 14 after challenge. The secondary DNP-specific IgG3 response was also significantly diminished in *C1qA*$^{-/-}$ mice (1 μg/ml ± 0.4) in contrast to the mean control response (3 μg/ml ± 0.5) (P <0.05) at 10 d after challenge (Fig. 3).

Cytokine Production and Precursor Frequency of Antigen-specific T Cells in C1qA$^{-/-}$ *Mice.* Gene-targeted and control mice were immunized intraperitoneally with 10 μg DNP-KLH in alum. The frequency of antigen-specific splenic T cells was assessed 15–16 d after priming in limiting dilution analysis. The mean frequency of antigen-specific T cells primed after immunization with KLH was similar in wild type: 1/17,316 (n = 4) and *C1qA*$^{-/-}$: 1/15,983 (n = 4)

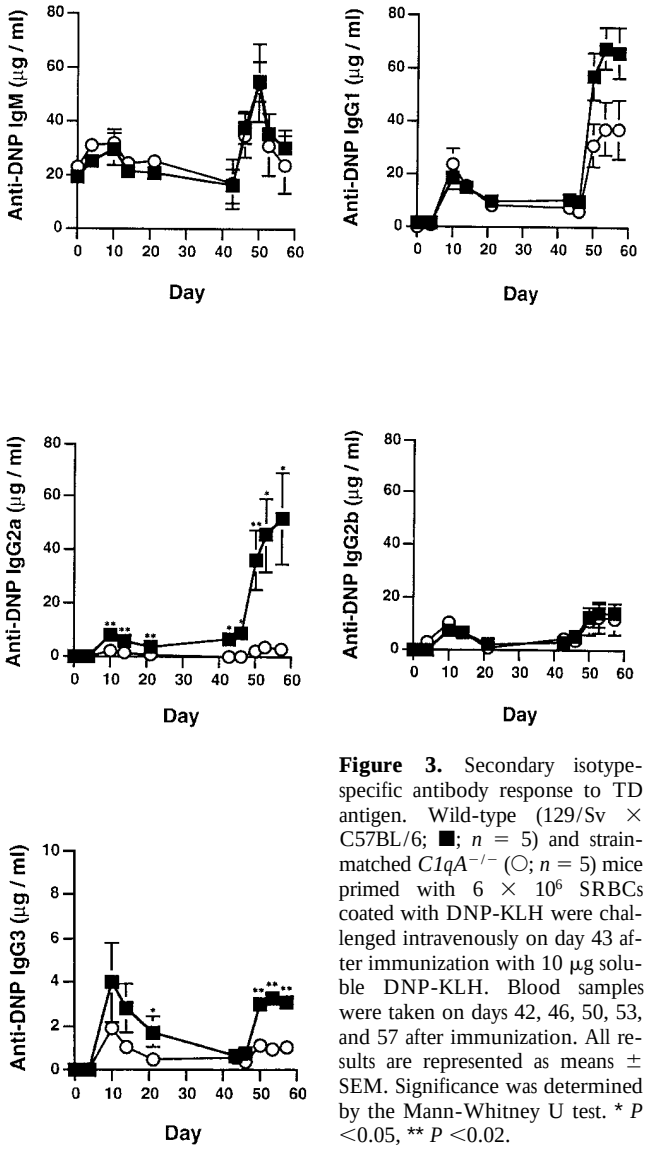

Figure 3. Secondary isotype-specific antibody response to TD antigen. Wild-type (129/Sv × C57BL/6; ■; n = 5) and strain-matched *C1qA*$^{-/-}$ (○; n = 5) mice primed with 6 × 10^6 SRBCs coated with DNP-KLH were challenged intravenously on day 43 after immunization with 10 μg soluble DNP-KLH. Blood samples were taken on days 42, 46, 50, 53, and 57 after immunization. All results are represented as means ± SEM. Significance was determined by the Mann-Whitney U test. * P <0.05, ** P <0.02.

mice (Fig. 4). Antigen-specific cytokine production by primed T cells was studied further. Mice were immunized intraperitoneally with 10 μg DNP-KLH in alum. Whole splenic cell suspensions were pulsed with 10 μg/ml KLH and cytokine secretion was assessed on days 4 and 7 of culture. IFN-γ production (Fig. 5 *A*) was significantly diminished in splenic cells from *C1qA*$^{-/-}$ mice when compared to controls (P <0.01). In contrast, IL-4 (Fig. 5 *B*) production, when detectable after 7 d of culture, was equivalent in the two experimental groups. Similar results were obtained in two further experiments (data not shown).

Proliferation of B Cells from C1qA$^{-/-}$ *Mice.* Considering the aberrant B cell responses in *C1qA*$^{-/-}$ mice after immunization with TD antigen, we asked whether these observations were a result of a defect within the B cell population resulting from the absence of functional C1q. B cells purified from wild-type and C1q-deficient mice were incubated with a number of B cell mitogens. B lymphocytes

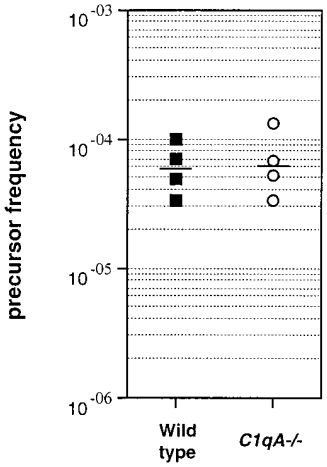

Figure 4. Analysis of T cell priming. Wild-type (129/Sv; ■; $n = 4$) and strain-matched $C1qA^{-/-}$ (○; $n = 4$) were immunized intraperitoneally with 10 μg DNP-KLH precipitated in alum. Precursor frequencies of KLH-specific splenic T cells were determined by IL-2 limiting dilution analysis as detailed in the experimental procedures section.

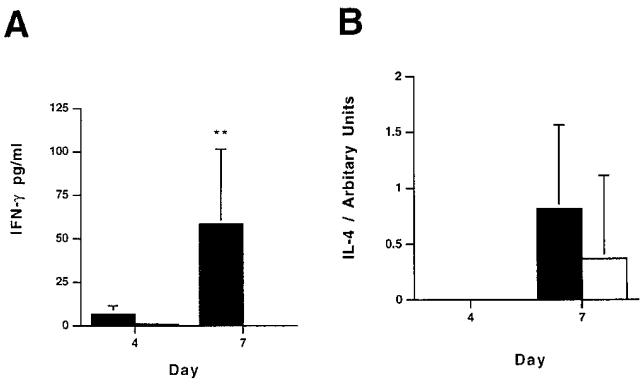

Figure 5. Cytokine production by antigen-specific T cells. 129/Sv (*filled bars*; $n = 5$) and strain-matched $C1qA^{-/-}$ (129/Sv; *open bars*; $n = 5$) mice were immunized intraperitoneally with 10 μg DNP-KLH in alum. T cell cytokine production was assessed day 14 after priming. Whole splenic cell suspensions were pulsed with KLH (10 μg/ml) and supernatants were harvested on days 4 and 7. (*A*) IFN-γ production was measured by ELISA and (*B*) IL-4 production was measured by bioassay as detailed in Materials and Methods. All results are represented as means ± SD. Significance was determined by the Mann-Whitney U test. (** $P < 0.01$).

from both groups proliferated to an equal degree in response to 1 μg of LPS (Fig. 6). Cross-linking of surface immunoglobulin molecules by an anti-Ig κ chain mAb in the presence of IL-4 also induced comparable proliferative responses in wild-type and $C1qA^{-/-}$ mice. Furthermore, stimulation of B cells through CD40, mimicking T–B cell interaction, induced equivalent proliferative B cell responses in both experimental groups (Fig. 6).

Localization of Immune Complexes. The role of the classical pathway of complement in the localization of model immune complexes was investigated using FITC-HAGG. FITC-HAGG was located within the splenic follicles of wild type mice 24 h after injection (Fig. 7 *A*), whereas no equivalent follicular trapping was observed in $C1qA^{-/-}$ mice (Fig. 7 *B*). Trapped FITC-HAGG was focused at the apex of germinal centers within the follicle, revealed by PNA staining (*red*; Fig. 7 *A*) and colocalized with FDCs (*yellow*; Fig. 7 *C*) in wild-type mice, but not C1q-deficient mice (Fig. 7 *D*). There was, however, clear uptake of FITC-HAGG in the red pulp of $C1qA^{-/-}$ mice (Fig. 7, *B* and *D*) as there was in wild-type mice.

Discussion

Activation of the classical pathway of complement is well known to be critical for the induction of IgG antibody responses after immunization with suboptimal doses of TD antigen. Initial experiments using SRBCs confirmed this immunological phenotype in $C1qA^{-/-}$ mice. However, it was apparent that the complement-deficient mice had the capacity to produce antigen-specific class-switched IgG antibody. Isotype-specific analysis of the primary antibody response revealed a novel pattern of IgG isotype production to both particulate and soluble protein TD antigen. Antigen-specific IgG2a and IgG3 production was significantly reduced in gene-targeted mice, whereas production of other isotypes was similar to that of controls. The phenotype was consistent between $C1qA^{-/-}$ mice from a mixed (129/Sv × C57/BL6) or a pure (129/Sv) genetic background.

No primary defect was detected in the B cell compartment, as B cells from $C1qA^{-/-}$ mice proliferated normally to TD stimuli in vitro. The selective reduction in IgG2a and IgG3 production therefore suggested that cytokine production by antigen-specific T cells may be abnormal in $C1qA^{-/-}$ mice. Ig class switching is thought to be induced after contact with antigen-specific T cells (38). Furthermore, cytokine production by antigen-specific T cells is known to influence antibody isotype production (39). IL-4 promotes the class switch to IgG1, whereas IFN-γ stimulates IgG2a isotype production (40) and possibly IgG3 production (41). Although similar frequencies of antigen-specific T cells were primed in $C1qA^{-/-}$ and wild-type mice, T cells in $C1qA^{-/-}$ mice produced diminished levels of IFN-γ, whereas secretion of IL-4 was equivalent in control and gene-targeted mice. Reduced IFN-γ production and normal IL-4 production could explain the differences in antibody isotype profiles observed.

Recent data suggest that cytokine production by antigen-specific T cells may be influenced by interaction with different APC populations. Activated B cells promote IL-4 secretion from antigen-specific T cells (42, 43), but cannot produce IL-12 in a similar context (44). In contrast, T cell–

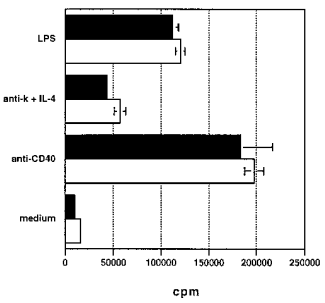

Figure 6. B cell proliferation. Purified B cell suspensions (2×10^5/ml) from wild-type (129/Sv × C57BL/6; *filled bars*; $n = 2$) or strain-matched $C1qA^{-/-}$ (*open bars*; $n = 2$) mice were plated in triplicate. B cells were then stimulated with LPS (1 μg/ml), anti-Ig κ chain (10 μg/ml) plus IL-4, or anti-CD40 treatment. B cell proliferation was assessed at 48 h by the uptake of [^{3}H]thymidine.

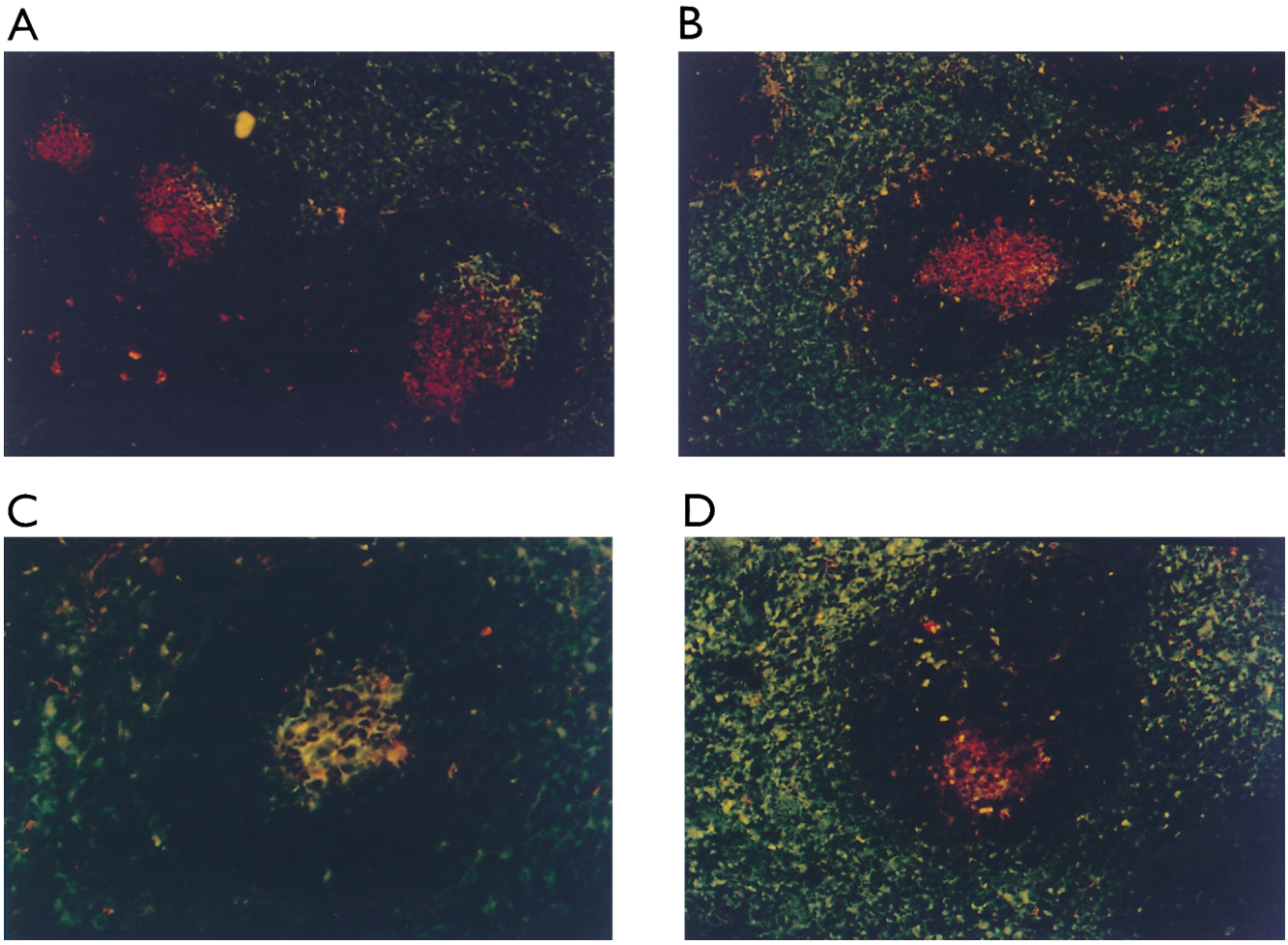

Figure 7. Absence of follicular localization of immune complexes in $C1qA^{-/-}$ mice. Spleens from wild-type (129/Sv × C57BL/6; $n = 5$) and strain-matched $C1qA^{-/-}$ ($n = 5$) mice were taken 24 h after intravenous injection with 500 μg FITC-labeled HAGG. FITC localization (*green*) was assessed in splenic sections relative to germinal centers (PNA expression; *red*) in wild-type (*A*) and $C1qA^{-/-}$ (*B*) mice. FDCs were detected by mAb FDC-M1 (*red*) in wild-type (*C*) and $C1qA^{-/-}$ (*D*) mice. Original magnification: 20 (*A*, *B*, and *D*) and 40 (*C*).

interdigitating dendritic cell (IDC) interaction promotes IL-12 production by IDCs and subsequent IFN-γ production by antigen-specific T cells (45), as does T cell–monocyte interaction (46). Notably, only IDCs pulsed with antigen in vitro can induce production of antigen-specific IgG2a in vivo (47, 48). In addition, IDCs in the presence of activated T cells have been shown directly to induce activated B cells to secrete IgG in vitro (49). It is of interest that dendritic cells identified within murine germinal centers (GCDCs) express CD21 (50). However, it is not known whether these GCDCs exhibit similar functions or secrete a similar cytokine profile as IDCs. Antigen-specific $CD4^+$ T (51) and B cells with class-switched Ig CH chains (52) accumulate within the germinal center during the follicular phase of the antibody response. Interaction between antigen-specific T cells, B cells, and GCDCs could be defective in $C1qA^{-/-}$ mice.

A direct interaction between C1q and APCs may be deficient in $C1qA^{-/-}$ mice. A number of cell types have been shown to bind C1q (53). Furthermore, several receptors for C1q have been proposed (54). Previous reports have suggested that aggregated C1q can enhance Ig production in B cells via interaction with C1q receptors (55). Notably, macrophages also express a receptor for C1q, which has been proposed to be involved in the enhancement of phagocytosis of antigen (56, 57). It has also been tentatively suggested that C1q synthesized by macrophages may play a role in antibody production at sites of inflammation (53). However, the function of C1q receptors in the immune response remains controversial and will require further study.

Fischer et al. have recently studied the immune response to TD antigen in C4 "knockout" mice. TD antigen-specific total IgG and IgM production was reduced in $C4^{-/-}$mice. However, antigen-specific isotype production was not evaluated in this model of classical complement pathway deficiency (8). In agreement with results in our study, T cell priming was unaffected in $C4^{-/-}$ mice (8) and in mice with complement receptors 1 and 2 blocked by specific

mAbs (23). However, cytokine production by antigen-specific T cells has not been reported previously in complement-deficient mice. This study constitutes the first demonstration that classical complement pathway activation is essential for normal IFN-γ production by antigen-specific T cells and subsequent IgG2a and IgG3 production.

Localization of immune complexes to splenic lymphoid follicles requires activation of the classical complement pathway. Localized FITC-HAGG was concentrated on FDC adjacent to germinal center B cells in wild-type mice. FDCs express both Fc and complement receptors (58, 59). Fc-γ chain–deficient mice exhibited enhanced trapping of immune complexes on FDCs (60). Clearly, activation of the classical complement pathway and complement receptors are essential for trapping immune complexes on FDCs. Nevertheless, FITC label was detected in the splenic red pulp of *C1qA*$^{-/-}$ mice. A previous report showed that HAGG injected intravenously was located in the red pulp of the spleen and the marginal zone within 30 min of injection. At 8 h, HAGG localized to the splenic follicle (61). Marginal zone B cells are proposed to transport immune complexes to germinal centers via complement receptors (62). It follows that in *C1qA*$^{-/-}$ mice, FITC-HAGG captured by Fc receptors would not be transported to germinal centers and may be catabolized by red pulp macrophages.

The poor secondary humoral response observed in *C1qA*$^{-/-}$ mice may be due to deficient trapping of antigen–antibody complexes within the FDC network. FDCs retain immune complexes in their native form on dendrites for months after priming with antigen (63). Antigen is released as immune complex-coated bodies after challenge with recall antigen (64) allowing uptake by memory B cells (65). The retention and release of antigen by FDCs has been implicated in the maintenance of antibody levels (63) and in B cell memory (66). The defect in secondary antibody responses in *C1qA*$^{-/-}$ mice might lie in defective maintenance, but might also indicate failed selection of antigen-specific B cells within the germinal center. Antigen receptor engagement on B cells is known to be an important signal for rescuing B cells from apoptosis (67). This signal can be provided by antigen on retained FDCs (15). The failure in selection of antigen-specific B cells might not allow the formation of a B cell memory pool.

Recent studies using mice with targeted deletions of C3 and C4 (8) or *Cr2*$^{-/-}$ (26–28) emphasized the importance of complement receptors on B cells in humoral responses to TD antigens. Data from this study do not exclude the possibility that enhancement of signaling through complement receptors on B cells is important in the induction of normal humoral responses. However, complement-dependent signals to specialized APC populations appear to be of equal importance in the generation of normal antibody responses to TD antigens. Complement-dependent delivery of antigen to FDCs may also be critical in the generation of normal secondary antibody responses.

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