

Correlation of anti-viral B cell responses and splenic morphology with expression of B cell-specific molecules

Thomas Fehr^{1,4}, Constantino López-Macías¹, Bernhard Odermatt¹, Raul M. Torres⁵, Daniel B. Schubart^{2,6}, Theresa L. O’Keefe^{3,7}, Patrick Matthias², Hans Hengartner¹ and Rolf M. Zinkernagel¹

¹Institute of Experimental Immunology, Department of Pathology, University Hospital, Schmelzbergstrasse 12, 8091 Zürich, Switzerland

²Friedrich Miescher Institute, Maulbeerstrasse 66, 4058 Basel, Switzerland

³Medical Research Council Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK

⁴Present address: Department of Internal Medicine, University Hospital, Rämistrasse 100, 8091 Zürich, Switzerland

⁵Present address: Basel Institute of Immunology, Grenzacherstrasse 487, 4005 Basel, Switzerland

⁶Present address: Axxima Pharmaceuticals AG, Am Klopferspitz 19, D-82152 Planegg-Martinsried, Germany

⁷Present address: Leukosite Inc., 215 First Street, Cambridge, MA 02142, USA

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Abstract

This study attempted to evaluate and compare the role of various B cell-specific markers for anti-viral immune responses in mouse strains lacking molecules belonging to the B cell receptor (BCR) complex (IgM, Ig α and C κ), the co-stimulatory molecules (CD19 and CD22), the protein kinases [Bruton’s tyrosine kinase (Btk)] or the transcription factors (OBF-1). These mice were tested in two model infections [vesicular stomatitis virus (VSV) and lymphocytic choriomeningitis virus (LCMV)] using T cell-independent (TI) or T cell-dependent (TD) antigens. All mice controlled an LCMV infection indicating that cytotoxic T cell functions were within normal ranges. In contrast, OBF-1^{-/-} mice were partially protected and mb-1 ^{$\Delta c/\Delta c$} mice not at all protected against VSV infection, a virus that is controlled virtually exclusively by neutralizing antibodies. Susceptibility to VSV infection was correlated with structural defects in the spleen: absence of mature B cells and follicles with marginal zone macrophages and absence of germinal centers with follicular dendritic cells correlated with lack or substantial reduction of protective IgM and IgG responses respectively. The lack of κ light chain did not affect the neutralizing response, indicating that it could easily be replaced by the λ chain. Absence of the co-stimulatory molecules CD19 and CD22 or of the signaling molecule Btk had modulating effects, but did not increase susceptibility to VSV or LCMV. Our findings suggest that there are crucial molecules for B cell activation at the beginning (BCR complex) and the end (transcription) of the signaling cascade, whereas fine-tuning factors modulating the response in between exhibit considerable functional overlap.

Introduction

A great number of molecules that play a role in B cell development and activation have been identified. Whereas a majority of them are expressed in various cell types of the immune system, some are B cell specific. They either belong to the B cell receptor (BCR) complex, to a heterogeneous group of tyrosine kinases and phosphatases as well as

other signaling molecules, to a group of B cell-specific co-stimulatory or inhibitory molecules, or they are B cell-specific transcription factors. The functions of these molecules have mainly been investigated by blocking studies with mAb, oligonucleotides or protein kinase inhibitors using cell culture systems. The generation of gene-targeted animals has offered

a new potent tool to investigate their role. A series of excellent reviews on various aspects of B cell signaling have drawn an increasingly precise picture of the hierarchy of transcription factors during B cell development (1,2) and of the cascade of events occurring in a B cell after antigen binding, which finally lead to cellular activation (3–11).

Antibody responses in various newly generated gene-targeted animals are not directly comparable because experiments involve different types of antigens for immunization and different read-out systems for analysis. It is therefore difficult to extrapolate these findings and to understand the relative importance of certain molecules and pathways for generation of immune responses against pathogens. The aim of this study was therefore a comparative analysis of antiviral antibody responses in various mouse strains lacking distinct B cell-specific molecules.

As experimental systems we used three well-established virus infections in mice: (i) vesicular stomatitis virus (VSV), a close relative of rabies virus belonging to the family of *Rhabdoviridae*; VSV is a potent inducer of T cell-independent (TI)-1 neutralizing IgM antibody responses (12,13), switch to IgG is largely T help dependent and protection against VSV is virtually exclusively mediated by neutralizing antibodies (14). (ii) A recombinant vaccinia virus expressing the VSV glycoprotein (vacc-VSV-G) after cellular infection; protection against this virus is mainly mediated by cytokines (IFN, tumor necrosis factor); VSV-specific neutralizing antibodies of TI-2 type are also efficiently induced (12). (iii) Lymphocytic choriomeningitis virus (LCMV), a member of the *Arenaviridae*, which, in contrast to VSV and vaccinia virus, is a non-cytopathic agent; LCMV induces potent cytotoxic T cell responses, that are responsible for early elimination of the virus after acute infection by a perforin-dependent mechanism (15); in addition a T cell-dependent (TD) type of antibody response to LCMV nucleoprotein is induced and can be measured by ELISA.

In this study we compared six different mouse strains lacking B-cell specific molecules as summarized in Fig. 1. The already described characteristics of these strains and the References for their initial publication are given in the summary Table 1. With standardized schemes of immunizations the following parameters were analyzed: (i) protection against primary and secondary infections, (ii) quantitative aspects of the antibody responses to TI-1, TI-2 and TD viral antigens, and (iii) splenic morphology by immunohistochemistry 1 month after VSV infection.

Methods

Mice

CD22^{-/-}, OBF-1^{-/-} and mb-1^{Δc/Δc} mice were obtained from the breeding colonies of the Medical Research Council Laboratory (Cambridge, UK), the Friedrich Miescher Institute (Basel, Switzerland) and the Basel Institute of Immunology (Basel, Switzerland) respectively. CD19^{-/-}, C_κ^{-/-} and C57BL/6 mice were obtained from the breeding colony of the Institut für Labortierkunde, Veterinary Hospital, Zurich, Switzerland. CBA and CBAXid mice were purchased from Harlan Nederlael (NM Horst, The Netherlands). Mice were bred and kept

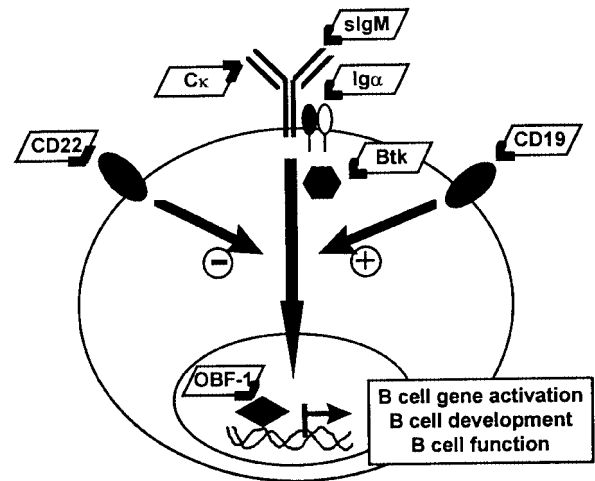


Fig. 1. Mutant mice used for this study. B cell-specific molecules coded by genes that were inactivated in the mouse strains used for this study are displayed in black. The function of these molecules is schematically demonstrated. ⊕, Co-stimulatory molecule; ⊖, co-inhibitory molecule; Btk, Bruton's tyrosine kinase (lacking in *xid* mice); OBF, Oct-binding factor; sIgM, surface IgM.

under specific pathogen-free conditions. Immunizations were performed in a conventional animal facility for infectious studies. Mice were used at 8–12 weeks of age. Note that animals are of different genetic backgrounds as indicated in Table 1. Therefore absolute antibody titers are not directly comparable, but only with the control group of the same genetic background.

Viruses

VSV serotype Indiana (Mudd-Summers isolate) was originally obtained from Professor D. Kolakowsky (University of Geneva, Switzerland). It was grown on BHK cells in MEM supplemented with 5% FCS at 37°C. LCMV (isolate WE) had originally been obtained from Dr F. Lehmann-Grube (Hamburg, Germany). The recombinant baculovirus expressing VSV-G was a generous gift from Dr D. H. L. Bishop (NERC Institute of Virology, Oxford, UK). It was derived from nuclear polyhedrosis virus and was grown at 28°C in *Spodoptera frugiperda* cells in spinner cultures in TC-100 medium (16). The recombinant vaccinia virus expressing VSV-G (vacc-VSV-G) was generated according to standardized protocols and grown on BHK cells in MEM supplemented with 5% FCS at 37°C (17).

Immunizations

For immunizations with live virus or baculovirus-derived VSV-G (Bac VSV-G), 2 × 10⁶ p.f.u. of VSV or vaccinia virus, 200 p.f.u. of LCMV or 10 μg of Bac VSV-G respectively were injected in 200 μl of BSS i.v. into the tail vein. For UV inactivation of VSV a small volume of high titer virus preparation was exposed in a thin layer in a Petri dish to a UV lamp (Philips; 15 W) for 3 min at a distance of 8 cm (18).

VSV neutralization assay

Neutralizing titers of sera were determined as described (19). Briefly, the sera were prediluted 40-fold in MEM supplemented

Table 1. Characteristics of the various mutant mouse strains

Strain	Background	Lacking molecule	Postulated function	B cell development	B cell function	Lymphoid architecture	Reference
μ MT ^a	129Sv	IgM heavy chain	part of BCR: antigen recognition and signal transduction	total block at the pro-B cell step	BCR is totally lacking on B cells; no antibody in serum of naive and immunized mice	disrupted, B cells absent	49,50
mb-1 ^{Δc/Δc}	C57BL/6	Cytoplasmic tail of Ig α	part of BCR: signal transduction	subtotal inhibition at the pro-B to pre-B cell step; <u>B1</u> : absent	BCR with truncated Ig α is expressed; <u>N</u> : ^b Ig concentration 4–10 \times ↓; <u>TD</u> : anti-nucleoprotein antibody 100 \times ↓; <u>TI</u> : anti-nucleoprotein-Ficoll antibody absent	small B cell clusters, no follicles, normal T cell zones	51
OBF-1 ^{-/-}	C57BL/6	OBF-1 (= OCA-B = Bob-1)	transcriptional activator of Oct factors	subtotal inhibition at the immature B to mature B cell step	<u>N</u> : IgM concentration normal; IgG/A concentration ↓; <u>TD</u> : anti-NIP-OVA 9 (M)–40 (G) \times ↓; <u>TI</u> : anti-NIP-Ficoll 3 (M)–300 (G) \times ↓	no. of B cells ↓, GC and FDC absent, normal T cell zones	45,46,52
CD19 ^{-/-}	129Sv/ C57BL/6	CD19	co-stimulatory molecule	<u>B2</u> : normal; <u>B1</u> : almost absent	<u>N</u> : Ig concentration normal; <u>TD</u> : anti-nucleoprotein/LCMV ↓; <u>TI</u> : anti-LPS/VSV normal, anti-Bac VSV-G ↓	no. of B cells, GC and FDC ↓, normal T cell zones	31,43,53
CD22 ^{-/-}	C57BL/6	CD22	co-inhibitory molecule	<u>B2</u> : shift from IgM ^{hi} /IgD ^{hi} to IgM ^{lo} /IgD ^{hi} ; <u>B1</u> : 2 \times ↑	<u>N</u> : IgM concentration 2 \times ↑, IgG/A normal; <u>TD</u> : anti-nucleoprotein- γ -globulin 2 \times ↑; <u>TI</u> : anti-DNP-Ficoll 5 \times ↓	normal	34,35
xid	CBA	Bruton's tyrosine kinase	signal transduction	<u>B2</u> : shift from IgM ^{hi} /IgD ^{hi} to IgM ^{hi} /IgD ^{lo} ; <u>B1</u> : absent	<u>N</u> : IgM and IgG3 concentration ↓; <u>TD</u> : anti-nucleoprotein antibody 10 \times ↓; <u>TI</u> : anti-nucleoprotein-Ficoll antibody ↓	no. of GC reduced in naive and immunized mice (primary response)	54–57
C κ ^{-/-}	C57BL/6	κ light chain	part of BCR: antigen recognition	normal, but only λ light chain expression	not tested	no. of B cells ↓	24,58

^a μ MT mice are added for direct comparison. They were not used for experiments in this study.

^bN, naive mouse; M, IgM; G, IgG.

with 5% FCS, then heat inactivated for 30 min at 56°C. Serial 2-fold dilutions were mixed with equal volumes of VSV diluted to contain 500 p.f.u./ml. The mixture was incubated for 90 min at 37°C in an atmosphere with 5% CO₂. Then, 100 μ l of serum-virus mixture was transferred onto Vero cell monolayers in 96-well plates and incubated for 1 h at 37°C. An overlay of 100 μ l DMEM containing 1% methylcellulose was added. After incubation for 24 h at 37°C the overlay was flicked off, and the monolayer was fixed and stained with 0.5% crystal violet. The highest dilution of serum that reduced the amounts of plaques by 50% was taken as the neutralizing titer. Titers are indicated as $-\log_2$ of 40-fold prediluted sera. To determine IgG titers, undiluted serum was first pretreated with an equal volume of 0.1 M 2-mercaptoethanol in saline. This treatment destroys the neutralizing capacity of the monoclonal and polyclonal IgM preparation and leaves IgG titers unchanged (20).

LCMV nucleoprotein-specific ELISA

We used a sandwich ELISA with the following steps: (i) coating with baculovirus-derived LCMV nucleoprotein (1 μ g/ml), (ii) blocking with 2% BSA (Fluka, Buchs, Switzerland) in PBS, (iii) 20-fold prediluted mouse serum, titrated 1:3 over 10 dilution steps, (iv) IgM- or IgG-specific horseradish peroxidase-labeled

goat anti-mouse antibodies (0.5 μ g/ml; Southern Biotechnologies, Birmingham, AL), (v) substrate ABTS (2,2'-azino-di-[3-ethylbenzthiazolin-sulfonate]; Boehringer, Mannheim, Germany) and H₂O₂ (Fluka). Plates were coated overnight at 4°C, all other incubations were for 60–90 min at room temperature. Between incubations plates were washed 3 times with PBS containing 0.5 ml Tween 20/l. OD was measured at 405 nm in an ELISA reader.

Immunohistochemistry

Freshly removed organs were immersed in HBSS and snap frozen in liquid nitrogen. Tissue sections of 5 μ m thickness were cut in a cryostat, placed on siliconized glass slides, air dried, fixed with acetone for 10 min and stored at -70°C . Rehydrated sections were incubated with rat mAb against follicular dendritic cells (FDC) [4C11; (21)] and against marginal zone metallophilic macrophages (MZM; MOMA-1; Biomedicals, Augst, Switzerland). Primary rat antibodies were revealed by sequential incubation with goat antibodies to rat Ig (Caltag, San Francisco, CA) and alkaline phosphatase-labeled donkey antibodies to goat Ig (Jackson Immuno-Research, West Grove, PA). To stain for mouse Ig, sections were incubated with biotinylated monoclonal rat anti-mouse IgM (R6-60.2) or a mixture of rat anti-mouse IgG1 (G1-6.5),

IgG2a/2b (R2-40) and IgG3 (R40-82; all from PharMingen, San Diego, CA), followed by alkaline phosphatase-labeled avidin–biotin complexes (Dako, Glostrup, Denmark). Alkaline phosphatase was visualized using naphthol AS-BI phosphate and new fuchsin as substrate. Endogenous alkaline phosphatase was blocked by levamisole. Sections were counterstained with hemalum, and coverslips mounted with glycerol and gelatin. Germinal centers were additionally stained with peanut agglutinin (PNA) (22).

Results

Immunization with live and inactivated VSV (TI-1 antigens)

VSV induces a potent TI-1 IgM antibody response (12). A peak of the IgM response is observed around day 4–8, whereas switch to IgG occurs after day 6 (23). We therefore immunized the various mutant animals (closed symbols) and their corresponding control animals (usually littermates; open symbols) with 2×10^6 p.f.u. of live VSV and looked for protection and neutralizing antibody titers on day 4, 8 and 12 (Fig. 2, column 1). The antibody response in $C_{\kappa}^{-/-}$, $CD19^{-/-}$ and *xid* mice was comparable to that of the control mice. In naive adult mice the normal κ/λ ratio is >10 (24) and also in VSV-immunized C57BL/6 mice $>95\%$ of the primary anti-VSV antibodies express κ light chains (25). The results here indicate that the κ light chain was readily replaced by the λ chain without noticeable biological effects. In *xid* mice, the normal IgM and IgG responses to TI-1 VSV particles was expected from earlier studies, because mainly TI-2 responses (in the absence of T cells!) and TD responses have been shown to be reduced in these mice (12,26). In $CD22^{-/-}$ mice, the kinetics of IgM and IgG responses was delayed, but by day 20 titers equal to those of control animals were reached and the mice survived VSV infection. In contrast, the antibody response in $mb-1^{\Delta c/\Delta c}$ and $OBF-1^{-/-}$ mice was severely impaired. In the case of $mb-1^{\Delta c/\Delta c}$ mice, no neutralizing IgG was produced and all the animals died from primary infection (Fig. 3C). $OBF-1^{-/-}$ mice produced markedly reduced neutralizing IgM and IgG titers, and only 50% of the animals survived (Fig. 3C).

When mice of the different strains were immunized with 10^7 p.f.u. of UV-inactivated VSV (Fig. 2, column 2), that cannot produce infectious progeny, but still behave as a TI-1 antigen (12), again $C_{\kappa}^{-/-}$, $CD19^{-/-}$, $CD22^{-/-}$ and *xid* mice produced normal antibody titers by day 12. All the $mb-1^{\Delta c/\Delta c}$ and $OBF-1^{-/-}$ mice now survived, but upon primary immunization with UV-inactivated VSV $mb-1^{\Delta c/\Delta c}$ mice did not produce any neutralizing antibodies, whereas $OBF-1^{-/-}$ mice only generated IgM responses without switch to IgG. This result may be explained by the limited antigen dose and reduced stimulatory cytokine production after immunization with inactivated VSV compared to a live virus infection (27–30) or by a major disruption of lymphoid architecture (see below) with limited capacity of filtering antigen in the marginal zone.

Immunization with VSV-G recombinant vaccinia virus and baculovirus-derived VSV-G (TI-2 antigens)

In a next series of experiments the various mutant strains were immunized with a replicating and a non-replicating form

of a TI-2 antigen: vacc-VSV-G and baculovirus-derived VSV-G (Bac VSV-G) respectively. The antibody responses to 2×10^6 p.f.u. of vacc-VSV-G (Fig. 2, column 3) were quite comparable to the results obtained by VSV infection. $C_{\kappa}^{-/-}$, $CD19^{-/-}$, $CD22^{-/-}$ and *xid* mice produced antibody titers within control levels by day 12. In the case of $CD19^{-/-}$ mice the kinetics of the IgM response was delayed, but quantitatively within normal ranges, as already described earlier (31). Again, antibody responses in $mb-1^{\Delta c/\Delta c}$ and $OBF-1^{-/-}$ mice were severely reduced. Against vacc-VSV-G, $mb-1^{\Delta c/\Delta c}$ mice were able to generate low titers of neutralizing IgG by day 12. All the mice survived vacc-VSV-G infection; this was, however, not surprising since protection is not mediated by antibodies, but mainly by cytokines (32,33).

After immunization with an optimal dose of 10 μ g Bac VSV-G (Fig. 2, column 4), a non replicating TI-2 antigen, we had already found earlier that IgM and IgG response in $CD19^{-/-}$ mice were reduced (31). $mb-1^{\Delta c/\Delta c}$ mice generated no neutralizing response, $OBF-1^{-/-}$ mice only made a low IgM titer by day 4 that faded quickly. In contrast, the response in $CD22^{-/-}$ mice was slightly enhanced for IgM and IgG. This result fits the notion that CD22 is a B cell inhibitory molecule. A similar effect has been shown for the TD antigen (4-hydroxy-3-nitrophenyl-acetyl) $_{17}$ -chicken γ -globulin before (34), whereas another study using a different strain of $CD22^{-/-}$ mice reported reduced TI-2 responses (35). Finally, in $C_{\kappa}^{-/-}$ and *xid* mice the response was again comparable to control animals. This and the same result for vacc-VSV-G were expected, because the defect of TI-2 IgM responses in *xid* mice can only be demonstrated, if $CD4^+$ T cells are depleted (12) or if B cells are cultured without T cells (26,36), since the TI-2 effect is thought to rely on a non-cognate T–B interaction.

Immunization with LCMV (TD antigen)

Finally, the various mouse strains were immunized with 200 p.f.u. of live LCMV (Fig. 2, column 5). An infection with 200 p.f.u. is known to induce neutralizing antibodies only very late and to variable titers that are difficult to assess. Therefore the very efficient TD antibody response to the internal nucleoprotein of this virus (37) was measured by ELISA. Since protection against primary infection is mediated by cytotoxic T cells (15) and not by antibodies, survival could not be used as a read-out in this model situation. In the mouse strains with severely impaired responses to TI antigens ($mb-1^{\Delta c/\Delta c}$ and $OBF-1^{-/-}$), the TD response to LCMV nucleoprotein was almost absent. In all the other strains, a slight to moderate reduction of IgM and IgG antibodies could be observed with a delay in kinetics of the response. This may be explained by the fact TD responses need optimal interaction of between antigen presenting cells, T and B cells for induction of an immune response, and that already minor structural defects of lymphoid architecture impair this interaction (see below).

Secondary response in $mb-1^{\Delta c/\Delta c}$ and $OBF-1^{-/-}$ mice

To further evaluate whether the limited responses of $mb-1^{\Delta c/\Delta c}$ and $OBF-1^{-/-}$ mice to primary VSV infection (Fig. 3C, column 1) is due to a problem of the kinetics of the antibody response or due to a general disability to produce VSV neutralizing antibodies, we evaluated secondary antibody responses and protection against a challenge infection. $OBF-1^{-/-}$ mice primed

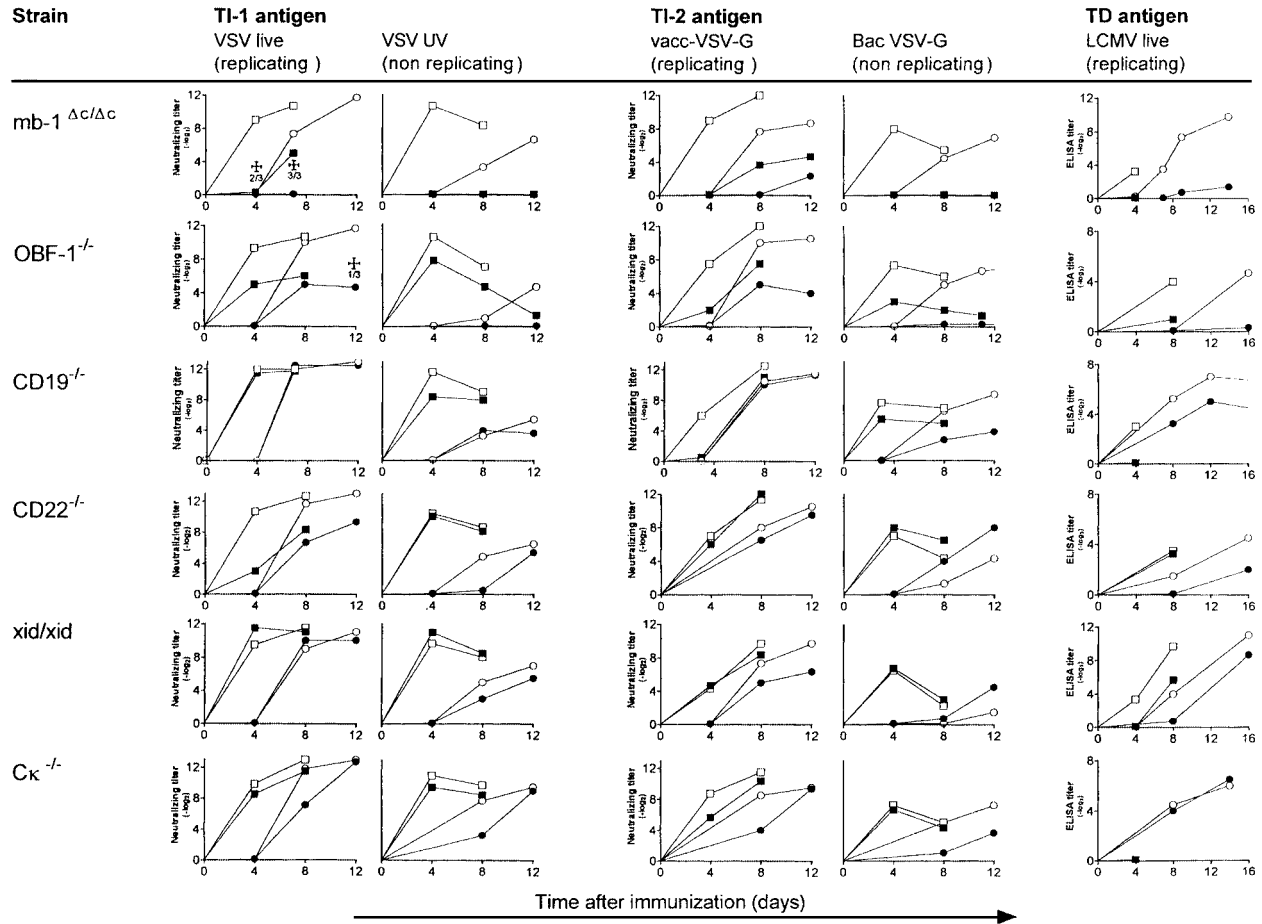


Fig. 2. Antibody responses of six mouse strains to viral antigens of TI-1, TI-2 and TD type. The six mutant mouse strains and control animals were immunized with 2×10^6 p.f.u. of live VSV (column 1), 10^7 p.f.u. of UV-inactivated VSV (column 2), 2×10^6 p.f.u. of vacc-VSV-G (column 3), $10 \mu\text{g}$ Bac VSV-G (column 4) or 200 p.f.u. of LCMV (column 5). Serum was taken on the indicated days after immunization, and VSV-neutralizing antibody titers of 40-fold prediluted serum (for column 1–4) or LCMV nucleoprotein-binding antibody titers of 20-fold prediluted serum (for column 5) were determined by a plaque reduction or an ELISA assay respectively. Squares, IgM; circles, IgG; closed symbols, mutant ($-/-$) animals; open symbols, control ($+/+$) animals. Data points indicate the mean of three animals per group. SD within ± 1 dilution step. One of two comparable experiments is shown.

with live VSV, of which 50% had survived, and a group of mice immunized with UV-inactivated VSV were challenged with 2×10^6 p.f.u. of live VSV i.v. All the animals generated secondary neutralizing IgG by day 6 (Fig. 3B) and survived the challenge (Fig. 3C). In $mb-1^{\Delta c/\Delta c}$ mice, which could not survive primary VSV infection, vaccination was performed either with vacc-VSV-G or Bac VSV-G. Thirty days later the animals were challenged with 2×10^6 p.f.u. of live VSV i.v. The animals primed with vacc-VSV-G survived, whereas those immunized with Bac VSV-G died (Fig. 3C). The survival correlated with the level of secondary IgG titers reached by day 6 after challenge, that were very low in the case of Bac VSV-G priming (Fig. 3A). These results show that mainly quantitative limitations (early kinetics as well as absolute titers reached) probably limit the protective capacity of the antibody response against this highly cytopathic virus in the severely B cell-compromised mouse strains $mb-1^{\Delta c/\Delta c}$ and $OBF-1^{-/-}$.

Splenic morphology after immunization with UV-inactivated VSV

In several studies it was shown that there is a correlation between structural impairment of secondary lymphoid organs and reduced immune effector functions (38–41). We therefore evaluated the morphology of the spleen 1 month after immunization with UV-inactivated VSV in the various mutant mouse strains (Fig. 4 and Table 2). Since not all the mouse strains survived an infection with live VSV for 1 month, we used UV-inactivated VSV for this study in order to obtain comparable sections from all groups. VSV has been shown earlier to induce long-lived germinal centers for >100 days (22). In parallel with the massively impaired B cell function, $mb-1^{\Delta c/\Delta c}$ mice revealed a totally disrupted splenic morphology without follicles, germinal centers (GC) and assembly of FDC. The number of IgM^+ B cells was severely reduced (Fig. 4), staining for IgD was almost absent (data not shown). In contrast, $OBF-1^{-/-}$

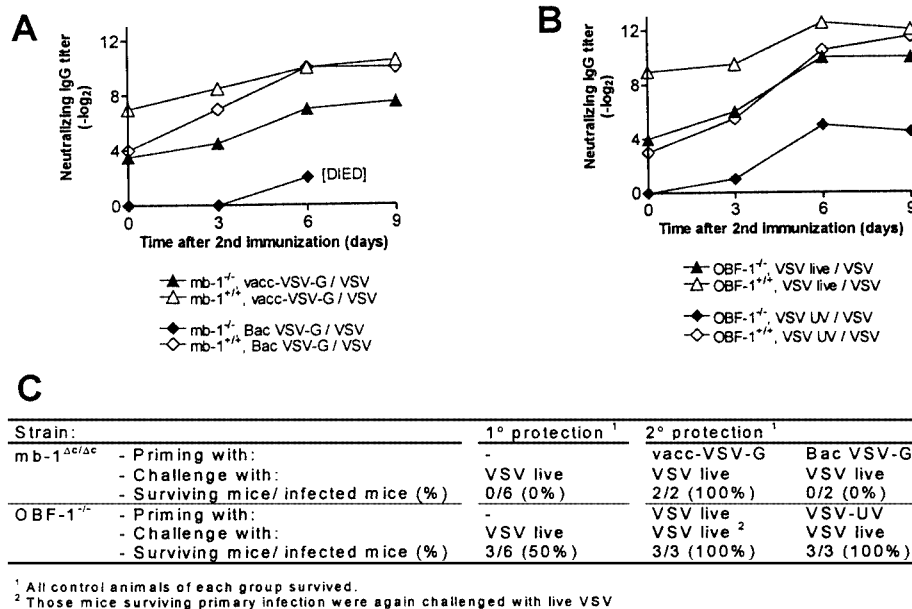


Fig. 3. Secondary antibody responses and protection against live VSV in mb-1^{Δc/Δc} and OBF-1^{-/-} mice. (A) Mb-1^{Δc/Δc} mice were primed either with 2×10⁶ p.f.u. vacc-VSV-G (triangles) or 10 μg Bac VSV-G (diamonds), and then challenged 30 days later with 2×10⁶ p.f.u. of live VSV. Neutralizing IgG titers of 40-fold prediluted serum are shown. (B) OBF-1^{-/-} mice were primed either with 2×10⁶ p.f.u. live VSV (triangles) or UV-inactivated VSV (diamonds), and then challenged 30 days later with 2×10⁶ p.f.u. of live VSV. Neutralizing IgG titers of 40-fold prediluted serum are shown. Closed symbols, mutant (-/-) animals; open symbols, control (+/+) animals. Data points in (A) and (B) indicate the mean of the number of animals indicated in (C). (C) Numbers of surviving mice after primary and secondary infection with live VSV. The respective control animals of each mouse strain all survived.

1^{-/-} mice showed rudimentary follicles with a marginal zone (MOMA-1 staining for MZM; Fig. 4) and a marginal sinus (staining for MadCAM, data not shown). IgM⁺ (Fig. 4) and IgD⁺ (not shown) B cells were only slightly reduced, but no GC and FDC were found. For CD19^{-/-} mice, we showed in an earlier study (31) that they are able to generate GC after VSV infection, although in reduced numbers. In CD22^{-/-} mice slightly reduced numbers of FDC and IgG⁺ B cells were found in GC; otherwise the splenic morphology and numbers of IgM⁺ and IgD⁺ B cells were within normal ranges. The spleen morphology of xid and C_κ^{-/-} mice was comparable to their control littermates. In all the strains immunohistology for GC (staining with PNA) and FDC (staining with 4C11) was almost perfectly correlated, therefore only GC are shown in Fig. 4.

Discussion

This study presents a systematic analysis of a series of mutant animals lacking B cell-specific molecules by testing them with a standardized scheme of immunizations with viral antigens. Protection of the animals after primary and secondary immunizations and the significance of the mutation in an infectious disease could therefore be evaluated. The results illustrate the following points.

First, there is a close relationship between splenic morphology and B cell function (see Table 2). Those strains with the most severe B cell deficiencies (mb-1^{Δc/Δc} and OBF-1^{-/-}) showed the most disrupted lymphoid architecture. The presence of B cell follicles with MZM and marginal sinuses

correlated well with generation of a substantial IgM response, whereas presence of GC and FDC correlated with an efficient IgG response. This immunohistochemical analysis was done after immunization with a TI-1 antigen, where T-B cooperation plays no limiting role for IgM responses. The fact that the T cell zones were within normal ranges in the spleen of all the strains (data not shown) also renders the influence of T cells on the observed changes as an explanation for our findings unlikely. An intact lymphoid tissue seems therefore also important for direct B cell priming.

Second, there are differences between a live virus infection and immunizations with proteinaceous antigens (42). For CD19^{-/-} mice we have already shown that these mice are able to generate GC after a virus infection (31), but not after immunizations with protein antigens (43). The present experiments show that mb-1^{Δc/Δc} mice are able to produce VSV neutralizing IgM antibodies, but only slowly and inefficiently. Therefore the mice were not protected against primary infection with a highly cytopathic virus. This reflects mainly quantitative limitations, because an early and sufficiently high neutralizing response is necessary to prevent lethal infection by VSV reaching the CNS (44). When these mice were primed with another virus expressing the same VSV-G antigen, but not leading to lethal CNS infection (vacc-VSV-G), neutralizing IgG titers were produced and the mice were protected against secondary challenge with VSV. For OBF-1^{-/-} mice severe defects in B cell responses have been described earlier (45,46). Nevertheless half of these mice survived primary VSV infection and all vaccinated mice survived secondary challenge. Therefore, these OBF^{-/-} mice seem just about

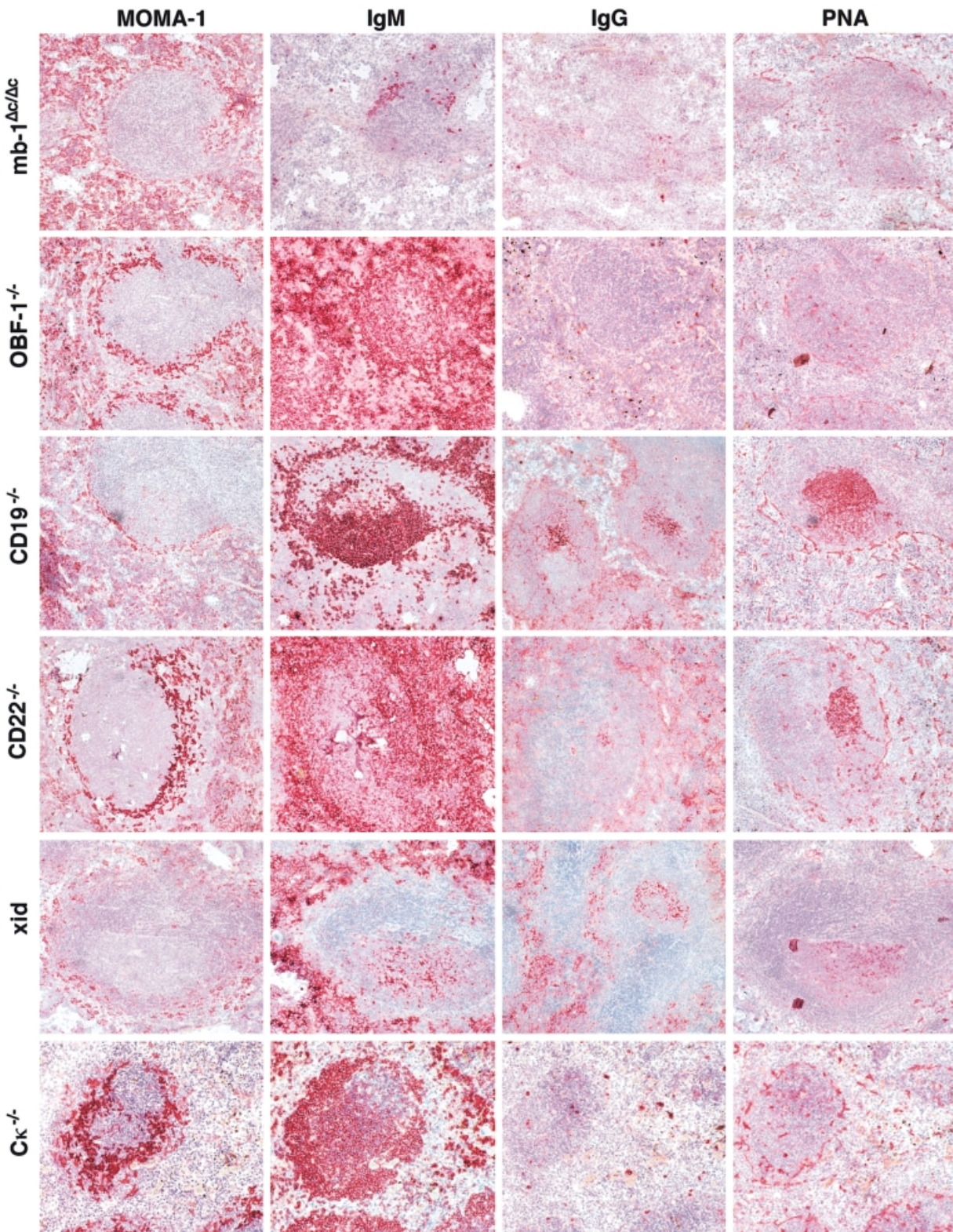


Fig. 4. Immunohistochemistry of spleen sections after immunization with UV-inactivated VSV. Groups of two to three individual mice of each strain and control animals were immunized with 10^7 p.f.u. of UV-inactivated VSV. After 1 month spleens were taken out, snap frozen, sectioned and stained. Stainings for MZM (MOMA-1), IgM⁺ and IgG⁺ B cells and GC (PNA) are shown for each strain.

Table 2. Summary table of antiviral immune responses of the various mutant mouse strains

Mouse strain	Anti-VSV neutralizing antibody (TI-1)		Anti-BacG antibody (TI-2)		Anti-LCMV antibody (TD)		Splenic architecture 30 days after VSV-UV immunization				Reference		
	Secondary protection	Primary protection	IgM	IgG	IgM	IgG	IgM	IgG	MZM	IgM		FDC	IgG
μ MT ^a	no	0%	0	0	0	0	0	0	NT	NT	NT	NT	50
mb-1 ^{Δc/Δc}	partial	0%	↓↓	0	0	0	0	0	↓	↓	0	0	this study, 51
OBf-1 ^{-/-}	yes	50%	↓↓	↓↓	↓↓	0	0	0	+	↓	0	0	this study, 45, 46
CD19 ^{-/-}	yes	100%	+	+	↓	↓	↓	↓	(↓)	↓	↓	↓	this study, 31, 43
CD22 ^{-/-}	NT	100%	↓ ^b	↓ ^b	+	+	↓	↓	+	+	+	+	this study, 35
xid	NT	100%	+	+	+	+	+	+	+	+	+	+	this study, 56
C _κ ^{-/-}	NT	100%	+	+	+	↓ ^b	+	+	+	+	+	+	this study

+ / ++ = normal/slightly enhanced; ↓ / ↓↓ = slightly/severely reduced; 0 = absent; bold text; severely impaired parameters; bold line: demarks normal from pathologic (= reduced).
^aμMT mice without BCR are added for direct comparison. They were not used for experiments in this study.
^bAntibody responses were delayed by about 4 days; equal neutralizing titers were reached by day 20.
 NT: not tested.

capable to mount a sort of 50% efficient B cell response, that provided a 50% protective primary immunity level.

Third, our experiments demonstrate a full redundancy for the use of κ or λ light chains. The normal κ/λ ratio in mice is >10 (24). A biased use of a certain light chain was described after immunization with several antigens [e.g. κ after VSV infection (25), λ after immunization with hydroxy-nitrophenyl-haptenated antigens (47)]. The results here demonstrate that the inability to generate κ chains had no influence on antibody titers against VSV or LCMV and on protection of the animals.

Fourth, the bottleneck for the signaling in B cells seems to be at the beginning (BCR complex) and at the end (B cell-specific transcription) of the cascade. Mutations in molecules belonging to these groups exerted the severest impairment of B cell function. A great number of molecules seem to be involved in fine tuning the signaling, some are co-stimulatory (as CD19 or B7), others are inhibitory (as CD22 or FcR γ II) (3). A complicated network of kinases and phosphatases transmit the signal (6). Three strains each lacking one of these modulating molecules were tested (CD19^{-/-}, CD22^{-/-} and xid) and neither of them exhibited major defects in B cell function that were limiting for survival in an antibody-dependent infection model as tested here. For CD22, positive and negative signaling properties have been described depending on the type of antigen (48). Results of this study indicating marginal effects of CD22 deficiency cannot shed more light onto this question.

This is the first study that attempts the analysis of antibody responses in several mutant mouse strains after immunization with infectious agents. Functional as well as morphological analysis allows direct comparison of the biological significance of a certain mutation. Limitations in the interpretation of our results may lay in the use of animals with different genetic backgrounds and in the general use of optimal doses of antigens known from earlier studies in order to save animals. Therefore smaller differences in antibody titers and kinetics may be overlooked, since no dose-response experiments were performed. This disadvantage was partly compensated by always using a control group of littermates with identical genetic background for each strain and antigen.

Taken together, there exist many functionally overlapping factors in B cells involved in fine regulation of antibody responses. The key B cell molecules that strikingly limit antibody responses are located at the initiation level of the BCR complex and at the level of B cell transcription (i.e. at the beginning and at the end of the signaling cascade). Their absence leads to severe structural defects in lymphoid architecture and therefore major impairment of antibody responses.

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Abbreviations

Bac	baculovirus derived
BCR	B cell receptor
Btk	Bruton's tyrosine kinase
FDC	follicular dendritic cell
GC	germinal center
LCMV	lymphocytic choriomeningitis virus
MZM	marginal zone macrophage
OBF	Oct-binding factor
TD	T cell dependent
TI	T cell independent
vacc	vaccinia virus
VSV	vesicular stomatitis virus
VSV-G	VSV glycoprotein
xid	x-linked immunodeficiency

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