CELLULAR IMMUNOLOGY **167,** 115–121 (1996)
Article No. 0014

Antiviral Immune Responses of Mice Lacking MHC Class II or Its Associated Invariant Chain

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Received June 12, 1995; accepted August 8, 1995

Induction of T-helper cells and T–B cell interaction have been considered to critically depend upon recognition of major histocompatibility complex (MHC) class II molecules by the T cell receptor. Mice lacking either MHC class II molecules (class $II^{0/0}$ mice) or its associated invariant chain ($Ii^{0/0}$ mice) provide new opportunities to test this premise. Immune responses to some protein antigens have been studied in these mice; little is known about their ability to withstand viral infections. We therefore tested $CD8^+$ effector T cells and $CD4^+$ T-cell-dependent B cell function during different viral infections. The vesicular stomatitis virus (VSV)-specific primary cytotoxic T cell response which is largely T-helper-dependent was diminished in $Ii^{0/0}$ and absent in class $II^{0/0}$ mice. The usually less T-helper-dependent cytotoxic vaccinia or lymphocytic choriomeningitis virus (LCMV)-specific $CD8^+$ T cell responses were reduced up to ninefold in class $II^{0/0}$ and up to threefold in $Ii^{0/0}$ mice. In class $II^{0/0}$ mice, the T-helper-independent neutralizing IgM response against the glycoprotein of VSV was within normal ranges but, in contrast to previous results on $CD4^{0/0}$ mice, the T-helper-dependent IgG response was absent. $Ii^{0/0}$ mice exhibited a normal neutralizing IgM response; in contrast to class $II^{0/0}$ mice, they mounted a significant, though reduced specific IgG response. Similar results were obtained for antibody responses against the nucleoprotein of VSV. Although the T-helper-cell response upon infection with VSV seemed diminished only a little in $Ii^{0/0}$ mice, presentation of VSV-G to a class II-restricted specific hybridoma was greater than 300-fold reduced in the absence of Ii. This suggests that local protein concentrations reached during viral infection in the host are high enough to override the Ii deficiency of antigen-presenting cells *in vivo.*

INTRODUCTION

Major histocompatibility complex (MHC) class II molecules play a crucial role in regulating the immune response. On professional antigen-presenting cells (APCs), such as dendritic cells and macrophages, they associate with antigenic peptides and present them to T lymphocytes expressing the CD4 differentiation marker. Class II molecules are also found on B cells and are crucial for contact-dependent collaboration with $CD4^+$ T-helper cells.

Newly generated class II molecules are intimately associated with the invariant chain (Ii), a type II transmembrane protein (for review see 1–4). The essential functions of the invariant chain involve the folding and/or assembly of class II heterodimers, their egress from the endoplasmic reticulum (ER) and their targeting to the endosomal compartments. It has also been suggested that Ii acts to segregate antigen presentation by MHC class I and II by blocking peptide binding to the latter in the ER; this notion is, however, controversial. Indeed the effect of Ii on class II-restricted presentation of intracellular antigens appears complex: Ii has a negative or no influence on presentation of some antigens or epitopes, but is required in other cases (5–8).

Several immune models of T-helper-cell deficiencies have been established and were used to study the role of class II antigens and of $CD4^+$ T cells in antiviral antibody or cytotoxic T cell (CTL) immunity. $CD4^+$ T cells were first eliminated by specific antibodies *in vitro* and assayed by adoptive transfer (9); when more potent monoclonal antibodies became available, $CD4^+$ T cells were depleted *in vivo.* These studies and experiments with T-cell-deficient nude mice (10) established that some antiviral neutralizing IgM antibodies were T-independent, whereas against other viruses they were T-dependent (10–12). The IgG responses against all viruses tested were strictly $CD4^+$ T-cell-dependent. Similarly, some antiviral $CD8^+$ T cell responses were generated against lymphocytic choriomeningitis virus (LCMV) or vaccinia WR virus without T help (11, 13), whereas CTL responses to vesicular stomatitis virus (VSV), influenza viruses, or avirulent vaccinia virus depended more strictly upon T help (12, 14, 15). Mice

0008-8749/96 $12.00

lacking $CD4^+$ T cells during their entire development because of inactivation of the CD4 gene by homologous recombination confirmed the findings from mice whose $CD4^+$ T cells had been depleted rather acutely by repeated antibody treatment in thymectomized mice. One major surprise and difference was, however, that $CD4^-$ $CD8^-$ T cells could provide T help in $CD4^{0/0}$ mice to generate neutralizing anti-VSV IgG (16) or anti-*Leishmania* immune effector T cells with helper phenotype (17). However, these functional double-negative cells have only been demonstrated in CD4-deficient mice and thus do not negate the notion that in general only $CD4^+$ T cells mediate help in normal mice.

Mice lacking the class II antigens (class $II^{0/0}$ mice) or the invariant chain (Ii) gene ($Ii^{0/0}$ mice) generated by homologous recombination offered new possibilities to assay the role of class II and of $CD4^+$ T cells in antiviral immunity (18–21). This seems important because it represents a less interventive approach than antibody treatment or adoptive transfer; also questions can be raised as to whether the antibody treatments remove all $CD4^+$ T cells, particularly activated ones and those in sites remote from the circulation (22). Moreover, nude mice (23) and, as mentioned above, $CD4^{0/0}$ mice may not be completely devoid of helper activity.

Class $II^{0/0}$ mice showed a massive reduction in the $CD4^+$ T cell population while the $CD8^+$ subset was proportionally increased (19, 21). In these mice, B lymphocytes were present in normal numbers. Nevertheless, several abnormalities in the B cell effector function were demonstrated, e.g., the lack of germinal centers, reduced production of IgG_1, and an inability to respond to T-dependent antigens (19). $Ii^{0/0}$ mice display aberrant intracellular transport of MHC class II molecules and class II antigens are expressed at reduced levels on the cell surface (18, 20). Interestingly, class II molecules on cells of $Ii^{0/0}$ mice formed less compact dimers than Ii-positive controls and the antigen presentation was severely affected; e.g., antigen-presenting cells from $Ii^{0/0}$ mice showed a strongly reduced ability to present several exogenously supplied proteins such as hen egg lysozyme or chicken ovalbumin to hybridomas, while they could efficiently present the corresponding optimal peptides (18, 20). In particular, efficient presentation of proteins in association with class II was only possible at high antigen concentrations.

The present studies aimed at evaluating the effects of complete class II deficiency and of abnormal class II transport, stabilization, and expression due to lack of Ii on diverse antiviral responses. The results provide evidence for the essential role of class II antigens in antiviral IgG responses, confirm the variable importance of T help in CTL responses against various viruses, and suggest that local *in vivo* concentrations during viral infections in the host may be surprisingly high and sufficient to overcome the Ii defect.

MATERIALS AND METHODS

Mice

Inbred C57BL/6 ($H\text{-}2^b$) were purchased from the Institut für Zuchthygiene, Tierspital, University of Zürich, Switzerland. The generation of class $II^{0/0}$ ($H\text{-}2^b$) and $Ii^{0/0}$ mice ($H\text{-}2^b$) by homologous recombination has been described in detail (18, 19).

Viruses

Lymphocytic choriomeningitis virus isolate WE (LCMV-WE) was obtained as a second passage from a triply purified LCMV-WE 350 isolate from Dr. F. Lehmann-Grube, Hamburg (24) and used for the foot pad swelling reaction (3×10^2 PFU into the foot pad), for virus elimination, and for cytotoxicity assays. LCMV-WE was quantified using an immunological focus formation assay (25).

VSV Indiana (VSV-IND) (Mudd–Summers isolate) seeds had been originally obtained from Dr. D. Kolakofsky, University of Geneva, and were grown on BHK 21 cells infected with low multiplicity of infection and plaqued on Vero cells.

Vaccinia virus (Lancy isolate; Serum und Impfinstitut, Bern, Switzerland) was used as described in detail elsewhere (11).

Vaccinia virus expressing the glycoprotein (G; vacc-G) or nucleoprotein (N; vacc-N) of VSV was a generous gift of Dr. B. Moss (Laboratory of Viral Diseases, National Institutes of Health, Bethesda, MD (26)). Recombinant viruses were grown at low multiplicity of infection on BSC cells and plaqued on BSC cells.

Recombinant baculovirus expressing the G or N of VSV has been described (27, 28). All recombinant baculoviruses were derived from nuclear polyhedrosis virus and were grown at 28°C in *Spodoptera frugiperda* cells in spinner cultures in TC-100 medium.

Preparation of Recombinant Viral Proteins

To produce viral proteins, *S. frugiperda* cells at a density of 2×10^6 cells/ml were infected with recombinant baculoviruses expressing G or N of VSV with a multiplicity of infection of 10 for 24 hr at 28°C. Infected cells were harvested, disrupted by sonication, and stored at −20°C.

Serum Neutralization Test

Serum was collected from mice at specific time points after VSV infection. The sera were prediluted 40-fold in minimal essential medium containing 5% FCS and then heat-inactivated for 30 min at 56°C. Serial twofold dilutions were mixed with equal volumes of VSV-containing medium (500 PFU/ml). The mixture was incubated for 90 min at 37°C in an atmosphere with 5% CO_2. One hundred microliters of the serum–virus mixture was transferred onto Vero cell monolayers in 96-

well plates and incubated for 1 hr at 37°C. The monolayers were then overlaid with 100 μl of DMEM containing 1% methyl cellulose. After incubation for 24 hr 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 number of plaques by 50% was taken as titer. Due to the addition of an equal volume of virus, the titer of serum was considered to be one step higher (29). To determine IgG titers, undiluted serum was pretreated with an equal volume of 0.1 m*M* 2-mercaptoethanol in saline (30).

ELISA

The VSV G and VSV N-specific ELISA was performed as follows: 96-well plates (Petra Plastic, Switzerland) were incubated with recombinant purified VSV in 0.1 *M* NaH_2PO_4, pH 9.6, at 4°C. Plates were then blocked with 2% BSA in PBS for 2 hr and washed, and serial dilutions of serum samples were added to the wells and then incubated for 1 hr. Plates were washed and incubated with goat anti-mouse IgG (Sigma). After 1 hr, plates were washed and developed with ABTS (5 mg 2.2′-azino-di-3-ethylbenzthiazolinesulfonate and 20 μl H_2O_2 in 50 ml $NaHCO_3$, pH 4.0). Optical density was determined at 405 nm.

^{51}Cr Release Assay

Vaccinia virus. Vaccinia virus (Lancy isolate, Schweizerisches Serum und Impfinstitut) was injected (2×10^6 PFU per mouse) intravenously. Spleen cells were tested 6 days after infection in a standard cytotoxicity assay. The test was performed in round-bottomed 96-well plates for 5 hr at 37°C in a 5% CO_2 incubator. Target cells (1×10^4) were mixed with prediluted effector cells at effector to target ratios of 70:1, 23:1, 8:1, and 3:1 ratios and the plates were spun for 4 min before incubation. Supernatant aliquots (70 μl) were analysed in a gamma counter (Packard, Zürich, Switzerland).

LCMV. Eight days after iv infection of mice with 200 PFU LCMV-WE, effector spleen cells were tested on LCMV-infected MC57G target cells as summarized above (5 hr).

VSV. Six days after iv infection of mice with VSV-IND (2×10^6 PFU), spleen cells were tested on EL-4 cells transfected with the nucleoprotein of VSV or mock-transfected (31, 32).

IL-2 Release

Hybridomas were stimulated with VSV-G for 18 hr in IMDM 10% FCS in a total volume of 200 μl. Fifty microliters of supernatant was transferred to a new 96-well plate and 1×10^4 CTLL cells were added per well in a volume of 150 μl of IMDM 10% FCS. Quantification of viable CTLL cells was performed by adding 1/10 vol per well of Alamar Blue solution (Biosource, International) for 4–6 hr. Color reaction was measured by fluorescence emission at 590 nm using the CytoFluor 2350 (Millipor) fluorimeter.

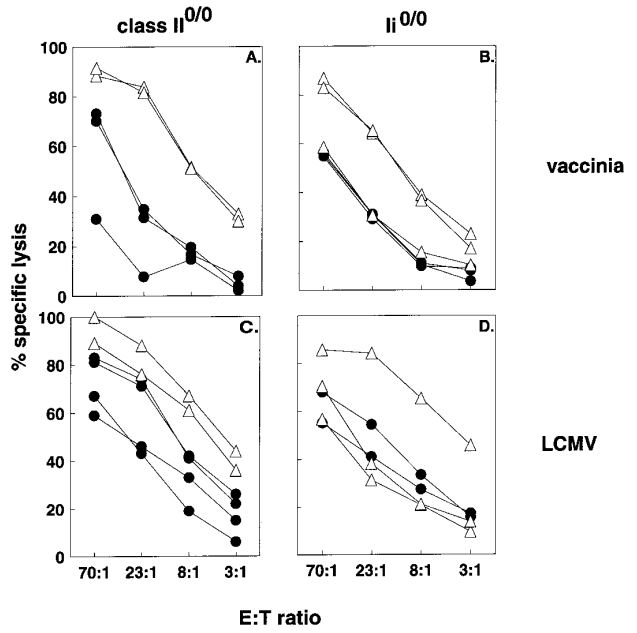

FIG. 1. Upper graphs: Antivaccinia ^{51}Cr release in MHC class II$^{0/0}$ (A) and invariant chain$^{0/0}$ mice (B). Knock-out mice (closed circles) and heterozygous control mice (open triangles) were challenged iv with vaccinia–Lancy 2×10^6 PFU. Cytotoxicity of spleen cells was tested against vaccinia-infected MC57G target cells in a 5-hr ^{51}Cr release assay 6 days after infection. Each single line corresponds to one mouse. Uninfected target cells were not lysed significantly (not shown). Lower graphs: Anti-LCMV ^{51}Cr release in class II$^{0/0}$ (C) and invariant chain$^{0/0}$ mice (D) after infection iv with 2×10^2 PFU LCMV-WE. Effector spleen cells were tested against LCMV-infected target cells in a 5 hr ^{51}Cr release assay 8 days after infection. Uninfected target cells were not lysed significantly.

RESULTS

Antiviral Cytotoxic T Cell Responses in Class II$^{0/0}$ and Ii$^{0/0}$ Mice

Primary CTL function was assessed against vaccinia virus, LCMV and VSV. The antivaccinia virus-specific CTL response measured 6 days after intravenous infection with vaccinia virus was reduced by a factor of about 9 in class II$^{0/0}$ and a factor of up to threefold in Ii$^{0/0}$ mice. LCMV-specific cytotoxicity was assessed 8 days after infection. CTLs generated by Ii$^{0/0}$ mice were within ranges similar to those in controls, whereas class II$^{0/0}$ mice demonstrated a CTL response which was three- to ninefold diminished compared with controls (Fig. 1). This relative decrease in specific CTL response was of no obvious biological significance, as revealed by both an efficient CD8$^+$ T-cell -dependent footpad swelling reaction by Days 7 to 8 and by virus elimination; no virus was detected on Day 8, 10, or 20

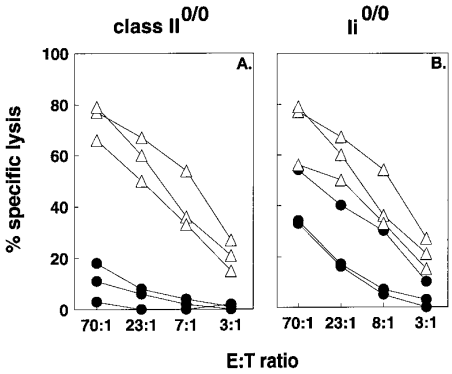

FIG. 2. Anti-VSV cytotoxic T cell response in class $II^{0/0}$ (A) and invariant $chain^{0/0}$ mice (B). Knock-out (closed circles) and control (open triangles) mice were infected iv with 2×10^6 PFU VSV-IND. Cytotoxicity of spleen cells was tested against EL-4 cells transfected to express the N of VSV in a 5-hr ^{51}Cr release assay 6 days after infection. Mock-transfected target cells were not lysed significantly. Each single line corresponds to one mouse. Spontaneous lysis was below 15%.

after primary infection with 2×10^2 PFU of LCMV-WE (not shown).

Generation of anti-VSV cytotoxic T cell responses was most dependent upon T help when compared with responses against LCMV or vaccinia virus. Primary CTL responses to VSV were reduced >20-fold and virtually absent in class $II^{0/0}$ mice, and reduced about 3- to 9-fold in $Ii^{0/0}$ mice (Fig. 2).

VSV-G-Specific Neutralizing Antibody Responses in Class $II^{0/0}$ Mice

VSV induces a strong neutralizing antibody response. All neutralizing antibodies are directed against the G of VSV and most bind to one immunodominant epitope (33–35). The early neutralizing IgM response peaking around Day 4 is T-helper-cell-independent, whereas the switch from IgM to IgG strictly requires the presence of T help (10, 11). The ability of class $II^{0/0}$ mice to mount neutralizing immunoglobulin (Ig) responses was assessed after intravenous infection with 2×10^6 PFU of VSV. The T-helper-cell-independent IgM response was only slightly reduced in class $II^{0/0}$ mice. Induction of B cells is therefore within normal ranges in these mice. In contrast, no T-helper-cell -dependent IgG response was detectable in class $II^{0/0}$ mice (Fig. 3). Thus, class $II^{0/0}$ mice offer an excellent control for the T help independence of IgM responses and T dependence of neutralizing IgG.

VSV-G-Specific Antibody Response in $Ii^{0/0}$ Mice

Antibody responses of $Ii^{0/0}$ mice were evaluated upon infection with 2×10^6 PFU of VSV. As expected, neutralizing IgM responses generated by the mutant mice were normal. However, surprisingly and in contrast to class $II^{0/0}$ mice, the neutralizing IgG response of $Ii^{0/0}$ mice was efficient and kinetics were similar to control animals; IgG levels were reduced by only 2–4 titer steps (Fig. 4A). Since VSV viral particles induce an extremely efficient B cell response due to the highly organized G in the envelope (36), this model antigen needs little T help for the switch to IgG and may thereby lead to an overestimation of T help (37). Therefore, a less immunogenic, but nevertheless infectious form of VSV-G was used for immunization, i.e., recombinant vaccinia virus expressing the G of VSV (vacc-G); this antigen also induced high IgG responses in $Ii^{0/0}$ mice (Fig. 4B).

Upon subcutaneous immunization at the base of tail with recombinant baculovirus-derived VSV-G protein in incomplete Freund's adjuvants, $Ii^{0/0}$ mice mounted a slower and more strongly reduced IgG response (Fig. 4C).

These results were confirmed when a VSV-specific ELISA was used as a readout rather than virus neutralization. Three weeks after immunization with vacc-G or recombinant VSV-G in adjuvants, $Ii^{0/0}$ mice had mounted significant, though reduced, levels of VSV-specific IgG antibody titers (Fig. 5).

VSV-N-Specific Binding Antibody Response in $Ii^{0/0}$ Mice

Since VSV-G is a membrane protein, it most probably can reach the class II pathway via an endogenous route as has been shown for other proteins (see e.g., 38). We therefore analyzed the antibody response against an internal component of VSV, i.e., the N of VSV. The N of VSV is a cytoplasmic protein and does not pass through the ER and therefore most probably does not reach the class II pathway efficiently via an endogenous pathway.

$Ii^{0/0}$ and control mice were immunized with a vaccinia recombinant virus expressing the N of VSV (vacc-N) (2×10^6 PFU) or with recombinant VSV-N (10 μg) in incomplete Freund's adjuvants and specific IgG titers were determined (Fig. 6). VSV-N induced strong spe-

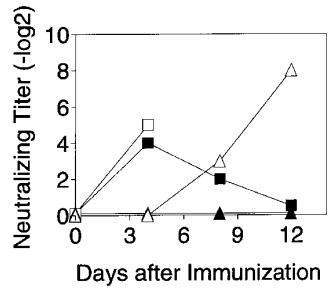

FIG. 3. Neutralizing antibody responses to VSV in MHC class $II^{0/0}$ mice. MHC class $II^{0/0}$ (closed symbols) and control mice (open symbols) were infected intravenously with 2×106 PFU of VSV IND and neutralizing IgM (squares) and IgG (triangles) titers were determined in 40-fold prediluted sera. Three mice were used per group. Variations were less than two dilution steps.

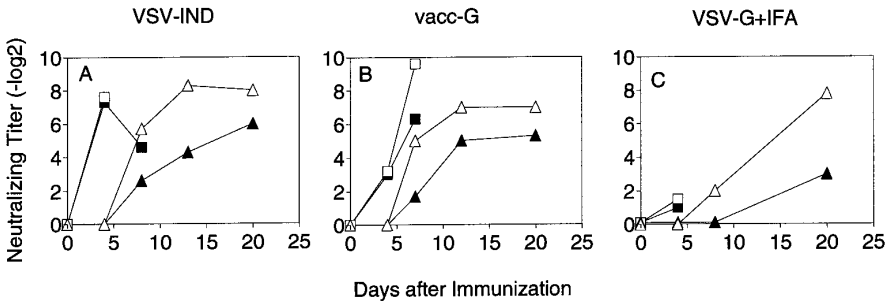

FIG. 4. Neutralizing antibody responses against VSV-G in Ii$^{0/0}$ mice. Ii$^{0/0}$ mice (closed symbols) or control mice (open symbols) were immunized with 2×10^6 PFU VSV-IND wild-type (A) or 2×10^6 PFU of vacc-G (B) or with 10 μg of baculovirus-derived VSV-G protein mixed with incomplete Freund's adjuvant (IFA) (C). Neutralizing IgM (squares) and IgG (triangles) titers were determined in 40-fold prediluted sera from three mice per group. Variations were less than two dilution steps.

cific IgG responses also in mutant mice even if applied in a noninfectious form; unlike the situation with VSV-G, no reduction of responses was evident.

Class II-Associated Presentation of VSV-G in the Absence of Ii

To reveal whether exogenous VSV-G exhibited special properties to reach the class II pathway in the absence of Ii, spleen cells from Ii$^{0/0}$ mice and control mice were loaded with graded amounts of VSV-G and used to stimulate a VSV-G-specific, class II-restricted T cell hybridomas (39) (Fig. 7). Presentation by Ii$^{0/0}$ spleen cells was more than 300-fold reduced compared to control cells. This strong reduction is comparable to previously reported values for other antigens (18).

DISCUSSION

The importance of class II molecules and their associated invariant chain for antiviral immune responses was tested in class II$^{0/0}$ and Ii$^{0/0}$ mice with respect to cytotoxic T, helper T, and B cell responses.

The class II deficiency led to slightly reduced CTL responses against LCMV and vaccinia virus similar to the previously reported results on influenza CTL responses (40, 41), but virtually no response against VSV. Lack of Ii had a less drastic effect on CTL responses against all three viruses; this correlated with the relatively efficient T-helper cell responses measured in these mice (see below). Overall, the results on CTL responses reflect the known differences in the dependence upon T help for these viruses and correlate with viral parameters: viruses that extensively replicate in the host, such as LCMV and vaccinia virus, probably induce a stimulatory interleukin milieu and upregulate necessary accessory molecules (40) as usually provided by noncognate T help (42). Poorly replicating VSV infects these cells only abortively and this apparently correlates with CTL responses being considerably more T-helper-dependent.

The T-helper-cell-independent B cell response of class II$^{0/0}$ and Ii$^{0/0}$ was studied by assessing the neutralizing IgM response against the G of VSV. B cell responsiveness was, as expected, normal in both mutant mouse strains. In contrast, the T-helper-cell-dependent IgG response was completely absent in class II-deficient mice. This is consistent with the almost total absence of helper T cells in these mice and con-

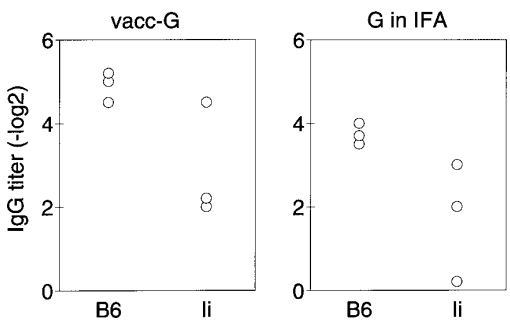

FIG. 5. VSV-G specific antibody responses in Ii$^{0/0}$ mice assessed by ELISA. Ii$^{0/0}$ and control mice were immunized with 2×10^6 PFU of vacc-G or with 10 μg of baculovirus-derived VSV-G protein mixed with incomplete Freund's adjuvant. VSV-G-specific titers were determined 3 weeks later on purified virus from 40-fold prediluted sera.

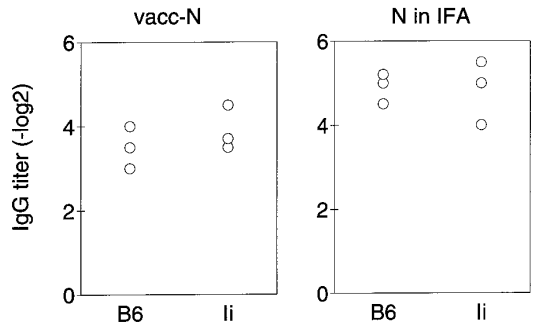

FIG. 6. VSV-N-specific antibody responses in Ii$^{0/0}$ mice assessed by ELISA. Ii$^{0/0}$ and control mice were immunized with 2×10^6 PFU of vacc-N or with 10 μg of baculovirus-derived VSV-N protein mixed with incomplete Freund's adjuvant. VSV-N-specific titers were determined 3 weeks later on purified virus from 40-fold prediluted sera.

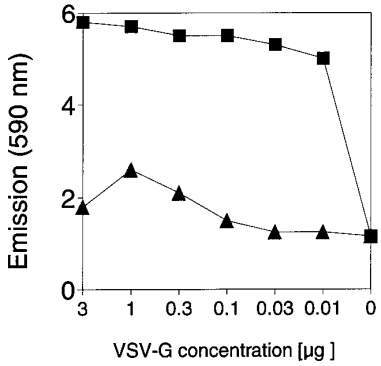

FIG. 7. Class II-associated presentation of VSV-G by Ii-deficient spleen cells. Spleen cells from $Ii^{0/0}$ and control mice were loaded with graded amounts of VSV-G and used to restimulate a class II-restricted and VSV-G-specific $CD4^+$ T cell hybridoma. IL-2 release was determined using the IL-2-sensitive cell line CTLL-2.

firms earlier results with monoclonal antibodies (11) and transgenic mice (43, 44). It is noteworthy that the relatively efficient IgG response measured in $CD4^{0/0}$ mice (16) reflects the presence of a particular $CD4^-CD8^-$ T cell population that is apparently not detected in normal mice, since no antibody class switch occurs after depletion of $CD4^+$ T cells (11).

In contrast to the mice lacking class II expression, $Ii^{0/0}$ mice mounted surprisingly efficient IgG responses against both the G and N of VSV. Although the infectious forms of VSV-G appeared to be particularly efficient, recombinant VSV-G in adjuvants also induced a significant response in $Ii^{0/0}$ mice. In addition, recombinant VSV-N induced an equally strong response also if applied in a noninfectious form. Despite these efficient *in vivo* responses, presentation of VSV-G to a specific T-helper-cell hybridoma was reduced by a factor of more than 300, which is comparable to previously reported results and shows that VSV-G is not generally presented independently of Ii. It has been reported that detection of the efficiency of antigen presentation in the absence of Ii apparently depends upon the particular hybridoma used for the experiments (18). It is probably unlikely that the surprisingly strong T-helper-cell response revealed here in $Ii^{-/-}$ mice *in vivo* reflects special T-helper-cell clones that respond in the absence of Ii because the T-helper-cell response observed *in vivo* depends upon the form of antigen applied (see below). This prompts the suggestion that, *in vivo,* local antigen concentrations during a viral infection may be sufficient to load class II molecules of both professional APCs and B cells in the absence of Ii. How might such a high local antigen concentration upon viral infection come about? Several possibilities exist. Virally infected cells are lysed either by cytolytic properties of the virus or by cytotoxic T cells. Thus, viral antigens will usually appear in association with cell membranes, preventing rapid diffusion of the antigen. Since APCs will take up the membrane fragments by phagocytosis, high antigen concentrations will eventually be reached in the endosome. Such cell debris in antigen preparations have also been shown to act as an adjuvant for the loading of class I molecules by exogenous proteins (45). Since the infectious form of the antigen seems to be particularly efficient, at least in the case of VSV-G, a second pathway of antigen presentation may facilitate the induction of T help in the absence of Ii. It is conceivable that endogenously produced viral antigen in the APCs efficiently load the class II molecules within the cells as has been reported for many antigens. This may be particularly important for the G of VSV, since membrane proteins seem to be much more efficient at exploiting this pathway than cytosolic proteins (7, 38). Furthermore, many reports suggested that this pathway also operates in the absence of Ii (7, 20). Yet, this pathway obviously cannot operate for specific B cells unless cytopathic virus replication is abortive in these cells. The data presented are, however, compatible with the finding that B cells take up antigen via their specific receptors (46). Thereby, B cells may concentrate the specific antigen sufficiently on their surface to load the class II molecules even in the absence of Ii. Finally, both recombinant VSV-G and VSV-N were able to induce T help in $Ii^{0/0}$ mice in the absence of an infection. It is important to note that both VSV-G and VSV-N were produced in a recombinant baculovirus system and were injected in association with cell debris from *in vitro* lysed cells. Thus, this form of antigen aggregates probably represents a similarly concentrated form of antigen as produced locally *in vivo* upon viral infection. This notion is supported by the finding that soluble proteins injected in adjuvants did not induce a measurable primary B cell response in $Ii^{0/0}$ mice (18).

Taken together, T-helper-cell-independent cytotoxic T cell and B cell responses were normal in the absence of class II or Ii. In contrast, a T-helper-cell -dependent class switch from IgM to IgG was absent in class $II^{0/0}$ mice but surprisingly efficient in $Ii^{0/0}$ mice. This suggests locally high intra- and/or extracellular protein concentrations caused by viral infections and destruction of infected cells which may be sufficient to overcome the Ii deficiency.

ACKNOWLEDGMENT

We thank A. Althage for excellent technical and Y. Deflorin for excellent secretarial assistance and A. Oxenius for helpful discussions. This work was supported by grants from the Swiss National Science Foundation, the Kanton Zürich, the EMDO Stiftung Zürich, and the Human Frontiers Science Program.

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