confirmed by hybridization of the same chromosome to a probe (*KEX2*) known to reside on chromosome 14 and by hybridization of a different nearby band to a probe (*CYC1*) known to reside on chromosome 10.
32. Multicopy plasmid pPHY52 (*18*), which carries the *VPS34* gene and a *URA3* marker, was introduced into the *pik1Δ1::LEU2/PIK1* heterozygous diploid strain, which was then subjected to sporulation. No Leu$^+$Ura$^+$ spores were recovered from 20 tetrads dissected. Conversely, neither of two multicopy plasmids carrying the *PIK1* gene, YEp-*PIK1/URA3* and YEp*PIK1/TRP1* (*15*), rescued the temperature-sensitive lethality of a *vps34* mutant [J. H. Stack and S. D. Emr, personal communication].
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42. We thank G. Anderson for the chromosome blot; P. Hieter and F. Spencer for the yeast genomic library in the centromere vector, pSB32; M. Moore and J. Onuffer for oligonucleotides; and J. Brabson and J. Flick for careful reading of the manuscript. Supported by NIH predoctoral traineeships GM07232 and CA09041 (to C.A.F. and E.A.S.); by a salary from the Howard Hughes Medical Institute (to A.A.); by NIH research grant GM21841 (to J.T.); by funds provided from the Lucille P. Markey Charitable Trust through the Berkeley campus Program in Biomolecular Structure and Design; and by facilities provided by the Berkeley campus Cancer Research Laboratory.

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The Influence of Antigen Organization on B Cell Responsiveness

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The influence of antigen epitope density and order on B cell induction and antibody production was assessed with the glycoprotein of vesicular stomatitis virus serotype Indiana [VSV-G (IND)]. VSV-G (IND) can be found in a highly repetitive form in the envelope of VSV-IND and in a poorly organized form on the surface of infected cells. In VSV-G (IND) transgenic mice, B cells were unresponsive to the poorly organized VSV-G (IND) present as self antigen but responded promptly to the same antigen presented in the highly organized form. Thus, antigen organization influences B cell tolerance.

Several mechanisms to prevent autoantibody production have been proposed: autoreactive B cells may be clonally deleted (*1*), functionally silenced (*2–5*), or they may simply ignore self antigen (*6–8*). It is not clear why some self antigens are ignored and others lead to clonal deletion of B cells or induce B cell anergy. Possibly, membrane-bound self antigens [for example H-2K^k (*1*), CD8 (*9*), and membrane-associated hen egg lysozyme (*10*)] induce clonal deletion, whereas soluble hen egg lysozyme (*2*) or single-stranded DNA (*4*) usually induce clonal anergy. These findings have been tentatively correlated with receptor cross-linking (*11–13*) and obviously impinge on the old debate regarding the role of density and the order of antigenic epitopes for the induction of B cells (*14*).

The antibody response to vesicular stomatitis virus [VSV serotype Indiana (VSV-IND)] has been analyzed extensively. All antibodies that neutralize VSV-IND infectivity in vitro bind to the glycoprotein VSV-G (IND) (*15, 16*). The early response (days 3 to 5) is of immunoglobulin M (IgM) isotype and can be induced in the absence of T cells (*17*), whereas the switch to IgG (days 6 to 8) is dependent on help from T cells (*18*). In addition, VSV has no mitogenic effects on B cells in vivo or in vitro (<30 μg/ml) (*19*).

To determine whether neutralizing IgM antibodies were induced predominantly by VSV-G (IND) in a highly organized form, as found in the envelope of the viral particle, or after fusion of virus and infection of cells, we performed the following experiment. Peritoneal macrophages (1×10^7) were infected with VSV-IND particles that had been inactivated with ultraviolet (UV) radiation (*20*). UV-inactivated VSV-IND infects cells to the extent that viral antigens are detectable by immunofluorescence on the cell surface but no progeny are produced. Separate groups of mice were injected with either the supernatant containing unadsorbed UV-inactivated virus or with abortively infected cells (*20*). Injected cells were barely able to induce neutralizing IgM antibodies, whereas the supernatant containing unadsorbed viral particles induced a considerable response (Fig. 1A). VSV-IND particles inactivated with formaldehyde, which apparently fail to fuse with target cells (*20*), also induced a good response (Fig. 1B). Thus, inactivated VSV-IND particles that displayed highly organized VSV-G (IND) were alone able to induce a T cell–independent IgM response, apparently even better than poorly organized VSV-G (IND) on infected cells (Fig. 1, A and B).

To evaluate the influence of antigen organization on B cell tolerance, we generated transgenic mice expressing VSV-G (IND) under the control of the *H-2K^b* promotor (KINDG mice). The VSV-G (IND)–specif-

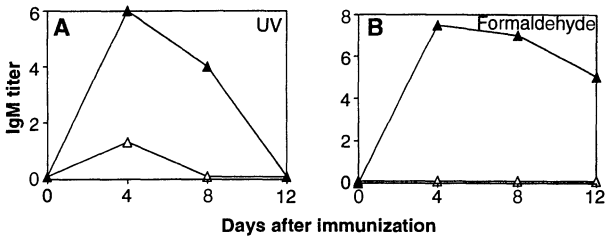

Fig. 1. More efficient induction of B cells by VSV-IND virions than by VSV-G (IND) expressed on infected cells. Peritoneal macrophages were incubated with VSV-IND inactivated by either (**A**) UV or (**B**) formaldehyde. Supernatant containing unadsorbed virus (closed triangles) or 10^7 virus-adsorbing cells (open triangles) were injected into the spleens of ICR mice (*20*), and neutralizing IgM antibody titers (*34*) were determined thereafter (*35*). Mean neutralizing IgM titers of three mice are shown; individual titers differed less than ± 1 dilution step of 2. Titers are indicated as $\log_2$ of 40-fold prediluted sera.

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Table 1. Average avidity of VSV-G (IND) binding antibodies of KINDG mice infected with VSV-IND 3 weeks previously (*38*).

Mice	ELISA titer^{-1}	Titer^{-1}/AFCs (±SD)	Glycoprotein concentration for 50% inhibition
KINDG	1430	47 ± 27	3×10^{-9} mol/liter
Control	3900	173 ± 3	10^{-9} mol/liter

ic neutralizing antibody response (*15*) of KINDG and normal mice against VSV-G (IND) was assessed after immunization with 10 μg of a soluble VSV-G (IND) preparation derived from a baculovirus expression system or with 2×10^6 plaque-forming units (PFU) of a recombinant vaccinia virus expressing VSV-G (IND). The recombinant vaccinia virus does not exhibit VSV-G (IND) in its envelope; therefore, VSV-G (IND) is expressed only after infection of cells and, similar to soluble VSV-G (IND), represents a poorly organized form of antigen. Control animals developed neutralizing IgM on days 4 to 8 and neutralizing IgG with high titers from day 8 after both immunizations. In contrast, KINDG mice produced no measurable IgM or IgG antibodies (Fig. 2, A and B), even after booster immunizations 30 days later. Because normal mice depleted of CD4$^+$ T helper cells with a monoclonal antibody to CD4 (*18*) develop neutralizing IgM titers within normal ranges (Fig. 2D), the lack of a measurable IgM antibody response in the transgenic KINDG mice reflected B cell, and not T helper cell, unresponsiveness (*21*).

This unresponsiveness was stable after transfer of transgenic spleen cells to irradiated, nontransgenic recipients infected with the recombinant vaccinia virus (Fig. 2F) and was specific; all mice responded comparably to a vaccinia recombinant expressing the glycoprotein of VSV serotype New Jersey (VSV-NJ) with neutralizing IgM antibodies, which do not cross-react with VSV-IND (*16*) (Fig. 2E). These results are in contrast to those found with a previously tested VSV-G (IND) transgenic mouse strain, which expressed G (IND) under the promotor of myelin basic protein (*7*) and which mounted a neutralizing IgM response after immunization with VSV-G (IND)–expressing vaccinia. However, comparable to the previously tested transgenic mice (*7*) KINDG mice possess potentially reactive B cells that can produce neutralizing antibodies, because KINDG mice immunized with 2×10^6 PFU of VSV-IND developed high neutralizing antibody titers (Fig. 2C).

To test the influence of the self antigen on the quality of the antibodies, we compared the avidities of antibodies to VSV-G (IND) in KINDG and normal mice. First, we estimated relative avidities by correlating the number of G (IND)–specific antibody-forming cells (AFCs) with the G (IND)–specific enzyme-linked immunosorbent assay (ELISA) titer (*2*) (Table 1). KINDG mice exhibited one-fourth the average avidity for IgG compared to controls. Second, the average avidities of sera were determined in a competition ELISA (*22*): The average avidities of VSV-G (IND) antibodies in KINDG mice were one-third of those of controls, but were comparable to the average avidities in normal mice (Table 1).

To define the responder status of VSV-G (IND)–specific T helper cells in KINDG mice, we performed an in vitro proliferation assay (*23*) 8 days after infection with VSV-IND. The absence of a proliferative response specific to VSV-G (IND) in KINDG mice (Fig. 3A) shows tolerance at the level of T helper cells. The fact that VSV-G–specific neutralizing IgG antibodies dependent on T helper cells were measurable in VSV-IND–infected KINDG mice suggested the involvement of linked T cell help specific for internal VSV-IND proteins (*7*, *24*). In a control experiment, KINDG and C57BL/6 mice were infected with a recombinant vaccinia virus that expressed the nucleoprotein (N) of VSV.KINDG and control mice exhibited similar N-specific IgG antibody titers 3 weeks after immunization, which shows normal N-specific T helper cell activity in KINDG mice (Fig. 3B).

Thus, IgM antibodies are induced in KINDG mice by wild-type or inactivated VSV-IND, but not by soluble G (IND) or G (IND) expressed by a recombinant vaccinia virus. Because both VSV-IND particles and VSV-G (IND) are filtered out by macrophages in the marginal zone of the spleen (*25*), the following differences in antigenicity may be relevant: (i) VSV-IND expresses the G (IND) in a highly repetitive way in its envelope (*26*), whereas the recombinant vaccinia virus does not (*7*); (ii) linked T cell help may be induced by VSV internal antigens but not by vaccinia virus antigens (*7*, *24*).

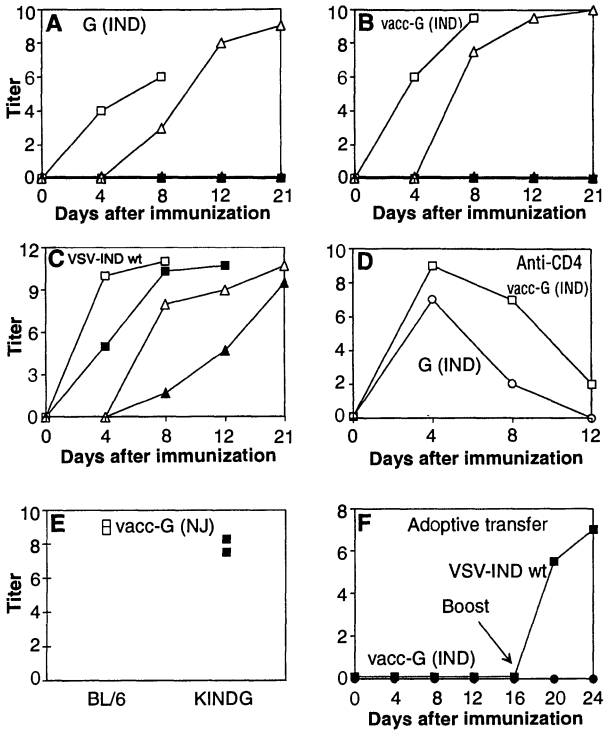

Fig. 2. Unresponsiveness of B cells in KINDG mice except against VSV particles. KINDG mice (closed symbols) and control C57BL/6 mice (open symbols) were immunized intravenously with 10 μg of recombinant VSV-G (IND) [G-(IND)] derived from a recombinant baculovirus (**A**), 2×10^6 PFU of recombinant vaccinia virus expressing the G (IND) [vacc-G (IND)] (**B**), or 2×10^6 PFU of wild-type VSV-IND (VSV-IND wt) (**C**), and neutralizing IgM (squares) and IgG (triangles) antibody titers were determined at various times as in Fig. 1 (*34*). Alternatively, C57BL/6 mice were depleted of CD4$^+$ T helper cells (*36*) and immunized with 2×10^6 PFU of recombinant vaccinia virus expressing the G (IND) (squares) or recombinant baculovirus–derived VSV-G (IND) (10 μg) (circles), and neutralizing IgM titers were determined thereafter as in Fig. 1 (*34*) (**D**). C57BL/6 (open symbols) and KINDG mice (closed symbols) were injected with 2×10^6 PFU of recombinant vaccinia virus expressing the glycoprotein of VSV-NJ [vacc-G (NJ)], and neutralizing IgM titers were determined on day 4 as in Fig. 1 (**E**). Finally, 10^8 transgenic spleen cells were transferred into irradiated normal C57BL/6 recipients (7.5 Gy, where 1 Gy = 100 rads), and the mice were challenged 3 days later on day 0 with 2×10^6 PFU of recombinant vaccinia virus expressing VSV-G (IND) and boosted 16 days later with either 2×10^6 PFU of the recombinant vaccinia virus (circles) or 2×10^6 PFU of wild-type VSV-IND (squares) (**F**). The mean neutralizing IgM titers of three mice are shown; individual titers differed less than ± 1 dilution step of 2.

These possibilities were evaluated as follows: KINDG mice were either depleted of T helper cells with a monoclonal antibody (*18*) and immunized with 2 × 10^6 PFU of infectious VSV-IND (Fig. 3C) or were immunized with 2 × 10^6 PFU equivalents of formalin-treated VSV-IND (Fig. 3D), which induces IgM antibodies but cannot induce T helper cells (*27*). Both experiments demonstrated that VSV-IND virus particles induce IgM in KINDG mice independently of T cell help. If higher doses of formaldehyde-treated VSV-IND were used (2 × 10^7 PFU), KINDG mice generated neutralizing IgM titers of 1:5120 on day 4. Therefore, the repetitive antigen arrangement on the viral envelope structure was essential for activation of unresponsive B cells. The B cells of KINDG mice primed with formalin-inactivated VSV-IND could be boosted by the recombinant vaccinia virus, which indicates that B cells from G (IND) transgenic mice, once activated by optimally organized antigen, may be restimulated by VSV-G (IND) on a cell surface (Fig. 3E) (*28*).

These results suggest that the organization of foreign antigen or of self antigen determines both immunogenicity and responsiveness of B cells. Antigens that are expressed in a poorly organized fashion on cell surfaces may induce IgM responses that are independent of T help, but this antigen may be much less efficient than densely packed, repetitively organized antigens on VSV-IND. In our transgenic mice, the differences between the response to less orderly and repetitively organized antigen are absolute: Mice are tolerant or unresponsive to the less organized form of the antigen, whereas they respond to antigen presented in a highly organized repetitive form (*29*). Interestingly, although the transgenic self VSV-G (IND) is membrane associated, it did not induce B cell deletion but rather some form of B cell anergy in KINDG mice. This fits the recently presented model that membrane-bound proteins at low density [as is the case for VSV-G (IND)] may induce anergy rather than deletion (*11, 12*).

These findings may be relevant to the understanding of the pathogenesis of the prototype antibody-mediated autoimmune disease in humans, myasthenia gravis, which is caused by acetylcholine receptor (AChR)–specific autoantibodies. As in the case of VSV-G (IND), AChRs exist in two forms: a randomly distributed form on the muscle cell surface during generation and regeneration of muscle cells and a highly organized form in the neuromuscular end plate (*30*). Interestingly, organized receptors exhibit a spacing of 5 to 10 nm (*31*), the same value as we calculated for VSV-G (IND) in the viral envelope and a distance that also has been found to be optimal for T cell help–independent IgM responses to dinitrophenyl (*32*). Our results here suggest that B cell unresponsiveness to AChRs may possibly be broken by a mechanism similar to that illustrated for VSV. Usually human B cells are tolerant to randomly expressed AChRs, which are present and accessible to B cells on myoid cells and during muscle (re)generation. The highly organized form of AChRs present in the neuromuscular end plate is usually hidden within the synapse and therefore is not accessible to B cells. However, such organized AChRs may become accessible to B cells after trauma or in tumors and may then possibly cause the induction of autoantibodies.

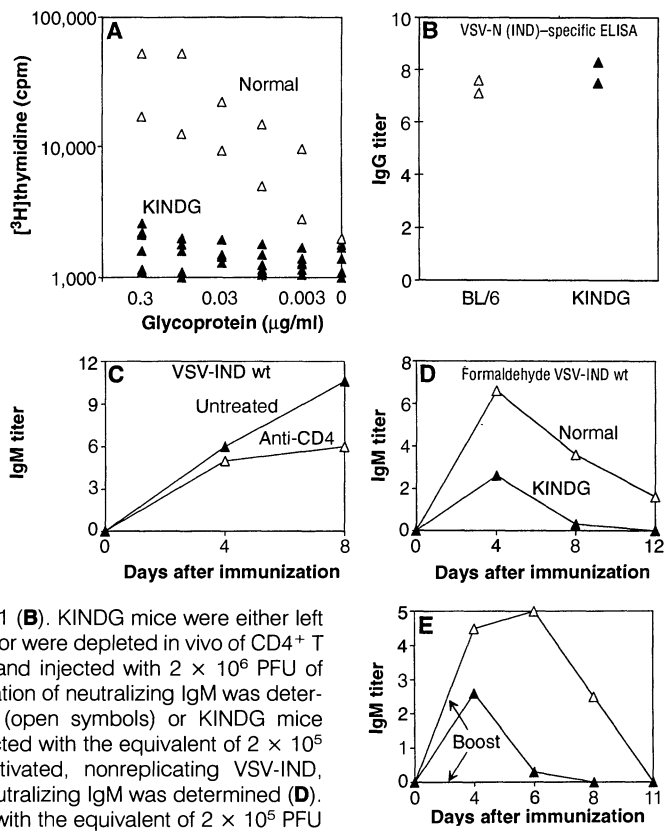

Fig. 3. Absence of VSV-G (IND)–specific T help in KINDG mice; KINDG mice (closed triangles) and control C57BL/6 mice (open triangles) were injected with 2 × 10^6 PFU of VSV-IND. Eight days later, proliferation of spleen cells upon stimulation with purified VSV-G (IND) was assessed in vitro (*23*) (**A**). Alternatively, KINDG mice (closed triangles) and C57BL/6 mice (open triangles) were immunized with 2 × 10^6 PFU of recombinant vaccinia virus expressing the N of VSV-IND [VSV-N (IND)]; N-specific IgG ELISA titers were determined 3 weeks later (*37*); titers are defined as in Fig. 1 (**B**). KINDG mice were either left untreated (closed symbols) or were depleted in vivo of $CD4^+$ T cells (*36*) (open symbols) and injected with 2 × 10^6 PFU of VSV-IND, and the concentration of neutralizing IgM was determined (**C**). C57BL/6 mice (open symbols) or KINDG mice (closed symbols) were injected with the equivalent of 2 × 10^5 PFU of formaldehyde-inactivated, nonreplicating VSV-IND, and the concentration of neutralizing IgM was determined (**D**). KINDG mice were injected with the equivalent of 2 × 10^5 PFU of formaldehyde-inactivated VSV-IND and boosted 2 days later with 2 × 10^6 PFU of recombinant vaccinia virus expressing VSV-G (IND) (open symbols) or left unchallenged (closed symbols), and the concentration of neutralizing IgM was determined (**E**). In (C) through (E), the mean neutralizing IgM titers of three mice are shown; individual titers differed less than ± 1 dilution step of 2.

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19. In contrast to others reporting mitogenic activity of both purified VSV-G (IND) and VSV-IND [G. Goodman-Snitkoff, R. J. Mannino, J. J. McSharrey, *J. Exp. Med.* **153**, 1489 (1981)], we could not find any B cell proliferation in vitro after stimulation with VSV-G (IND): neither purified VSV-G (IND) (< 30 μg/ml) nor recombinant baculovirus–derived VSV-G (IND) (< 30 μg/ml) nor formaldehyde-inactivated VSV-IND (< 10^7 PFU/ml) induced any proliferation in vitro.
20. VSV-IND (1 ml) was inactivated with UV for 2 min in a thin layer in a 100-mm petri dish under a UV lamp (7UV 15 W; Philips) at a distance of 10 cm. The intensity of this treatment was chosen so that no plaque formation in vitro was detectable anymore. For formaldehyde inactivation, VSV-IND was inactivated for 18 hours at 4°C in modified Eagle's medium, supplemented with 2% fetal calf serum and a final formaldehyde concentration of 0.064%. This treatment prevented plaque formation in vitro, and inactivated VSV failed to induce

surface-stainable VSV-G (IND) after incubation with cells or to prime mice for cytotoxic T cells or efficient T cell help (*27*). Peritoneal macrophages were incubated with UV- or formaldehyde-inactivated VSV-IND 90 min at 37°C at a multiplicity of 1. The supernatant containing unadsorbed virus was saved, and the cells were washed three times. Both cells and supernatant were injected directly into the spleen of ICR mice to avoid loss of cells in the lung.

21. Suppressor T cells are not involved because there are no CD8+ T cells that are glycoprotein-specific in *H-2*^{b} mice [J. W. Yewdell *et al.*, *J. Exp. Med.* **163**, 1529 (1986); T. M. Kündig *et al.*, *J. Virol.* **67**, 3680 (1993)]. Also, B cells able to be activated must be present in KINDG mice because they respond promptly to VSV-IND virus [which may be even more apt at inducing CD8+ T cells than soluble VSV-G (IND), but obviously this suppression does not happen here].
22. The concentration of glycoprotein needed to compete for 50% of antibody binding in the glycoprotein-specific ELISA was determined as described [S. Rath, C. M. Stanley, M. W. Steward, *J. Immunol. Methods* **106**, 245 (1988)]. In brief, graded amounts of purified viral particles were incubated with diluted sera of VSV-IND–immunized mice. Serum dilutions leading to less than the half-maximal absorbance without competing glycoprotein were used in order to avoid antibody excess.
23. Mice were immunized with 2 × 10^{6} PFU of VSV-IND, and spleens were removed 8 days later. Spleen cell suspensions were prepared, and 4 × 10^{5} cells were incubated in 96-well plates in the presence of various amounts of purified VSV-G (IND) in serum-free Ventrex medium (NBS Scientific Biologicals, Portland, ME) at 37°C. Three days later, the cells were pulsed for 8 hours with [^{3}H]thymidine (1 mCi per well).
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28. This experiment shows that neutralizing antibodies induced by VSV-IND particles in KINDG mice also react with the poorly organized form of VSV-G (IND) and thus do not represent new antibodies against determinants that are unique to the virion and therefore would be anti-foreign. This notion is supported by the fact that the neutralizing activity of neutralizing polyclonal and a collection of about 60 monoclonal VSV-IND antibodies can readily be absorbed by infected cells or purified G (IND). In addition, it appears that there is only one single neutralizing epitope on VSV [H. P. Roost, thesis, University of Zürich (1991)].
29. Thus, optimal B cell induction is achieved with repetitive antigens, and induction of tolerance with repetitive antigens, for example, flagellin [G. Möller, *Immunol. Rev.* **23**, 126 (1975)], may either reflect interaction with, and deletion of, immature B cells or exhaustive induction of mature B cells, particularly in the absence of T help.
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34. The serum neutralization test was performed as described [H. P. Roost, S. Charan, R. M. Zinkernagel, *Eur. J. Immunol.* **20**, 2547 (1990)]. To determine IgG titers, we pretreated undiluted serum with an equal volume of 0.1 M 2-mercaptoethanol in saline. Unreduced samples were taken as IgM titers only if the corresponding reduced samples had a titer at least one-quarter its size—that is, when the IgG present in the unreduced sample could be neglected.
35. Mice were kept and experiments were performed according to cantonal and federal law in Switzerland.
36. For the CD4 depletion of mice, on days 3 and 1 before immunization the mice were injected with two doses of 1 mg of YTS 191.1. The depleted CD4+ T cell population was determined to be below the detection level by fluorescence-activated cell sorter analysis. Functional depletion was confirmed in control mice by complete abrogation of the IgM to IgG switch of neutralizing antibodies against VSV (*18*).
37. VSV-N–specific IgG ELISA titers were determined in the serum (*33*).
38. Mice were immunized with 2 × 10^{6} PFU of VSV-IND. Twenty-one days later, blood was taken and spleen and bone marrow cells were isolated. VSV-G (IND)–specific ELISA titers were determined in the serum as described (*33*). VSV-G (IND)–specific AFCs were enumerated in spleen and bone marrow as described [D. Moskophidis and F. Lehmann-Grube, *J. Immunol.* **133**, 3366 (1984); plates (Greiner, Frickenhausen, Germany) were coated with purified VSV-G (IND)]. To calculate the reciprocal of the ELISA titer per the number of AFCs, we look for groups of three mice the sum of AFCs from spleen and bone marrow per 10^{6} lymphocytes. A similar method has been used by C. C. Goodnow and co-workers (*2*). Because in the VSV system AFC tests cannot be performed for the neutralizing determinant only, we determined AFCs and ELISA titers specific to the whole VSV-G (IND) molecule. We have found that if purified VSV-IND is used to coat plates for ELIspot assays to assess AFCs, more than 50% of VSV-specific AFCs are specific for the neutralizing determinant of VSV-G (M. F. Bachmann, unpublished results). Neutralization assays and ELISA tests against VSV-G therefore appear to measure the same antibodies and in fact give comparable results in the VSV system. To determine the glycoprotein concentration competing for 50% of antibody binding in the ELISA (*22*), we analyzed the diluted sera of three mice per group 21 days after immunization with VSV-IND.
39. The baculovirus expressing the G (IND) of VSV was a generous gift of D. H. Bishop, Institute of Virology and Environmental Microbiology, Oxford, UK. Recombinant vaccinia viruses expressing the G and N of VSV were generous gifts of B. Moss, Laboratory of Viral Diseases, NIH, Bethesda, MD. The hybridoma cell line YTS 191.1 was a generous gift of H. Waldmann, Cambridge, UK. Supported by grants from the Swiss National Science Foundation (31-32179.91) and the Kanton Zürich. We thank A. Althage and H. Pircher for critically reading the manuscript and Y. Deflorin for excellent secretarial assistance.

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Mechanism-Based Inactivation of Prostatic Acid Phosphatase

Jason K. Myers and Theodore S. Widlanski*

Protein phosphatases play important roles in the regulation of cell growth and metabolism, yet little is known about their enzymatic mechanism. By extrapolation from data on inhibitors of other types of hydrolases, an inhibitor of prostatic acid phosphatase was designed that is likely to function as a mechanism-based phosphotyrosine phosphatase inactivator. This molecule, 4-(fluoromethyl)phenyl phosphate, represents a useful paradigm for the design of potent and specific phosphatase inhibitors.

Many biological processes, such as signal transduction, nucleic acid repair and synthesis, phospholipid metabolism, and energy storage, involve the formation and cleavage of phosphate ester bonds. Protein phosphorylation in particular is an important mechanism for the regulation of cellular activity (*1*, *2*). In vivo, the steady-state level of protein phosphorylation is controlled by the opposing activities of protein kinases and protein phosphatases. Overexpression of protein tyrosine kinase activity can cause cell transformation (*3*), and it has been suggested that phosphotyrosine phosphatases (PTPases) may function as tumor suppressor genes (*4*). PTPases also exhibit less benign functions. The PTPase YOP 2b is an essential virulence determinant of the bacterium *Yersinia pestis*, the pathogen responsible for the bubonic plague (*5*). Another PTPase is encoded by vaccinia virus and thus may play a role in the pathogenesis of smallpox (*6*).

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Although there is much interest in PTPase inhibitors, efforts to rationally design such inhibitors have been hampered by our limited knowledge of the structural features of these enzymes. We have avoided this problem by designing a mechanism-based inactivator (also known as a suicide inhibitor). Mechanism-based inhibitors are enzyme substrates that undergo an enzyme-catalyzed transformation to give reactive intermediates that, before their release, inactivate the enzyme by forming a covalent bond to an active site residue (*7*). Such molecules are valuable because they are usually potent and specific inhibitors and because they can be used to introduce a radioactive label into the active site of the enzyme.

We used human prostatic acid phosphatase (PAP) as a model enzyme. Although PAP has a broad substrate specificity in vitro, it displays a preference for aryl phosphates (*8*). This phosphatase is an important diagnostic marker for prostate cancer (*9*), and there is circumstantial evidence that it may be involved in the regulation of androgen receptor activity in prostate cells (*10*).