IDIOTOPE REGULATION BY ISOTYPE SWITCH VARIANTS OF TWO MONOCLONAL ANTIIDIOTOPE ANTIBODIES

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The administration of antiidiotypic antibodies to an adult mouse influences the expression of the complementary idiotype in a subsequent immune response in a dose-dependent fashion. Nanogram doses enhance, and microgam doses suppress, the appearance of the idiotype-bearing antibodies (for review see 1). In addition, there is evidence indicating that the heavy chain class of the antiidiotype antibodies may influence their regulatory function (2–5). This evidence, however, is based on experiments involving the use of antibodies that were xenogeneic and/or differed from each other both in specificity and isotype.

For a systematic investigation of the class dependency of the regulatory properties of antiidiotope antibodies, we have isolated spontaneous isotype switch variants from the hybridomas Ac146 (6) and A39-40 (7) that secrete monoclonal antibodies specific for idiotopes in the variable (V)[1] region of the germline-encoded antibody B1-8 (8–10). B1-8 is an antibody derived from the primary immune response of C57BL/6 mice against the hapten (4-hydroxy-3-nitrophenyl)acetyl (NP), and all idiotopes so far defined on B1-8 monoclonal antiidiotopes are regularly expressed in the humoral anti-NP response of mice bearing the Igh^b allotype (6, 7). The family of switch variants isolated from the cell line A39-40 consists of antibodies of the IgG1, IgG2b, and IgG2a classes. The Ac146-variant family consists of IgG1, IgG2b, IgG2a, and IgE antibodies (11). The members of a family possess identical idiotope-binding specificity in combination with different isotypes and can thus be used for a formal analysis of the effector functions of the isotypes in idiotopic regulation. The results presented in this paper show that the antiidiotopes of a given family have indistinguishable regulatory properties with respect to both enhancement and suppression of the idiotypic target. In addition, we find that antibody variable regions differ in their sensitivity to regulation by antiidiotope antibodies, depending on their idiotopic makeup.

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[1] *Abbreviations used in this paper*: BSA, bovine serum albumin; CG, chicken gamma globulin; NIP, (4-hydroxy-3-nitro-5-iodo-phenyl)acetyl; NP, (4-hydroxy-3-nitro-phenyl)acetyl; PBS, phosphate-buffered saline pH 7.2; RIA, solid-phase radioimmunoassay; V region, variable region.

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Materials and Methods

Animals. C57BL/6 and (C57BL/6 × CBA)F_1 mice were obtained from the breeding colony at our institute. The animals were immunized against ectromelia 4–5 wk before the start of an experiment. For the regulatory experiments we used 10–14-wk-old mice. Within an experiment the animals were age matched.

Hybridoma Cell Lines and Monoclonal Antibodies. The hybridoma cell line B1-8 and its subclone B1-8.64, which secrete an antibody of the IgM class with specificity for NP, have been described (8, 11). Affinity-purified antibody B1-8.64 was kindly given to us by Dr. T. Takemori.

The hybridoma cell lines Ac146 ($IgG1^j$), Ac38 ($IgG1^j$), A39-40 ($IgG1^b$), A25-9 ($IgG1^b$), and A6-24 ($IgG2a^b$) secrete monoclonal antiidiotope antibodies with specificity for distinct determinants on the V region of antibody B1-8 (6, 7). We use the same designation for an antiidiotope antibody and the target idiotope; i.e., antibody Ac146 recognizes idiotope Ac146, etc. From the cell lines Ac146.8 and A39-40.5, two families of isotype switch variants were selected by either fluorescence-activated cell sorting or sequential sublining in vitro (11). Within a family, the antibodies express identical idiotope-binding specificities and most probably identical V regions. Their κ light chains are indistinguishable from each other in analytical isoelectric focusing. Serological and biochemical evidence indicates that class switching involved the complete exchange of the constant domains of the heavy chain. Purified antibody Ls136 ($IgG1^b$) specific for λ1 light chains (6) and antibody X63 ($IgG1^a$) (12) were gifts of Dr. T. Takemori.

Purification of Antibodies and Immunosorbents. Antiidiotope antibodies were purified from ascites fluid by precipitation with saturated ammonium sulfate and subsequent ion exchange chromatography on DEAE cellulose (Whatman, Ltd., Maidstone, United Kingdom) as described previously (13). Purified antibodies were examined by analytical sodium dodecyl sulfate-polyacrylamide electrophoresis and found to contain <10% material that was not co-migrating with immunoglobulin (Ig) light or heavy chains. In addition the antiidiotopes were titrated in solid-phase radioimmunoassay (RIA) (see below) on B1-8-coated plates. Antibody concentrations determined in RIA differed by <10% from the concentrations determined from the absorbance at 280 nm. Before we injected the antiidiotope antibodies into mice, the specific idiotope-binding activity was again determined to assure that the mice received the desired antiidiotope dose.

Immunosorbents were prepared by coupling purified antibodies to cyanogen bromide-activated Sepharose 4B (Pharmacia Fine Chemicals, Freiburg, FRG) (14). Absorption of pooled immune sera was done as described by Takemori et al. (15). Serum pools were diluted with phosphate-buffered saline pH7.2 (PBS) containing 1% bovine serum albumin (BSA). The pools were divided into aliquots and each aliquot was absorbed twice with an equal volume of X63-, A39-40-, Ac146-, or A25-9-coupled Sepharose at 4°C overnight. Each absorbed serum aliquot was then titrated in RIA for residual idiotope-expressing antibodies.

Immunizations. Antiidiotope antibodies were diluted in PBS containing 5 μg mouse serum albumin (gift of T. Takemori) per ml. Groups of five mice were injected intraperitoneally with 0.2 ml antiidiotope dilution. Control mice received only diluent. At various times after injection of antiidiotope, the mice were immunized with 100 μg alum-precipitated NP_{17}-CG (chicken gamma globulin) (16). The animals were bled 12 d later and the titers of (4-hydroxy-3-nitro-5-iodo-phenyl)acetyl (NIP)-binding or idiotope-expressing antibodies determined by RIA.

Radioimmunoassay. Titration of λ1-bearing anti-NP or idiotope-expressing antibodies was done as described (15, 17). Plastic plates (Dynatech Deutschland GmbH, Plochingen, FRG) were coated with 35 μg of the various antiidiotope antibodies or NIP_9-BSA (gift of T. Imanishi-Kari) per ml and incubated with the appropriately diluted serum samples. (Lambda chain-bearing anti-NP antibodies are titrated on BSA coupled to NIP because their affinity for NIP is higher than for NP [16]). Bound λ1-bearing antibodies were detected with ^{125}I-labeled antibody Ls136. Antibody isotypes were detected with purified ^{125}I-labeled goat antibodies specific for mouse IgG1 and IgG2a, or with monoclonal rat antibodies R14-50 (anti-IgG2b) (11) and R33-24.12 (anti-IgM) (18).

Results

Nanogram Doses of Antiidiotope Antibodies of the IgG1, IgG2b, and IgG2a Classes Enhance Idiotope Expression. It was shown previously (17, 19) that the antiidiotope antibodies Ac38 and Ac146 of the IgG1 isotype enhanced the expression of their target idiotope when injected in nanogram doses into adult C57BL/6 mice several weeks before immunization with the NP hapten. A dose of 10 μg, however, had a suppressive effect. In contrast, an IgG2a antiidiotope, A6-24, was suppressive at both microgram and nanogram doses, although suppression was less efficient in the latter than in the former case (5). This suggested that the isotype of an antiidiotope antibody may determine its regulatory function, in line with earlier data obtained with guinea pig IgG1 and IgG2 antiidiotype antibodies in mice (2–4). In subsequent, unpublished experiments, we found some irregularity in the induction of idiotope enhancement in C57BL/6 mice, whereas enhancement was regularly found in (C57BL/6 × CBA)F_1 animals. In Fig. 1 we depict the results of an experiment in which (C57BL/6 × CBA)F_1 mice were pretreated with 10 ng of three antiidiotope antibodies, Ac146 ($IgG1^j$), A39-40 ($IgG1^b$), and A6-24 ($IgG2a^b$), and immunized 6 wk later with NP-CG. It is obvious that in all three cases the expression of the target idiotope was enhanced 5–10-fold as compared with the controls. In each case, not only the expression of the target idiotope, but also that of all other B1-8 idiotopes tested was enhanced, whereas the total anti-NP response was unaffected. This accords with previous data (17, 19). It is thus clear that enhancement of idiotope expression can be mediated by IgG1 antiidiotopes of the j and b allotype and by an $IgG2a^b$

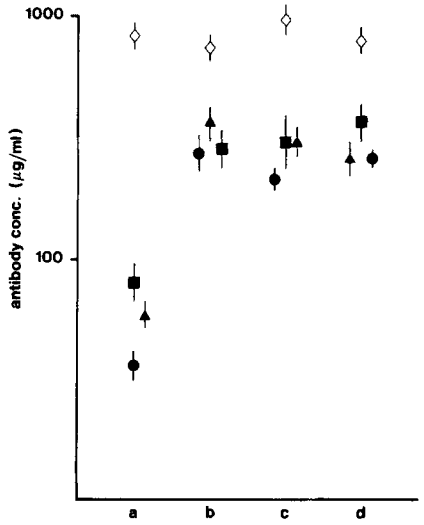

FIGURE 1. Enhancement of idiotope expression in (C57BL/6 × CBA)F_1 mice. Mice were injected with diluent (*a*) or 10 ng of the antiidiotopes Ac146 (*b*), A39-40 (*c*), or A6-24 (*d*) 6 wk before standard immunization with NP-CG. The animals were bled 12 d after immunization and the concentration of λ1-bearing antibodies (ordinate) that bound NIP (◇) or expressed idiotopes Ac146 (●), A39-40 (▲), or A6-24 (■) were determined by RIA. Each experimental point represents the geometric mean with standard deviations of antibody titers determined in the sera of five mice.

antiidiotope, in a situation where the allotype of the injected antibodies is also expressed on the Ig of the recipients.

In the experiments illustrated in Fig. 2, we induced enhancement with antiidiotopes differing from each other only in isotype, by using class switch variants of the Ac146 (Igh^j) and A39-40 (Igh^b) antiidiotope-secreting cell lines (11). The IgG1, IgG2b, and IgG2a antibodies of either variant family caused similar enhancement of the expression of the target idiotope. Enhancement was ~10-fold for idiotope Ac146 (Fig. 2.1) and ~5-fold for idiotope A39-40 (Fig. 2.2).

Microgram Doses of Antiidiotopes of the IgG1, IgG2b, and IgG2a Class Suppress Idiotope Expression. The phenomenon of idiotope suppression by microgram doses of antiidiotopes was regularly observed in both C57BL/6 and (C57BL/6 × CBA) F_1 mice. In Fig. 3 we depict the expression of idiotope Ac146 in C57BL/6 mice injected with the IgG1, IgG2b, or IgG2a antiidiotopes of the Ac146 family. Profound suppression of similar duration was induced by all three antiidiotope antibodies, although the curves obtained for each antibody differ somewhat from each other. Similarly, injection of 10 μg of the IgG1, IgG2b, and IgG2a antibodies of the A39-40 family 4 wk before immunization with NP suppressed the expression of idiotope A39-40 to the same extent (Fig. 4). We have also treated C57BL/6 mice with a 1 μg dose of the various IgG antibodies of the Ac146 or the A39-40 family. Again the result was similar in all cases in

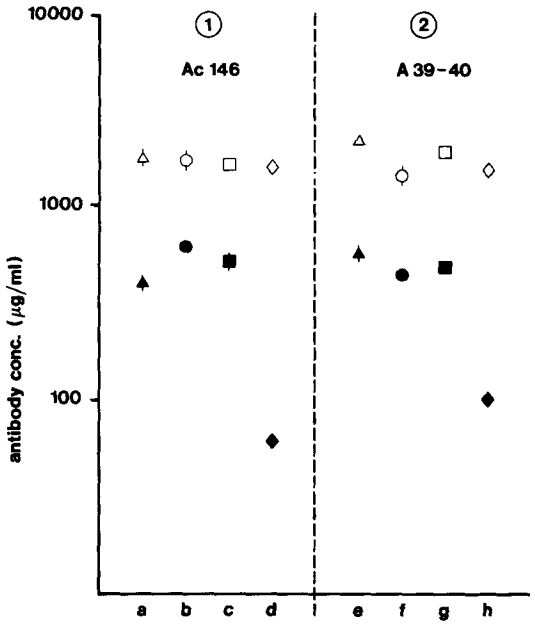

FIGURE 2. Enhancement of the expression of idiotopes Ac146 (*1*) and A39-40 (*2*) by injection of 10 ng IgG1 (*a, e*), IgG2b (*b, f*), or IgG2a (*c, g*) antibodies of the Ac146 (*1*) or A39-40 family (*2*) in (C57BL/6 × CBA)F_1 mice. Lanes *d* and *f* represent control responses. For experimental details see legend to Fig. 1. The ordinate gives the concentration of λ1-bearing antibodies that bind NIP (open symbols) or express idiotope Ac146 or A39-40 (closed symbols). Each experimental point represents the geometric mean with standard deviations of antibody titers determined in the sera of five mice. In cases where the deviation is not indicated, the symbols are bigger than the deviation.

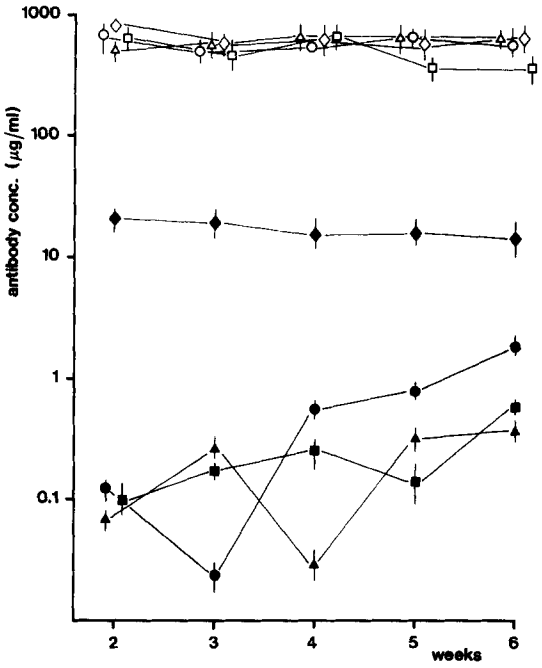

FIGURE 3. Suppression of idiotope Ac146 by the antiidiotope antibodies of the Ac146 family. C57BL/6 mice were injected with 10 μg IgG1 (▲), IgG2b (●), or IgG2a (■) antibody of the Ac146 family or with diluent (♦) at various times (abscissa) before standard immunization with NP-CG. The animals were bled 12 d after immunization and the concentration of λ1-bearing antibodies (ordinate) that bound NIP (open symbols) or expressed idiotope Ac146 (closed symbols) were determined by RIA. Each experimental point represents the geometric mean with standard deviations of antibody titers determined in the sera of five mice.

that marginal (two to threefold) suppression of the target idiotopes were obtained (data not shown).

In the above experiments the effects of the antiidiotopes were monitored at the level of total λ1-bearing serum antibodies. To extend our analysis, we investigated the isotype distribution of the antibodies expressing idiotope Ac146 in mice pretreated with 10 μg IgG1 or IgG2b antiidiotopes of the Ac146 family, 4 wk before immunization with NP-CG. In accord with previous results (5, 17), the antiidiotopes affected almost exclusively the expression of IgG1 and IgG2b antibodies, while antibodies of the IgM class were only marginally (two to threefold) reduced. Idiotope Ac146-expressing antibodies of the IgG2a class were below the limit of detection in control and suppressed mice (data not shown).

Antiidiotopes of the IgE Class Enhance and Suppress Idiotope Expression like IgG Antiidiotopes. The isolation of an IgE-expressing class switch variant in the Ac146 family allowed us also to compare antiidiotope antibodies of the IgE and IgG class with respect to their regulatory effects on idiotope expression. The data in Fig. 5 show that both the IgE and the IgG1 antiidiotopes of the Ac146 family enhanced expression of idiotope Ac146 ~10-fold and suppressed it ~100-fold. Again, the regulatory effects were dependent on the dose and not the

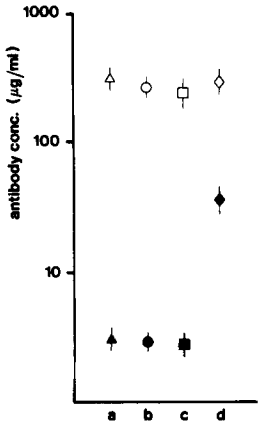

FIGURE 4. Suppression of idiotope A39-40 by the antiidiotope antibodies of the A39-40 family. C57BL/6 mice were injected with 10 μg IgG1 (*a*), IgG2b (*b*), or IgG2a (*c*) antibody of the A39-40 family or with diluent (*d*) 4 wk before immunization with NP-CG. The animals were bled 12 d after immunization. The ordinate gives the concentration of λ1-bearing antibodies that bound to NIP (open symbols) or expressed idiotope A39-40 (closed symbols). Each experimental plot represents the geometric mean with standard deviations of antibody titers determined in the sera of five mice.

isotype of the injected antiidiotope. Idiotope A25-9 was suppressed to the same extent as idiotope Ac146 by injection of antiidiotope Ac146 of the IgG1 class, while idiotopes A39-40 and Ac38 were suppressed only about twofold (Fig. 5*A*). The effect of a suppressive dose of the IgE antiidiotope on the expression of nonhomologous idiotopes was not analyzed. In the case of enhancement, however, both antiidiotopes co-enhanced the expression of the nonhomolgous idiotopes A25-9, Ac38, and A39-40 (Fig. 5*B*)

Idiotopes Appear to Differ in their Sensitivity to Antiidiotopic Regulation. The extent of suppression caused by 10 μg of the antiidiotopes of the Ac146 and the A39-40 families differed drastically. While the antiidiotopes of the Ac146 family induced a 100-fold suppression of idiotope Ac146 (Fig. 3), the antiidiotopes of the A39-40 family suppressed idiotope A39-40 only 5–10-fold (Figs. 4 and 6). However, when the titers of other B1-8 idiotopes in the latter sera were examined (Fig. 6), idiotopes Ac146 and A25-9 were found to be suppressed by more than two orders of magnitude, much more than the target idiotope A39-40 itself. In fact, the A39-40 antibody suppresses idiotope Ac146 as efficiently as the homologous antiidiotope, antibody Ac146.

In a certain way, a similar effect had been seen in the sera of mice pretreated with enhancing doses of antiidiotope: antiidiotope A39-40 enhanced the expression of idiotope Ac146 as well as that of the homologous idiotope A39-40 (Fig. 1). However, in contrast to the situation in suppressed mice, enhancement appeared always to affect the expression of *all* B1-8 idiotopes tested (Figs. 1 and 5). Because of these puzzling results, we investigated the idiotopic makeup of anti-NP antibodies in control and idiotypically manipulated animals in more detail.

Target of Regulation by Antiidiotope Antibodies. We know from previous experiments that certain B1-8 idiotopes can be found expressed together (as on antibody

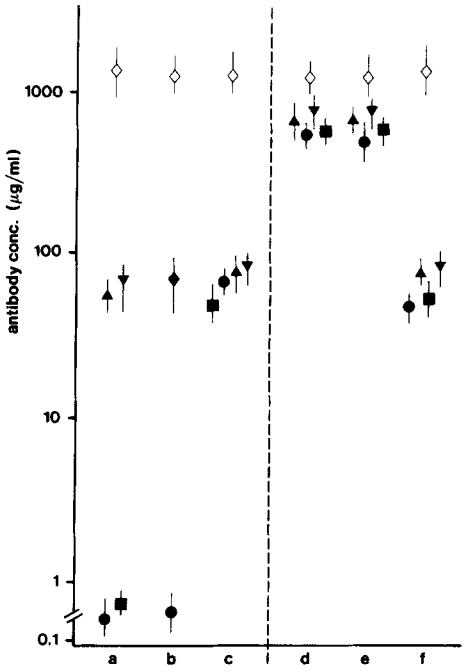

FIGURE 5. Idiotope regulation by antiidiotope antibodies of the IgE class. In one experiment, (C57BL/6 × CBA)F_1 mice were injected with 10 μg IgG1 (*a*) or IgE (*b*) antiidiotope antibody of the Ac146 family, or with diluent (*c*) 4 wk before immunization with NP-CG. In a second experiment, the animals received 10 ng IgG1 (*d*) or IgE (*e*) antiidiotope or diluent (*f*) 6 wk before immunization with NP-CG. The animals were bled 12 d after immunization and the concentrations of λ1-bearing antibodies (ordinate) that bound to NIP (◇) or expressed idiotopes Ac146 (●), A25-9 (■), Ac38 (▼), or A39-40 (▲) were determined by RIA. Experimental points represent geometric means with standard deviations of antibody titers determined in the sera of five mice.

B1-8) or independently of each other on the V regions of antibodies (6, 7). We extended this analysis and absorbed sera from control mice, immunized with NP-CG, on immunosorbents coupled with the antiidiotopes A39-40, Ac38, Ac146, or A25-9. After absorption, the sera were assayed for residual idiotope-expressing antibodies. In Table I, we list the percentages of idiotope-bearing antibodies that could be absorbed by the various immunosorbents. We had found in previous experiments that antibodies bearing idiotope Ac146 mostly coexpress idiotopes Ac38 and A6-24 (7). The data in Table I confirm the former result and show in addition that Ac146-positive antibodies also express idiotope Ac39-40. Furthermore, idiotope A25-9 is also mostly (80–90%) molecularly associated with idiotope Ac146 and to >90% included in A39-40-bearing antibodies. Since, in addition, absorption with antibody Ac146 or A25-9 removes roughly half (35–60%) of the Ac38- or A39-40-bearing antibodies, and the latter two idiotopes are molecularly associated to ~50%, we can define three almost equally sized sets of anti-NP antibodies bearing B1-8 idiotopes: antibodies bearing idiotopes Ac38, A39-40, A6-24, Ac146, and, the majority (70–90%), A25-9 (subset *a*); antibodies bearing idiotope Ac38 but lacking A39-40, Ac146, and A25-9 (subset

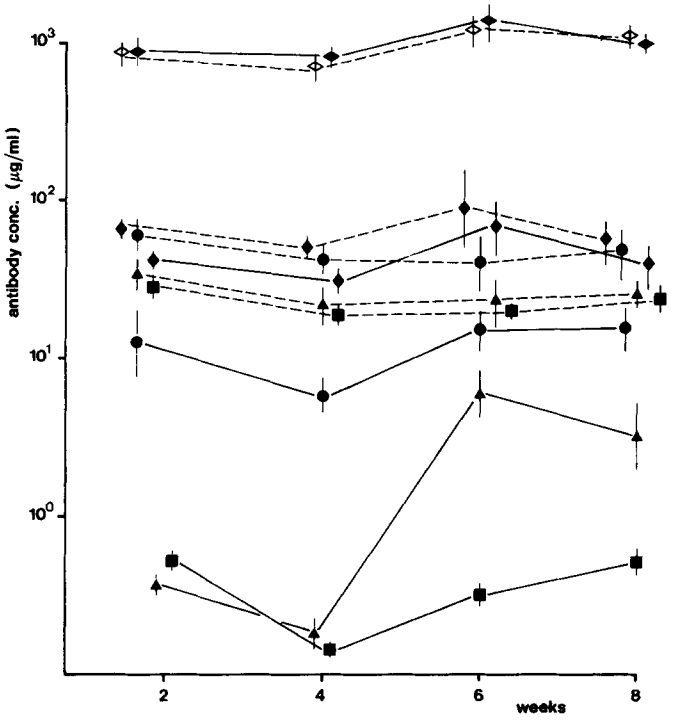

FIGURE 6. Expression of idiotopes A39-40, A25-9, Ac146, and Ac38 in mice suppressed with antiidiotope A39-40. C57BL/6 mice were injected with 10 μg antiidiotope antibody A39-40 of the IgG1 class (unbroken lines) or diluent (broken lines) and immunized with NP-CG at various times (abscissa) after this treatment. The concentrations of λ1-bearing antibodies (ordinate) that bound NIP (♦) or expressed antiidiotopes A39-40 (●), A25-9 (■), Ac146 (▲), or Ac38 (◆) were determined by RIA 12 d after immunization. Experimental points represent geometric means with standard deviations of the antibody titers determined in the sera of five mice.

b); and antibodies bearing idiotope A39-40 but lacking Ac38, Ac146, and A25-9 (subset *c*) (20).

The results of absorption experiments using sera from mice in which idiotope expression had been enhanced by nanogram doses of antiidiotope show that enhancement is essentially restricted to antibodies of subset *a* (Table I). This is demonstrated by the ability of any of the four antiidiotopes A39-40, Ac38, Ac146, and A25-9 to absorb from those sera most and in most cases essentially all material bearing any of the four corresponding idiotopes, in striking contrast to what was found in control anti-NP sera. In one case, a fifth idiotope, A6-24, also characteristic for subset *a*, was included in the analysis and found to behave like the four other idiotopes.

Looking back at the suppression experiments depicted in Figs. 5 and 6 it seems that also there the antibodies of subset *a* are the main target of regulation. This is particularly strongly suggested by the data obtained with antibody A39-40, which profoundly suppresses idiotopes Ac146 and A25-9, whereas suppression of the target idiotope A39-40 is only marginal (Fig. 6). The interpretation that microgram doses of antibody A39-40 selectively suppress the expression of subset

TABLE I

Linkage of Idiotopes in λ_1-Bearing Anti-NP Antibody Populations

Mice injected with:*	Step 2: Detecting antibodies	Step 1: Absorbing antibodies			
		A39-40	Ac38	Ac146	A25-9
		%			
Diluent‡	A39-40	>99§ >99 >99	52	41, 39, 36, 40	40, 35
	Ac38	51, 50	>99	57, 60	52, 49, 40
	Ac146	>99, >99, >99	>99	>99, >99, >99, >99	91, 83, 76
	A25-9	92, 96	71	80, 90, 83	>99, >99, >99
	A6-24	ND	ND	84	74
10 ng Ac146‡	A39-40	>99	99	99	99
	Ac38	97	>99	95	94
	Ac146	>99	>99	>99	>99
	A25-9	>99	>99	99	>99
10 ng A39-40‡	A39-40	ND	ND	95	97
	Ac38	ND	ND	80	80
	Ac146	ND	ND	>99	99
	A25-9	ND	ND	99	>99
	A6-24	ND	ND	>99	>99

* Pooled sera of five to six mice were analyzed.

‡ Given 6 wk before immunization with NP-CG.

§ Percent absorption, corrected for nonspecific absorption on X63-coupled Sepharose. ND, not done.

a anti-NP antibodies was verified by the absorption experiments depicted in Fig. 7.

In mice injected with the antiidiotope 4 wk before immunization, antibodies expressing idiotopes Ac146 and A25-9 (subset *a*) are hardly detectable. However, antibodies expressing idiotope A39-40 are again only slightly reduced, in accord with the data in Fig. 6. One can calculate from the data in Fig. 6 and Table I that antibodies expressing idiotope A39-40 but lacking the Ac146 and A25-9 determinants (subset *c*) are only reduced 3.5-fold at this point in time and represent >90% of the total A39-40-bearing population, in contrast to the 60% found in controls (Table I and Fig. 7). In the sera of mice injected with antiidiotope A39-40 6 wk before immunization with NP-CG, the titers of antibodies of subset *c* are only about twofold lower than in the controls. Antibodies of subset *a* have partially recovered from suppression, but recovery from suppression is more rapid for those subset *a* antibodies that lack idiotope A25-9. The preferential suppression of A25-9-bearing antibodies of subset *a*, suggested by the data in Fig. 6, is strikingly born out by the absorption experiments (Fig. 7). While in control sera, 80–90% of Ac146-bearing antibodies coexpress idiotope A25-9, only ~20% of Ac146-positive antibodies do so in anti-NP sera obtained 6 wk after suppression the A39-40 idiotope. Again, however, the A25-9 idiotope is largely confined to Ac146-positive antibodies (Fig. 7). These results demonstrate that even within the subset *a* antibodies, idiotypic phenotypes can be ordered on the basis of their sensitivity to idiotypic regulation: the more the antibodies idiotypically resemble antibody B1-8, the better they are suppressed.

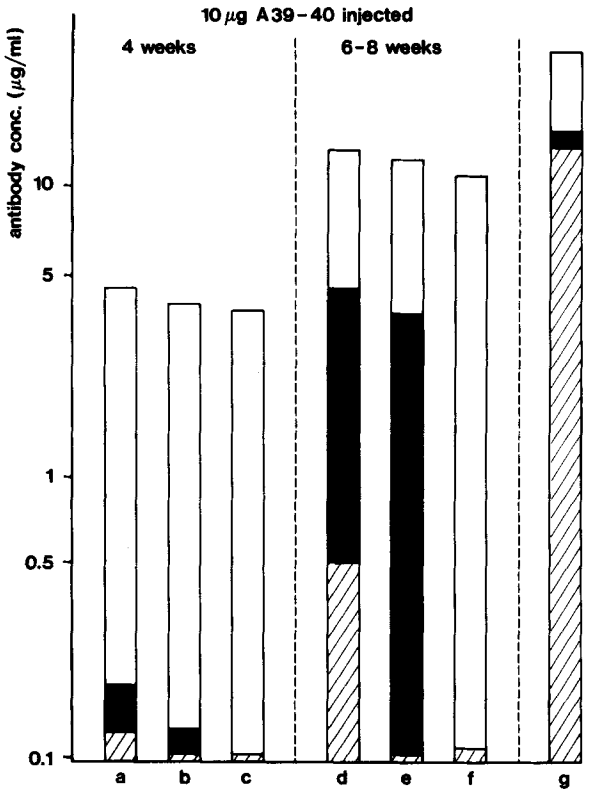

FIGURE 7. Linkage of idiotopes Ac146, A25-9, and A39-40 on serum antibodies of C57BL/6 mice suppressed with 10 μg of antiidiotope A39-40. Pooled sera of mice injected with IgG1 or IgG2b antiidiotopes of the A39-40 family 4 wk (*a–c*) or 6–8 wk (*d–f*) before standard immunization with NP-CG were absorbed twice on Sepharose coupled to X63 protein (*a*, *d*) or the antiidiotope antibodies A25-9 (*b*, *e*) and Ac146 (*c*, *f*). The concentrations of λ1-bearing antibodies (ordinate) expressing idiotopes Ac146 (■), A25-9 (▨), or A39-40 (□) were determined in the absorbed sera. Column *g* shows the concentrations of these idiotopes in control sera (from NP-CG-immunized mice preinjected with diluent) absorbed with X63 protein. For an analysis of idiotope linkage in such sera see Table I.

Discussion

Regulatory Properties of Antiidiotopes of Different Isotypes. The isolation of families of class switch variants from hybridoma cell lines secreting monoclonal antiidiotope antibodies (11) enabled us to compare the regulatory properties of antiidiotope antibodies that differ from each other only in heavy chain isotype, but share all other properties, including binding specificity. The cell lines Ac146 and A39-40, from which we selected isotype variants, secrete antibodies specific for idiotopes on the germline-encoded V region of antibody B1-8. Both antiidiotopes regulate the expression of their target idiotopes in a subsequent immune response against NP. The antiidiotope antibodies of the IgG1, IgG2b, IgG2a, and IgE class of the Ac146 or A39-40 families enhance or suppress the expression of their target idiotopes, depending on the dose but not the isotype of the injected antiidiotope. We thus conclude that for the function of an antiidiotope in

idiotypic regulation of the humoral response, it is irrelevant whether it expresses the IgG1, IgG2b, IgG2a, or IgE class.

In the course of the present experiments, we found an irregularity of the enhancing phenomenon in C57BL/6 but not in (C57BL/6 × CBA)F_1 mice. The expression of idiotope-bearing antibodies in idiotypically manipulated animals may depend on an interaction of suppressive and enhancing forces. In C57BL/6 mice injected with 1 μg antiidiotope antibody Ac38, the expression of the target idiotope is neither enhanced nor suppressed (17, 19). An analysis of the in vitro immune response to NP-Ficoll of spleen cells from these mice revealed that the removal of T cells bearing the Lyt-2 marker resulted in an enhanced expression of the target idiotope (G. Kelsoe, T. Takemori, and K. Rajewsky, unpublished data). The balance between enhancement and suppression may often be shifted towards suppression in the C57BL/6 mice in our colony. The variability of the enhancing phenomenon in C57BL/6 mice allows a reinterpretation of previous results pointing to isotype-dependent regulation. In these experiments, antiidiotope A6-24 ($IgG2a^b$), in contrast to antiidiotope Ac38 ($IcG1^j$), did not enhance, but slightly suppressed the target idiotope at nanogram doses in the C57BL/6 strain (5). In unpublished experiments we have reproduced this result. However, antiidiotope A6-24 enhances its target idiotope in (C57BL/6 × CBA)F_1 mice as well as any other antiidiotope so far tested (Fig. 1).

Our results accord with data from the T15 system (21), indicating that antiidiotypic antibodies of the IgG3, IgG1, IgG2b, and IgG2a classes of a conventional antiidiotypic serum suppressed an immune response against phosphorylcholine to the same extent in vitro. However, distinct effector functions in idiotypic regulation have been assigned to the isotypes of antiidiotypic antibodies in the A5A system. Guinea pig antiidiotypic antibodies of the IgG1 class enhanced or suppressed A5A idiotype expression in A/J mice in a dose-dependent fashion, while IgG2 antibodies of the same origin were suppressive at all doses (2–4). The discrepancy between these and the present results could have several reasons. First, the different isotypes of guinea pig IgG antibodies may indeed induce different regulatory mechanisms in the mouse. As long as murine antibodies of different isotypes do not have the same effects, the physiological significance of such a phenomenon would remain in doubt, in particular since IgG1 and IgG2 antibodies are distinguished from each other in a similar way in both mice and guinea pigs in terms of their effector functions in complement activation and Fc receptor binding (22). Alternatively, as the guinea pig anti-A5A sera contained presumably a variety of antiidiotope specificities, antisera of different idiotope-binding specificity and affinity might have been generated by separating the two isotypes. Although we are unable to spell out the possible regulatory consequences of such a situation in terms of a mechanism, the question remains whether different distributions of idiotype-binding specificities might have been responsible for the distinct regulatory functions of the guinea pig IgG1 and IgG2 antibodies, rather than the two isotypes themselves.

However, a few words about the limitations of our analysis should be added. Thus, we have not investigated the cellular basis of idiotype suppression and enhancement for the various antiidiotope isotypes, except that of suppression by IgG1 antibodies (1, 23; T. Takemori and K. Rajewsky, submitted for publica-

tion). Although suppressor T cells have been identified in the latter case, in contrast to the results with guinea pig IgG1 antibodies, it remains a possibility that the regulatory mechanisms induced by antiidiotopes of different isotype differ from each other in detail. Furthermore, we have not yet excluded the possibility that antiidiotope antibodies of isotypes other than IgG1, IgG2b, IgG2a, and IgE may have regulatory properties other than antibodies of the former isotypes. Antiidiotopes of the IgM, IgD, IgA, and IgG3 class are not available in our system.

The Target of Idiotypic Regulation. As an unexpected result, we found that both enhancement and suppression by antiidiotope antibodies was predominantly expressed in a certain subset (subset *a*) of anti-NP antibodies bearing the target idiotope, namely antibodies coexpressing with the target idiotope other idiotopes of antibody B1-8 against which the antiidiotope antibodies had originally been raised. The present data show that this subset is selectively regulated by two different antiidiotopes (Ac146 and A39-40) and suggest that this is also true for a third one, antibody A6-24 (Fig. 1). In a separate study (T. Takemori and K. Rajewsky, submitted for publication; see also reference 1.) it was shown that neonatal suppression induced by yet another antiidiotope, antibody Ac38, was again predominantly expressed in subset *a* antibodies. The fact that idiotypic regulation of the expression of a set of antibody V regions can be induced by antiidiotope antibodies recognizing any of the idiotopes shared by those V regions supports the view (24) that the V regions themselves are the primary target of the injected antiidiotope. The interaction of the antiidiotope antibody with the target V regions would initiate the chain of events leading in the end to idiotypic suppression or enhancement (20). A similar conclusion was reached by Cerny et al. (25) in their study of idiotypic suppression in the T15 system. In our experimental system, as in many others (see reference 1 for review), T cells are involved in the regulatory process at least in the case of suppression (23), and recent studies indicate that these T cells indeed specifically regulate the expression of subset *a* antibodies (1; T. Takemori, and K. Rajewsky, submitted for publication).

Ongoing work aims to elucidate the peculiar specificity of idiotypic regulation observed in our experiments. Why are subset *a* antibodies preferentially enhanced or suppressed by antiidiotypic manipulation? One can imagine that antibody-mediated idiotypic regulation exhibits a high degree of selectivity, based on the affinity of the antiidiotope for its idiotypic target. Thus, antibodies raised against B1-8 idiotopes may best fit to V regions whose overall shape resembles closely that of antibody B1-8, and this may define anti-NP antibodies of subset *a*. This simple view is not supported by experimental evidence demonstrating a similar affinity of antibody Ac38 for its original target (antibody B1-8) and for Ac38-binding antibodies in anti-NP sera, irrespective of whether they coexpress (subset *a*) or lack (subset *b*) idiotope Ac146 (T. Takemori and K. Rajewsky, submitted for publication). One might therefore consider an alternative model, namely that the regulatory T cells like to see B1-8-like molecules in anti-NP responses, possibly because of peculiarities of the T cell receptor repertoire. These considerations lead us into the problem of the control of the antibody repertoire by T cells. One might wonder whether such a control has played a

role in the generation of the plasma cell that was immortalized in the B1-8 hybridoma.

Summary

Previous work has shown that the injection of antiidiotope antibodies specific for idiotopes of the germline-encoded anti-(4-hydroxy-3-nitro-phenyl) acetyl (NP) antibody B1-8 enhanced or suppressed the expression of B1-8 idiotopes in subsequent humoral anti-NP responses, depending on the dose and perhaps also the isotype of the injected antibody. To formally answer the question of whether the isotype of an antiidiotope determines its effector function in this type of idiotypic control, we have performed regulatory experiments with isotype switch variants selected from two hybridomas secreting anti-B1-8 idiotopes of CBA (Igh^j) and C57BL/6 (Igh^b) origin. The antibodies of each variant family differ from each other only in the constant region of the heavy chain. The results show that, irrespective of whether an antiidiotope antibody belongs to the IgG1, IgG2b, IgG2a, or IgE class, a 10-ng dose enhances idiotope expression whereas a dose of 10 μg exerts a suppressive effect.

It emerges from the present and parallel data that the expression of antibody V regions resembling idiotypically that of antibody B1-8 can be enhanced and suppressed by any of four antiidiotope antibodies that recognize distinct idiotopes on those V regions. This suggests that the initial step in the regulatory process induced by an antiidiotope is its binding to antibody V regions carrying the target idiotope. The antiidiotopes preferentially regulate the expression of antibodies that coexpress with the target idiotope other B1-8 idiotopes, despite the fact that some B1-8 idiotopes are also expressed independently of each other in anti-NP responses of idiotypically unmanipulated mice. This finding may reflect high affinity binding of the antiidiotopes to the target against which they were originally raised (i.e., antibody B1-8) or, more likely, a preferential recognition of B1-8-like V regions by regulatory T cells.

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References

1. Rajewsky, K., and T. Takemori. 1983. Genetics, expression and function of idiotypes. *Annu. Rev. Immunol* 1:569.
2. Eichmann, K. 1974. Idiotype suppression. I. Influence of the dose and effector functions of anti-idiotypic antibody on the production of an idiotype. *Eur. J. Immunol.* 4:296.
3. Eichmann, K. 1975. Idiotype suppression. II. Amplification of a suppressor T cell with anti-idiotypic activity. *Eur. J. Immunol.* 5:511.
4. Eichmann, K., and K. Rajewsky. 1975. Induction of T and B cell immunity by anti-idiotypic antibody. *Eur. J. Immunol.* 5:661.
5. Reth, M., G. Kelsoe, and K. Rajewsky. 1981. Idiotopic regulation by isologous monoclonal anti-idiotope antibodies. *Nature (Lond.).* 290:257.
6. Reth, M., T. Imanishi-Kari, and K. Rajewsky. 1979. Analysis of the repertoire of

anti-(4-hydroxy-3-nitro-phenyl)acetyl (NP) antibodies in C57BL/6 mice by cell fusion. II. Characterization of idiotopes by monoclonal anti-idiotope antibodies. *Eur. J. Immunol.* 9:1004.

7. Rajewsky, K., T. Takemori, and M. Reth. 1981. Analysis and regulation of V gene expression by monoclonal antibodies. *In* Monoclonal Antibodies and T Cell Hybridomas—Perspectives and Technical Advances. G. J. Hämmerling, U. Hämmerling, and J. F. Kearney, editors. Elsevier/North-Holland Biomedical Press, Amsterdam. 399–408.
8. Reth, M., G. J. Hämmerling, and K. Rajewsky. 1978. Analysis of the repertoire of anti-NP antibodies in C57BL/6 mice by cell fusion. I. Characterization of antibody families in the primary and hyperimmune response. *Eur. J. Immunol.* 8:393.
9. Bothwell, A. L. M., M. Paskind, M. Reth, T. Imanishi-Kari, K. Rajewsky, and D. Baltimore. 1981. Heavy chain variable region contribution to the NP^b family of antibodies: somatic mutation event in a γ 2a variable region. *Cell.* 24:625.
10. Bothwell, A. L. M., M. Paskind, M. Reth, T. Imanishi-Kari, K. Rajewsky, and D. Baltimore. 1982. Somatic variants of murine light chains. *Nature (Lond.).* 298:380.
11. Müller, C. E., and K. Rajewsky. 1983. Isolation of immunoglobulin class switch variants from hybridoma lines secreting anti-idiotope antibodies by sequential sublining. *J. Immunol.* 131:877.
12. Potter, M. 1972. Immunoglobulin-producing tumors and myeloma proteins of mice. *Physiol. Rev.* 52:631.
13. Liesegang, B., A. Radbruch, and K. Rajewsky. 1978. Isolation of myeloma variants with predefined variant surface immunoglobulin by cell sorting. *Proc. Natl. Acad. Sci. USA.* 75:3901.
14. March, S. C., I. Parikh, and P. Cuatrecasas. 1974. A simplified method for cyanogen bromide activation of agarose for affinity chromatography. *Anal. Biochem.* 60:149.
15. Takemori, T., H. Tesch, M. Reth, and K. Rajewsky. 1982. The immune response against anti-idiotope antibodies. I. Induction of idiotope-bearing antibodies and analysis of the idiotope repertoire. *Eur. J. Immunol.* 12:1040.
16. Imanishi, T., and O. Mäkelä. 1973. Strain differences in the fine specificity of mouse anti-hapten antibodies. *Eur. J. Immunol.* 3:323.
17. Kelsoe, G., M. Reth, and K. Rajewsky. 1981. Control of idiotope expression by monoclonal anti-idiotope and idiotope-bearing antibodies. *Eur. J. Immunol.* 11:418.
18. Grützmann, R. 1981. Vergleichende idiotypische Analyse von Rezeptoren mit Spezifität für Histokompatibilitätsantigene. PhD Thesis. University of Cologne, FRG. p. 74.
19. Kelsoe, G., M. Reth, and K. Rajewsky. 1980. Control of idiotope expression by monoclonal and anti-idiotype antibodies. *Immunol. Rev.* 52:75.
20. Rajewsky, K., T. Takemori, and C. E. Müller. 1984. Self tolerance through idiotype suppression. *In* Progress in Immunology, Vol. V. Y. Yamamura and T. Tada, editors. Academic Press Japan, Inc., In press.
21. Köhler, H., B. C. Richardson, D. A. Rowley, and S. Smyk. 1977. Immune response to phosphorylcholine. III. Requirement of the Fc portion and equal effectiveness of IgG subclasses in anti-receptor antibody-induced suppression. *J. Immunol.* 119:1979.
22. Winkelhake, J. L. 1978. Immunoglobulin structure and effector functions. *Immunochemistry.* 15:695.
23. Kelsoe, G., T. Takemori, M. Reth, and K. Rajewsky. 1981. Generation of specific regulatory T cells with monoclonal anti-idiotope antibody: induction of suppressor T cells. *In* B Lymphocytes in the Immune Response: Functional, Developmental, and Interactive Properties. N. R. Klinman, D. Mosier, I. Sher, and E. Vitetta, editors. Elsevier North-Holland, New York. 423.

24. Rajewsky, K., M. Reth, T. Takemori, and G. Kelsoe. 1981. A glimpse into the inner life of the immune system. *In* The Immune System, Vol. 2. C. M. Steinberg and I. Lefkovits, editors. S. Karger AG, Basel, Switzerland. 1.
25. Cerny, J., R. Cronkhite, and C. Heusser. 1983. Antibody response of mice following neonatal treatment with a monoclonal anti-receptor antibody. Evidence for B cell tolerance and T suppressor cells specific for different idiotopic determinants. *Eur. J. Immunol.* 13:244.