INDUCTION OF A CROSS-REACTIVE IDIOTYPE DEXTRAN-POSITIVE ANTIBODY RESPONSE IN TWO Igh-C^b MOUSE STRAINS TREATED WITH ANTI-J558 CROSS-REACTIVE IDIOTYPE ANTIBODIES*

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It has been well established that the expression of a particular idiotype (Id)[1] on antibodies of a given specificity is often linked to the Igh-C gene complex that determines the allotype markers of the Ig heavy chain constant region (1). This has been particularly well documented in the anti-α(1–3) dextran (Dex) system which in fact serves as a model reference system for this rule (2, 3). Previous studies have thus indicated that BALB/c (Igh-C^a) mice immunized with Dex B1355 [35% α(1–3), 8% α(1–4), and 57% α(1–6)] (4) respond with λ_1-bearing antibodies directed almost exclusively against the α(1–3) linkage (2). As much as 50% of these antibodies express the cross-reactive idiotype (IdX) (5) that has been defined with reference to α(1–3) Dex-binding BALB/c myeloma and hybridoma proteins (6). The structural correlates of this Id have been recently defined by a comparison of the amino-acid sequence of the heavy chain variable region (V_H) of a panel of IdX-positive and IdX-negative α(1–3) Dex-binding myeloma and hybridoma proteins from BALB/c mice (6). These elegant studies have identified two amino acids in positions 54 and 55 of the second V_H hypervariable region which determine IdX expression.

The strict linkage of IdX expression with the Igh-C^a allotype marker has been demonstrated by the incapacity of mice of other heavy chain linkage groups to synthesize anti-α(1–3) Dex antibodies expressing this Id (2, 3). For example, C57Bl/6 (Igh-C^b) mice do not respond with detectable amounts of such antibodies. Their anti-α(1–3) Dex antibodies bear the κ chain in the primary response to B1355, and although they switch to λ_1-bearing antibodies when hyperimmunized, these antibodies still do not express IdX (7). It has been proposed that this immune status reflects an absence of the structural V_H^{Dex} gene in these mice (7). In contrast, this gene would be present in all Igh^a haplotype strains, where it codes for IdX.

* Supported by a grant from the Centre de la Recherche Scientifique, A.T.P. No. 60-80-846.

[1] *Abbreviations used in this paper:* Ab1, BALB/c anti-α(1–3) Dex antibodies; Ab1′, IdX-positive anti-α(1–3) Dex antibodies induced by Ab2 antibodies; Ab2, anti-IdX 558 antibodies; Ab3, IdX-positive Ig having no measurable anti-α(1–3) Dex-binding activity; Ar, *p*-azophenylarsonate; BSA, bovine serum albumin; CFA, complete Freund's adjuvant; Con A, concanavalin A; Dex, dextran; IBC_{50}, idiotype-binding capacity; Id, idiotype; IdX, cross-reactive idiotype; KLH, keyhole limpet hemocyanin; M21, MOPC-21; M104E, MOPC-104E; M173, MOPC-173; M245, MOPC-245; NRabIgG, normal rabbit IgG; PC, phosphorylcholine; RIA, radioimmunoassay; V_H, variable region of Ig heavy chain.

Related studies in other idiotypic systems indicate however, that these relations may not always obey strict rules (8, 9). The nonexpression of gene products may only be apparent when they are probed by standard immunization procedures. Mechanisms based on Id recognition (10) may in some instances activate clones that otherwise remain silent or nonexpressed (11–14). In some idiotypic systems in the mouse, recent studies have demonstrated that after immunization by anti-Id it was possible to induce detectable amounts of Ig (13) or antibodies (11, 12) expressing a reference Id in animals that could not express this Id upon conventional immunization with antigen.

In the present study we tested this approach using as a model the α(1–3) Dex system. Our results indicate that substantial amounts of λ_1-bearing, IdX-positive Ig (Ab3), and α(1–3) Dex-binding antibodies (Ab1′) appear in C.B20 and C57Bl/6 (Igh-C^b) mice after treatment with rabbit anti-IdX antibodies (Ab2). Contrary to BALB/c mice immunized with Dex B1355, a significant fraction of this response was of the IgG1 class. The purified C.B20 Ab1′ antibodies differed however from the BALB/c antibodies (Ab1) in Dex-binding specificity, and the IdX-positive IgG1 antibodies expressed the $Igh4^b$ allotype determinants.

Materials and Methods

Mice. C57Bl/6 and C57Bl/10 (Igh-C^b) mice were obtained from the Centre de sélection des animaux de laboratoire, Orléans, France. The C.B20 (Igh-C^b, $H2^d$) congenic strain was obtained from Dr. M. Potter, National Cancer Institute, National Institutes of Health, Bethesda, MD. BALB/c (Igh-C^a, $H2^d$) and SJL/J (Igh-C^b) mice were obtained from our breeding facilities at the Institut de Recherche Scientifique sur le Cancer. BAB/14 (Igh-C^b) mice were provided by Dr. M. Seman (Institut Jacques Monod, Paris). Mice aged 12–15 wk were used in this study.

Antigens. Dex B1355 [35% α(1–3), 8% α(1–4), and 57% α(1–6)] (4) was obtained from Dr. Allene Jeanes (Northern Regional Research Laboratory, U. S. Department of Agriculture, Peoria, IL). Dex B512 [96% α(1–6), 4% α (1–3)] (4) was obtained from Pharmacia, Uppsala, Sweden. Dextrans B742L, B742S(C3R), B1501S, B1498S, B1255, B1375, and B1254S(L) were kindly donated by Dr. E. A. Kabat, College of Physicians and Surgeons, Columbia University NY. Panose [α(1–6), α(1–4)] was purchased from Sefochem Fine Chemicals, Paris, France. Escherichia coli, strain B was purchased from Sigma Chemical Co., St. Louis, MO, and concanavalin A (Con A) from Serva Feinbiochemica GmbH & Co., Heidelberg, Federal Republic of Germany (FRG).

Myeloma and Hybridoma Proteins. The α(1–3) Dex-specific MOPC-104E (M104E) and J558 myeloma proteins were maintained in our laboratory by subcutaneous transfer of BALB/c mice. The proteins were purified from ascites fluids by immunoabsorption on columns of Dex B1355-Sepharose as described previously (15). The following myeloma and hybridoma proteins were generous gifts from various sources.

The BALB/c α(1–3) Dex-binding hybridoma proteins Hdex1, Hdex3, Hdex8, Hdex12, and Hdex14 were obtained from Dr. B. Clevinger, Washington University School of Medicine, St. Louis, MO. The proteins were purified from ascites fluids on columns of B1355-Sepharose. Purified W3129, a BALB/c α(1–6) Dex-binding myeloma protein, was from Dr. M. Scharff, Albert Einstein College of Medicine, NY. The purified inulin-binding myeloma protein J606 was from Dr. M. Cohn, Salk Institute, San Diego, CA. Ascitic fluids containing the β(1–6) galactan-binding proteins X24, X44, and J539, the α(1–3) Dex-binding protein TEPC-1072, and the phosphorylcholine (PC)-binding protein HOPC-8 and TEPC-15 were from Dr. M. Potter. The PC-binding proteins were purified on a column of cytidine 5′-diphosphocholine coupled to AH-Sepharose as described (16). Purified (4-hydroxy-3-nitrophenyl)acetyl (NP)-binding hybridoma proteins B1-8 and S43 were from Dr. K. Rajewsky, Institut fur Genetik, Koln, FRG. Ascites fluids containing the *p*-azophenylarsonate (Ar)-binding hybridoma protein 36-65, 45-49, 44-1-3, and 31-65 were from Dr. M. N. Margolies, Massachusetts General Hospital, Boston, MA; the hybridoma proteins 91A3 and 93G7 were from Dr. J. D. Capra, University of

Texas, Dallas, TX, and hybridoma R16-7 was from Dr. A. Nisonoff, Brandeis University, Waltham, MA. These proteins were purified on columns of protein A-Sepharose. The pH of the eluting buffer was optimal for the respective classes of these proteins, as specified by Ey et al. (17). Purified UPC-10 was purchased from Litton Bionetics Inc., Kensington, MD. Ascites fluids containing the $Igh4^b$ myeloma protein MOPC-245 (M245) was from Dr. M. Potter. The protein was purified on a column of protein A-Sepharose (17).

Purification of Induced Anti-α(1-3) Dex Antibodies. Dextran B1355- or Ab2-induced antibodies were purified on mini-columns consisting of 1-ml capacity disposable plastic tips packed with 200–500 μl of B1355-Sepharose or B512-Sepharose gels. After passing through 0.2–1 ml of the test serum and washing until OD (280 nm) was ≤0.02, the bound antibodies were eluted with 0.2 N glycine buffer, pH 2.8, and collected into 2% bovine serum albumin, 0.05 M phosphate buffer (BSA-PO_4), pH 8.0. All procedures were carried out at 4°C.

Anti-IdX Antibodies. An anti-IdX reagent specific for the public Id shared by IdX-positive α(1–3) Dex-binding antibodies was prepared from the serum of rabbit 314 immunized with the J558 myeloma protein. Antiisotype antibodies were removed by absorption with the following myeloma proteins coupled to cyanogen bromide-activated Sepharose 4B: Fab MOPC-173 (M173) (γ_{2a}, κ); MOPC-21 (M21) (γ_1, κ); HOPC-1 (γ_{2a}, λ_1); HOPC-8 (α, κ); A2 (γ_1, κ). The purification of Fab M173, M21, HOPC-1, and A2 was as described elsewhere (14). The antibodies in the effluent serum were purified on a column of M104E-Sepharose. They were dialyzed against phosphate-buffered saline and concentrated to 3 mg/ml on Amicon CF50 membranes (Amicon Corp., Danvers, MA). Some batches of antibodies were reabsorbed on the HOPC-1 column to remove residual anti-λ_1 antibodies.

The idiotype-binding capacity (IBC_{50}) of these antibodies, as determined by the indirect precipitation method described by Kuettner et al. (18), was 0.77 IBC_{50}/μg of purified antibodies. Binding-site inhibition assays indicated 40% inhibition by Dex B1355 of the binding of ^{125}I-labeled J558 to microplates coated with these antibodies. Dex B512 or panose were not inhibitory.

Allogeneic anti-Id antibodies were prepared by immunizing C57Bl/6 mice with J558 myeloma protein coupled to the keyhole lympet hemocyanin (KLH) (19). The serum was first absorbed on a column of HOPC-8 (α, κ)-Sepharose to remove anti-$Igh2^a$ antibodies, and the antibodies were purified on a column of J558-Sepharose. 75% of the antibodies were directed to the J558 IdI, and 25% to the J558 IdX, as determined in an indirect hemagglutination assay (20) using J558- and M104E-coupled sheep erythrocytes. Binding-site inhibition assays gave 75% inhibition by Dex B1355. The IBC_{50} content was 1.86 IBC_{50}/μg of purified antibodies.

A syngeneic BALB/c anti-J558 serum was a gift of Dr. P. A. Cazenave, Institut Pasteur, Paris, France. The anti-J558 antibodies were purified on a column of J558-Sepharose.

Rabbit Anti-Mouse Heavy and Light Chain Reagents. Affinity-purified anti-κ, anti-Fcμ, anti-γ_1, and anti-γ_2 antibodies were obtained by previously described methods (15). Anti-λ_1 antibodies were purified from an anti-M104E (μ, λ_1) serum. The serum was preabsorbed on columns of Fab M173, M21, and TEPC-183 (μ, κ) coupled to Sepharose, and the antibodies were purified on a column of HOPC-1. Anti-γ_3 antibodies were obtained from an anti-J606 (γ_3, κ) serum preabsorbed on columns of Fab M173, M173, and M21, and the antibodies were purified on a column of FLOPC-21 (γ_3, κ). Anti-α antibodies were obtained from an anti-TEPC-15 (α, κ) serum preabsorbed on columns of Fab M173 and M21, and the antibodies were purified on a column of J558 (α, λ_1). The specificity of these reagents was verified in indirect radioimmune binding assays (18) using a panel of myeloma proteins representative of various Ig classes. Whenever required the purified reagents were reabsorbed on appropriate columns.

Immunization of Mice with Antiidiotype Antibodies. Anti-IdX J558 antibodies from rabbit 314 were coupled to KLH by means of glutaraldehyde according to Bona et al. (19). One group of five C.B20 mice was immunized with Ab2-KLH. The mice received a total of five injections, each containing 150 μg of Ab2-KLH, the first in complete Freund's adjuvant (CFA), the second in incomplete Freund's adjuvant, and the remaining three in saline. Two other groups of C.B20 mice, group 2 (five mice) and group 3 (eight mice), received only the first two injections of Ab2 antibodies, which were not coupled to KLH. The group 2 mice received 150 μg, and group 3 mice, 100 μg per injection. One group of five C57Bl/6 mice received two injections of 150 μg of Ab2-KLH. Equal numbers of control mice in each group were identically immunized with

normal rabbit IgG (NRabIgG) (Pentex Biochemicals, Kankakee, IL) absorbed on a column of J558-Sepharose. Lastly, one group of six C.B20 mice were immunized with two injections (80 μg) of allogeneic C57BL/6 anti-J558 antibodies coupled to KLH.

Radiolabeling. Myeloma proteins and antibodies were radiolabeled with ^{125}I according to the method of Greenwood and Hunter as modified by Cuatrecasas and Hollenberg (21). The specific radioactivity ranged between 0.5 and 3×10^6 cpm/μg.

Radioimmunoassay (RIA) for the IdX Idiotype. The IdX determinant was measured by a competitive inhibition RIA as described by Hansburg et al. (5). In brief, serial dilutions of test serum or affinity-purified antibodies were tested for their capacity to inhibit the binding of ^{125}I-labeled M104E (4 ng, ~4,000 cpm) to 96-well polyvinyl chloride microplates (Dynatech Laboratories, Inc., Alexandria, VA) coated overnight with affinity-purified anti-IdX 558 antibodies at 1.5 μg/ml in 10 μg/ml BSA-PO_4. After overnight incubation at 37°C, the microplates were washed and the radioactivity was estimated in a gamma counter. The use of the heterologous M104E protein with the anti-J558 reagent ensured that the assay measured the IdX determinant, since the two α(1–3) Dex-binding myeloma proteins share this public determinant but differ in their respective IdI determinants (6). The amount of IdX was estimated with reference to a standard inhibition curve constructed with unlabeled M104E. A maximum of 60–70% of added counts were bound to the wells, with background binding ~0.5% of added counts.

The IdX assay using the syngeneic anti-J558 antibodies was carried out by the competitive RIA described by Bosma et al. (22). This involved precoating the wells with M104E (0.25 μg/ml in BSA-PO_4), followed by incubation with anti-J558 antibodies (1.5 μg/ml in 0.5% BSA, pH 8.0). The remainder of the assay was performed as described above.

RIA for the $Igh4^b$ and $Igh4^a$ Allotype. $Igh4^b$ allotype determinants were estimated using a competitive RIA described by Bosma et al. (22). Microplates were coated with 10 μg/ml of M245 ($Igh4^b$) in BSA-PO_4 followed by a 3 d incubation with a 1:40 dilution of a BALB/c anti-C57Bl/6 Ig serum (a gift of Dr. M. Bosma, Fox Chase Institute, Philadelphia, PA). Radiolabeled M245 (2.5 ng, ~25,000 cpm) was added together with dilutions of test sera. A standard curve was constructed with unlabeled M245. The microplates were incubated overnight at 37°C, washed, and the radioactivity was estimated.

For $Igh4^a$ allotype determinants, microplates were coated overnight with specifically purified C57Bl/6 anti-BALB/c $Igh4^a$ antibodies (6 μg/ml in BSA-PO_4). Myeloma protein M21 ($Igh4^a$) was used as the standard and radiolabeled ligand (4 ng, ~4,000 cpm). From here on, the assay was carried out as above.

RIA for Anti-α(1–3) and Anti-α(1–6) Dex Antibodies. The titer of these antibodies was estimated by a solid-phase binding assay on 96-well microplates as described in detail by Geckeler et al. (23). For anti-α(1–3) determination, serial dilutions of sera or purified antibodies and the M104E myeloma protein standard (concentration range, 0.31–25 ng/100 μl) were incubated in duplicate (18 h, 24°C) on plates coated with 200 μg/ml of Dex B1355. This was followed by a 6-h incubation at 37°C with a predetermined saturating dose of ^{125}I-labeled anti-λ_1 antibodies (15 ng/100 μl, ~30,000 cpm). Independent experiments using ^{125}I-labeled M104E or J558 indicated that ~75% of the added counts were bound to the B1355-coated plates.

For anti-α(1–6) determination, B512-coated plates were used, and the standard was the W3129 (anti-α(1–6) Dex myeloma protein). In this case, ^{125}I-labeled anti-κ antibodies were used in place of the anti-λ_1 reagent.

The values were read on the linear portion of the standard binding curves and were expressed as μg/ml of anti-Dex antibodies. Threshold detectability of antibodies in the two assays was 0.5 μg/ml of serum. Background binding (omission of the sera or Dex) was ~2% of the added radiolabeled anti-light chain antibodies and was substracted from all values.

RIA for Class Composition of Purified Anti-Dex Antibodies. Whereas the preceding assay was designed to measure the total amount of either λ_1-bearing or κ-bearing anti-Dex antibodies, the present assay was designed to determine the relative heavy chain class representation of these antibodies. This assay was essentially similar to that above, except that the antibodies bound to the Dex-coated plates were detected using an excess of ^{125}I-labeled heavy chain class-specific reagents. The percentage of each antiisotype bound was calculated from the following formula: [(nanograms ^{125}I-antiisotype bound per milliliter of antibodies − background)/(nanograms ^{125}I-

anti-($\gamma_1 + \gamma_2 + \gamma_3 + \alpha + \mu$) bound per milliliter of antibodies − background)] × 100. The percentage of κ-bearing anti-Dex antibodies was calculated as follows: [(nanograms ^{125}I-anti-κ bound per milliliter of antibodies − background)/(nanograms ^{125}I-anti-($\kappa + \lambda_1$) bound per milliliter of antibodies − background)] × 100. The percentage of λ_1-bearing anti-Dex antibodies was calculated as follows: [(nanograms ^{125}I-anti-λ_1 bound per milliliter of antibodies − background)/(nanograms ^{125}I-anti-($\kappa + \lambda_1$) bound per milliliter of antibodies − background)] × 100.

RIA for Class Composition of Ab3 Ig. This was carried out using a microplate-binding assay as described above for the class composition of antibodies, except that the microplates were precoated with rabbit anti-IdX 558 antibodies (1.5 μg/ml in BSA-PO_4). Before incubation, the test sera were absorbed on columns of Dex B1355 and B512 to remove anti-Dex antibodies and additionally with NRabIgG-Sepharose gels to remove anti-rabbit IgG antibodies. Such antibodies were present in large amounts in all mice immunized with the rabbit Ab2. Their thorough elimination was essential in this assay and was verified in each experiment by a parallel incubation on NRabIgG-coated wells (control binding). The percentage of each antiisotype bound per milliliter of test serum was calculated from the same formulas as used for the anti-Dex antibodies (see above).

Results

Specificity of the Anti-IdX Antibodies. The specificity of the rabbit anti-IdX 558 reagent was verified using as competitors a panel of myeloma and hybridoma proteins that have various antigen-binding activities. As can be seen in Fig. 1, only the IdX-positive α(1–3) Dex-binding myeloma and hybridoma proteins M104E, J558, Hdex1, and Hdex3 completely inhibited the binding of ^{125}I-labeled M104E to anti-IdX 558 antibodies, whereas the IdX-negative hybridoma proteins Hdex8 and Hdex14 were not inhibitory. Previous work by Clevinger et al. (6) has provided the structural correlates of this idiotype. Amino acids in positions 54 and 55 in the second V_H hypervariable region were identified as immunodominant residues determining IdX

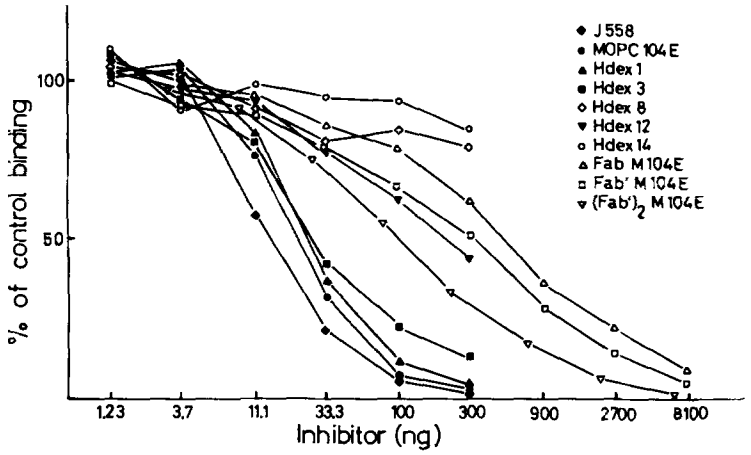

FIG. 1. Specificity of anti-IdX 558 antibodies using IdX-defined (6) myeloma and hybridoma α(1–3) Dex-binding proteins and M104E pepsin fragments. The relative inhibitory power of the proteins was measured in a competitive RIA as described in Materials and Methods. Proteins J558, M104E, Hdex1, Hdex3, and Hdex12 are IdX-positive (6, and B. Clevinger, private communication); protein Hdex8 has 20% relative IdX expression (6) and protein Hdex14 is IdX-negative (B. Clevinger, private communication). Note that in the present assay, Hdex8 typed as IdX-negative and Hdex12 as having only 10% IdX expression relative to the M104E ligand. This loss of idiotype expression may have resulted from some denaturation during purification on the B1355-Sepharose column (see Materials and Methods).

expression. The present reagent is thus similar to the one used by Clevinger et al. (6) which allowed the authors to distinguish IdX expression on these monoclonal antibodies.

The specificity of the reagent was further tested by using as competitors a panel of Ar-, NP-, galactan-, PC-, and inulin-binding myeloma and hybridoma proteins as well as a panel of 220 ascites fluids containing various myeloma proteins. A single ascites fluid was found inhibitory. This contained protein TEPC-1072, a μ, λ_1 weak anti-α(1-3) Dex-binding myeloma protein (M. Potter, personal communication).

The subsequent experiments were designed to test the in vivo functional properties of these antibodies with respect to their capacity to induce an IdX-positive response in C57Bl/6 and C.B20 mice.

Induction of IdX-positive Ig in C.B20 and C57Bl/6 Mice. Groups of C.B20 and C57Bl/6 mice (23 mice in all; see Materials and Methods) were injected with rabbit anti-IdX 558 (Ab2) in CFA. The controls consisted of equal numbers of mice injected with NRabIgG. 7 d after the last injection, the sera of these animals were tested in the IdX assay, and the results are shown in Fig. 2. The sera of all mice that received Ab2 completely inhibited the binding of ^{125}I-labeled M104E to anti-IdX 558-coated microplates, while no significant inhibition was obtained with either their pre-immune sera or the sera of mice immunized with NRabIgG. Similar results were obtained in an IdX assay that used syngeneic anti-J558 antibodies (not shown).

In view of the early collection time of the sera, there remained a possibility that residual rabbit anti-IdX was responsible for the inhibitions observed. Indeed, we as well as others (24), have shown that anti-Id antibodies successfully compete with the

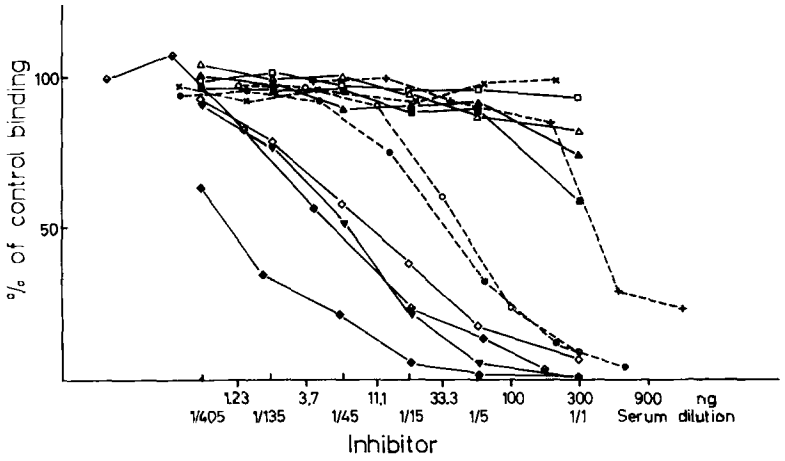

FIG. 2. Expression of IdX on purified antibodies and sera from anti-IdX 558-treated C.B20 and C57Bl/6 mice. The competitive RIA for IdX is described in Materials and Methods. Dotted lines, purified antibodies; full lines, whole sera. (○), M104E ligand; (●), purified C.B20 anti-α(1-3) Dex antibodies (Ab1'), pool of days 42-63 (see Fig. 4); (+), C.B20 anti-α(1-6) Dex antibodies purified from the same pool; (×), C.B20 anti-α(1-6) Dex antibodies purified from control NRabIgG-injected mice, days 42-63 (see Fig. 4); (▼), whole C.B20 sera, pool of days 42-63 (see Fig. 4); (◆), same pool absorbed on B1355-Sepharose; (◆), whole C.B20 sera, day 7 after the last injection of B1355-Con A (see Fig. 4); (▲), pre-immune C.B20 serum (pool of five mice); (■), C.B20 whole sera from the NRabIgG-injected control group (see Fig. 4); (◇), whole C57Bl/6 sera (pool of five mice), day 7 after the last injection of Ab2 (see Fig. 4); (△), pre-immune C57Bl/6 serum (pool of five mice); (□), whole C57Bl/6 serum from the NRabIgG-injected control group (see Fig. 4).

radiolabeled ligand in such assays. Independent measurements of residual Ab2 administered as two 23 μg injections per mouse of ^{125}I-Ab2 in CFA into six normal C.B20 mice, indicated a rapid clearance from the serum; 30 ng/ml remained at 2 d, and 2 ng/ml remained at 7 d after the last injection.

In other experiments, individual sera were preabsorbed with myeloma proteins or rabbit anti-mouse antibodies coupled to Sepharose before being tested in the IdX assay. The results shown in Fig. 3 indicate that whereas the M173- and J558-Sepharose gels removed marginal inhibitory activity, the rabbit anti-IdX and anti-λ_1 gels removed most of the inhibitory activity. Some inhibitory activity was also removed by the anti-κ and anti-α gels. The results strongly suggest that the small amounts of residual Ab2 did not compete in this assay since inhibitory activity would have been removed by the J558, not by the anti-IdX gel. Rather, these results suggest that Ig expressing a λ_1 chain and the IdX determinant were responsible for most of the inhibition observed in the IdX assay.

In all, these results indicate that λ_1-bearing, IdX-positive Ig were being generated soon after immunization with Ab2 antibodies. The titer and the kinetics of these IdX-positive Ig in individual weekly bleeds are shown in Fig. 4. It can be seen that IdX levels, undetectable in pre-immunization sera, abruptly reached a value of 100–300 μg/ml in C.B20 mice and 50–100 μg/ml in C57Bl/6 mice 7 d after the last injection of Ab2 antibodies. The titers remained at a plateau during the 6 wk (C57Bl/6) and 18 wk (C.B20) periods investigated.

When C.B20 mice were boosted with Dex B1355-Con A 18 wk after the last injection of Ab2, they responded with a sharp increase in the IdX titer (maximum, 150–350 μg/ml). This response however, was not observed in C57Bl/6 mice (Fig. 4). Control sera never contained >0.5–1 μg/ml of the IdX Id, values that are within the limits of the sensitivity of the assay.

The results indicate that the amount of IdX-positive Ig can be further increased upon boosting with B1355. Similar results were obtained in 13 other C.B20 mice

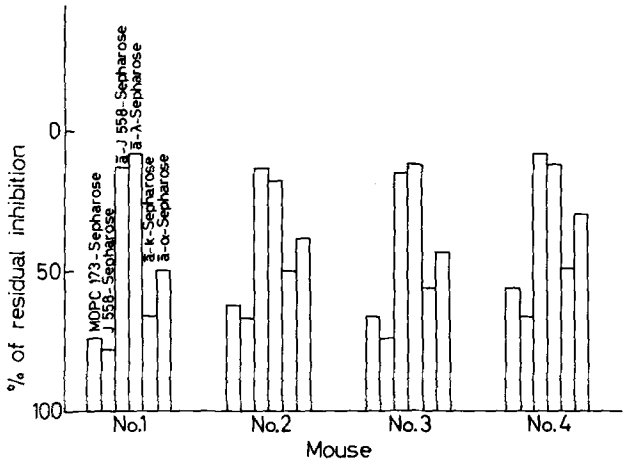

FIG. 3. Absorption of anti-IdX 558 (Ab2)-treated C.B20 sera (day 42, see Fig. 4) with myeloma proteins, antiisotype and anti-IdX 558 antibodies coupled to Sepharose 4B. The experiments were done on four individual sera. 100 μl of a serum dilution giving 60–80% inhibition in the competitive RIA for IdX were incubated with 50 μl of the indicated Sepharose gels for 1 h at 4°C. The supernatants were then retitrated to estimate the residual IdX content (see text).

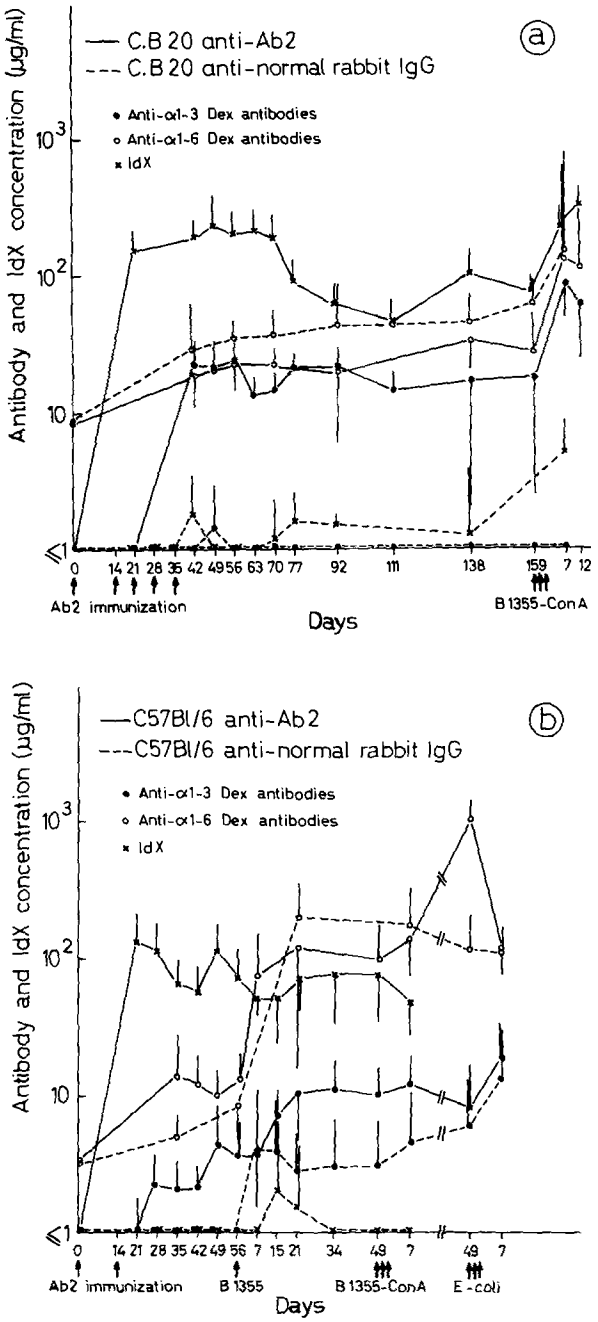

FIG. 4. Effect of anti-IdX 558 treatment on the serum anti-α(1–3) Dex, anti-α(1–6) Dex, and IdX responses of C.B20 (a) and C57Bl/6 (b) mice. Five mice were treated with anti-IdX (C.B20 anti-Ab2), and five control mice were treated with normal rabbit IgG (C.B20 anti-NRabIgG) on the days indicated by the arrows (see Materials and Methods). On day 159 (C.B20 mice), and on day 56 (C57Bl/6 mice), the animals were boosted with B1355 in CFA according to (2), B1355-Con A according to (7), or E. coli according to (44). The antibody titer was estimated in a binding assay, and the IdX concentration by competitive RIA as described in Materials and Methods. Each point is the arithmetic mean ± SD of individual determinations.

immunized with only two injections of anti-IdX antibodies that were not coupled to KLH (see Materials and Methods).

Induction of IdX-positive Anti-α(1-3) Dex Antibodies (Ab1') in C.B20 and C57Bl/6 Mice. The finding of this strong IdX-positive response suggested that some of the IdX-positive Ig carried α(1-3) Dex-specific activity. First, we tested the sera for total λ_1-positive anti-α(1-3) Dex antibodies. The results of these experiments are shown in Fig. 4.

Whereas anti-α(1-3) Dex antibodies were not detectable in significant amounts (≤1 μg/ml) in either pre-immune bleeds or in the NRabIgG-treated control groups, such antibodies appeared in small but significant amounts in each animal pretreated with Ab2. The antibodies were detectable with a delay of 1-3 wk with respect to the IdX-positive Ig. The titers ranged from 15 to 40 μg/ml in the three groups of C.B20 mice and from 2 to 5 μg/ml in C57Bl/6 mice. In control animals conventionally immunized with B1355 alone, some C57Bl/6 mice, but none of the other Igh-C^b mice, responded with high levels (30-100 μg/ml) of anti-α(1-3) Dex antibodies (not shown). However, as will be discussed below, these conventionally induced anti-α(1-3) Dex antibodies differed from the Ab2-induced antibodies in IdX expression, in agreement with a previous study (7).

These results indicate that immunization with Ab2 alone leads to the appearance of a low, but significant λ_1-positive anti-α(1-3) Dex response. The maximum titer of these antibodies is about one order of magnitude lower than the serum IdX titer.

Second, in order to determine whether these anti-α(1-3) Dex antibodies expressed the IdX determinant, the antibodies were affinity purified from pooled sera on columns of B1355-Sepharose, as described in Materials and Methods. The sera were preabsorbed on B512-Sepharose columns to eliminate anti-α(1-6) Dex antibodies that served as controls. Anti-α(1-6) Dex antibodies were also purified from control mice injected with NRabIgG. Each effluent serum fraction from these absorptions was also saved.

Fig. 2 shows that purified C.B20 anti-α(1-3) Dex antibodies completely inhibited the binding of ^{125}I-labeled M104E to rabbit anti-IdX 558. The inhibition curves overlapped with the standard M104E myeloma protein inhibition curve, as well as with hyperimmune BALB/c anti-α(1-3) Dex antibodies. This inhibition pattern is suggestive of a high degree of idiotypic similarity. The data furthermore suggested that most, if not all, of these antibodies express the IdX idiotype. This was indicated by the fact that the inhibition curves varied within a factor of 0.8-1.2 in terms of weight of added competitor compared to the M104E standard. An idiotypic deficiency would have been seen by a more pronounced shift of the curve to the right. Thus, Ab2-treatment generates an early IdX-positive anti-α(1-3) Dex response.

Anti-α(1-3) Dex antibodies purified from C.B20 mice boosted with B1355-Con A (Fig. 4) were indistinguishable from the above antibodies in IdX expression. In addition, Ab2 pretreatment in some way appeared to sensitize these animals to respond with substantial amounts of IdX-positive antibodies, as the antibody titer rose from an average of 20 to 30-150 μg/ml after B1355 boosting. In all, 10 pools of sera from three C.B20 groups that include pre- and post-B1355 boost bleeds and one pool from C57Bl/6 mice (a pre-B1355 boost bleed) were tested, with similar results. Control λ_1-positive anti-α(1-3) Dex antibodies purified from conventionally immune C57Bl/6 mice, some of which responded with up to 300 μg/ml of antibodies, were

devoid of inhibitory activity in the IdX assay.

Induction of IdX-positive Ig and Anti-α(1–3) Dex Antibodies in C.B20 Mice Immunized with Allogeneic Anti-J558 Ab2. A group of six C.B20 mice were injected twice at a 15 d interval with 80 μg/ml (150 IBC_{50}) of anti-J558 antibodies coupled to KLH. The magnitude of the IdX-positive Ig and the anti-α(1–3) Dex antibody responses was significantly lower in these animals compared with the groups immunized with rabbit Ab2. Only four mice responded, with an average of 8 μg/ml of IdX-positive Ig first detected as late as 6 wk after the second injection of Ab2. IdX expression in these sera was measured in the IdX assay that used the rabbit anti-IdX 558 reagent. Pre-immune sera contained no detectable IdX-positive Ig or anti-α(1–3) Dex antibodies. Only two mice responded with low titers (2–4 μg/ml) of λ_1-positive anti-α(1–3) Dex antibodies. However, all mice responded with anti-α(1–3) Dex antibodies when boosted with B1355-Con A 11 wk after the last Ab2-injection, but still with low titers (average 9 μg/ml). It was difficult to purify sufficient amounts of these antibodies for the determination of their IdX expression.

Induction of IdX-positive Ig Devoid of Dex-binding Activity (Ab3). The presence of such molecules was suggested by an inspection of the data presented in Fig. 4 which indicated a several-fold greater level of total IdX-positive Ig compared with IdX-positive anti-α(1–3) Dex antibodies. These Ig were assayed after thorough absorption of the sera on B1355 and B512 columns. The effluents, which were verified as lacking detectable Dex-binding activity in a binding assay, were nevertheless found inhibitory in the IdX assay. The Ig differed, however, from the Ab1′ antibodies by the slope of their inhibition curve, which was suggestive of an idiotypic deficiency in spite of their capacity to completely inhibit the binding of ^{125}I-labeled M104E to anti-IdX 558 (Fig. 2).

Independent calculations gave the following relative amounts of Ab3 Ig and Ab1′ antibodies in whole sera. In the pre-B1355-Con A boost bleeds of C.B20 and C57Bl/6 mice, ~90% of the IdX-positive Ig represented Ab3. In post-B1355 boost bleeds of C.B20 mice Ab3 and Ab1′ were about equally represented among IdX-positive Ig, while in C57Bl/6 mice the proportion of Ab3 remained at 90% of the total IdX-positive Ig. For comparison, BALB/c hyperimmune sera contain only ~3% of Ab3 Ig with the vast majority of the IdX-positive component being represented by the Ab1 anti-α(1–3) Dex antibodies.

Therefore, injection of Ab2 appears to generate at least two populations of IdX-positive Ig in these Igh-C^b mice. Molecules lacking detectable Dex-binding activity are defined as Ab3, and molecules with intrinsic α(1–3) Dex-binding activity are defined as Ab1′. Both definitions are used with reference to previous studies (24, 25).

Effect of Ab2-treatment on the Anti-α(1–6) Dex Response. The responder status of Igh-C^b mice with respect to the α(1–6) linkage of Dex is now well established (26, 27). It was thus of importance to assess whether anti-IdX 558 administration had any effect on the quantity and quality of this response.

As shown in Fig. 4 the pre-immune level of anti-α(1–6) Dex antibodies (10–30 μg/ml) increased two to threefold whether the C.B20 and C57Bl/6 mice were injected with Ab2 or NRabIgG. Boosting with B1355-Con A did not affect the relative antibody level, which rose to ~100 μg/ml in both groups.

The expression of IdX on these antibodies was examined in the IdX assay. As shown in Fig. 2, anti-α(1–6) Dex antibodies purified from C.B20 mice treated with

Ab2 or NRabIgG were not inhibitory. However, antibodies purified from four other pools of Ab2-treated mice (but from none of the NRabIgG-treated groups) gave up to 70% inhibition at high concentrations (Fig. 2).

Binding assays indicated that these sera contained ~5% of anti-α(1–3) Dex antibodies. Since, as mentioned above, all anti-α(1–3) Dex antibodies were IdX-positive, these results can be interpreted on the basis of a contamination by a small amount of copurified anti-α(1–3) Dex antibodies (Dex B512 contains 4% of α(1–3) glucosidic linkages [4]).

The results indicate that the in vivo administered rabbit anti-IdX 558 had no significant effect on the normal course of the anti-α(1–6) Dex response.

Specificity of Ab1′ Antibodies for Various Dex. The finding that Ab1′ antibodies expressed the IdX determinant prompted a study of their Dex-binding specificity using a panel of eight Dex having different glucosidic linkages compositions (4).

In all, six serum pools or individual bleeds from Ab2-treated C.B20 mice, and one purified anti-α(1–3) Dex antibody sample were tested. The binding pattern obtained with the purified antibodies was typical of the results obtained with the serum samples, and is shown in Table I. Also shown is the binding pattern of purified anti-α(1–3) Dex antibodies from hyperimmune BALB/c mice and from conventionally hyperimmunized C57Bl/6 mice, as well as the binding of the reference J558 and M104E myeloma antibodies (28, 29).

The C.B.20 and the BALB/c antibodies bound most strongly to Dex B1355, B1498S, and B1501S compared with the remaining five dextrans, B742S (C3R), B742L, B1375, B1255, B1254S(L). However, the C.B20 antibodies could be distinguished from the BALB/c antibodies in that they bound less strongly to these last five Dex. This is clearly evident from the calculations of their relative binding avidities. Table I also shows that the binding specificity of the J558 myeloma protein measured in the present assay is very similar to that of its reported specificity in a precipitin assay (28). In contrast, purified λ_1-positive IdX-negative conventional C57Bl/6 antibodies have a very different binding pattern; they do bind to B1355, B1498S, and B1501S, but they do not bind at all to the remaining five Dex.

An element to be considered in such comparisons would appear to be the degree of polymerization of the antibodies as documented by previous work (30). We have not detected significant differences in binding avidity in samples typed as high IgG1, low IgM vs. high IgM, low IgG1 Ab1′ content (see below for the class composition of Ab1′ antibodies).

These experiments indicate that the Dex-binding specificity of C.B20 Ab1′ can be distinguished from that of the BALB/c antibodies. The C.B20 antibodies are, however, more closely related to the BALB/c than to the IdX-negative C57Bl/6 antibodies.

Class Composition of the Ab1′ Antibodies and Ab3 Ig. Previous studies on the class composition of anti-α(1–3) Dex antibodies of BALB/c mice have demonstrated the striking class restriction of this response (31, 32). Since the IdX-positive Ab1′ antibodies were induced by a procedure that differed from conventional immunization with Dex, it seemed relevant to study their class composition.

The main finding was that the heavy chain class composition of the Ab1′ antibodies was highly restricted. IgG1 together with IgM represented the bulk of the response, with IgG1 by itself as the major class representing 70% of the Ab1′ antibodies (Fig. 5 a). This was in contrast to the BALB/c primary and hyperimmune anti-α(1–3) Dex

TABLE I

*Relative Binding of Ab1′ Antibodies to Dextrans**

Sera, purified antibodies, myeloma proteins	Heavy chain class composition‡	Dextrans								
		B1355	B1498S	B1501S	B742S(C3R)	B742L	B1255	B1375	B1254S (K)	B512
J558	IgA	0.7	0.8	0.8	0.1	0	0	0.15	0	ND§
J558 (precipitation)‖		+++	+++	+++	++	+	++	++	+	ND
M104E	IgM	0.7	0.7	0.7	0.7	0.4	0.2	0.6	0.1	ND
M104E (precipitation)‖		100	—	—	100	6	—	—	100	
W3129 (anti-α(1–6)Dex)	IgA	0.1	0	0	0	0	0	0	0	0.38 ± 0.14
BALB/c (anti-α(1–3)Dex)¶										
Serum	IgM, IgG3, IgA	1.0	1.0	1.0	0.75	0.6	0.4	0.55	0.1	ND
Antibodies	IgM, IgG3, IgA	1.55	1.1	1.25	1.15	0.95	0.85	0.85	0.3	ND
C.B20 (anti-Ab2)serum	IgM, IgG1	1.0 ± 0.17	1.2 ± 0.17	1.36 ± 0.33	0.32 ± 0.11	0.35 ± 0.17	0.28 ± 0.10	0.38 ± 0.14	0.083 ± 0.016	ND
C.B20 (anti-Ab2, anti-B1355-Con A)										
Serum	IgM, IgG1, IgG3	1.15 ± 0.21	1.3 ± 0.14	1.45 ± 0.21	0.4 ± 0.14	0.45 ± 0.07	0.4	0.65	0.3 ± 0.21	ND
Antibodies	IgM, IgG1, IgG3	0.9	0.75	0.9	0.55	0.45	0.55	0.6	0.15	ND
C57Bl/6 (anti-B1355-Con A) antibodies	IgM, IgG3	1.0	0.6	1.1	0	0	0	0	0	ND

* This was estimated in a solid-phase binding assay using microplates coated with the indicated Dex, followed by rabbit ^{125}I-anti-λ_1 antibodies for anti-α(1–3)Dex estimation or ^{125}I-anti-κ antibodies for W3129-binding estimation (see Materials and Methods). It was assumed that anti-α(1–3)Dex-specific antibodies bear exclusively the λ_1 light chain although exceptions were reported using different immunization protocols (33, 34). The values represent nanograms of ^{125}I-labeled antibodies bound when 2 ng of purified anti-Dex antibodies or myeloma proteins were added. When whole serum was used, the anti-α(1–3)Dex titer was predetermined. These values fall on the linear portions of binding curves.

‡ Determined from the data in Fig. 5.

§ Not determined.

‖ Binding specificity as reported in (28) for J558 and in (29) for M104E.

¶ BALB/c anti-α(1–3)Dex: pool of four mice immunized according to the E. coli protocol described by Hansburg et al. (44); C.B20 (anti-Ab2): anti-IdX 558-treated mice (days 42–63; see Fig. 4); C.B20 (anti-Ab2, anti-B1355-Con A): anti-IdX 558-treated mice boosted with B1355-Con A (see Fig. 4); C57Bl/6 (anti-B1355-Con A): anti-α(1–3)Dex antibodies purified from a single mouse hyperimmunized with B1355-Con A and bled at day 7.

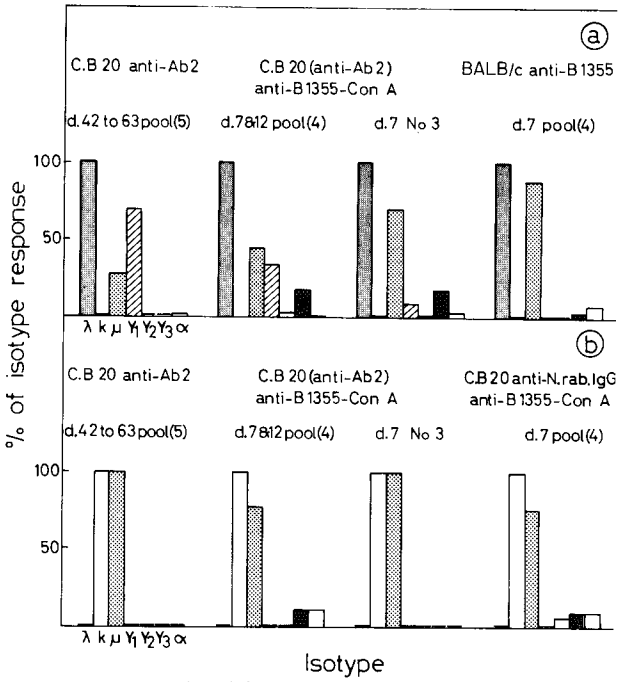

FIG. 5. Class composition of purified C.B20 anti-α(1-3) Dex antibodies (Ab1'), anti-α(1-6) Dex antibodies and BALB/c anti-α(1-3) Dex antibodies (Ab1). (a) Classes of anti-α(1-3) Dex antibodies. (b) Classes of anti-α(1-6) Dex antibodies. C.B20 anti-Ab2: anti-IdX 558-treated mice (see Fig. 4); C.B20 (anti-Ab2) anti-B1355-Con A: anti-IdX-treated and B1355-Con A boosted mice (see Fig. 4); BALB/c anti-B1355: 7 d primary response; C.B20 (anti-NRabIgG) anti-B1355-Con A: NRabIgG-treated mice boosted with B1355-Con A (see Fig. 4). In C.B20 mice, the two anti-Dex antibodies were purified from the same sera (see text).

response which was principally in the IgM class, whereas IgG1 was not detectable (Fig. 5 a).

After boosting of the C.B20 mice with B1355-Con A (see Fig. 4), the proportion of the IgG1 antibodies fell to 6–35% of the heavy chain classes, and was replaced by IgM as the major class (Fig. 5 a). However, even then IgG1 still represented a sizeable proportion of the response. This class was not detectable in any of the α(1-6) specific antibody samples purified from the same sera (Fig. 5 b), or from the sera of mice injected with NRabIgG. Here, the response was mainly in the IgM class, with some IgG3. As to the light chains, λ_1 was the only light chain type found on the Ab1' antibodies, except for one C.B20 pool in which 20% of the response was composed of κ chains. The occurence of a κ anti-α(1-3) Dex component was also reported on previous occasions (33, 34).

The class composition of Ab3 Ig is shown in Fig. 6. This was determined on seven individual C.B20 sera or serum pools from the post-B1355-Con A boost period. The sera were preabsorbed on columns of B1355 and B512, and on a column of NRabIgG before being titrated on anti-J558-coated microplates (see Materials and Methods). As in the case of the Ab1' antibodies, these IdX-positive Ig were also largely represented by the IgG1 class. Taken together, these results indicate that the IgG1 class represents a major component of the C.B20, C57Bl/6 Ab1', and Ab3 responses.

Allotype Assignment of C.B20 IgG1 Ab1' Antibodies. The exclusive linkage of IdX Dex

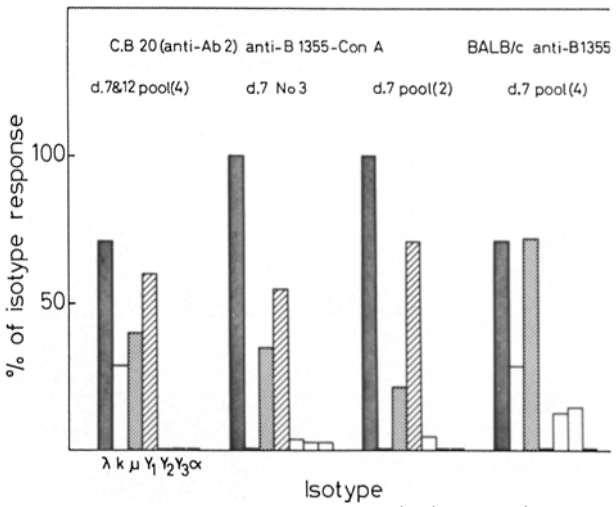

FIG. 6. Relative percentage of isotype composition of Ab3 Ig in whole sera (see text for assay procedure). C.B20 (anti-Ab2)anti-B1355-Con A: anti-IdX-treated and B1355-Con A boosted mice (see Fig. 4); BALB/c anti-B1355: hyperimmune BALB/c anti-B1355 serum pool.

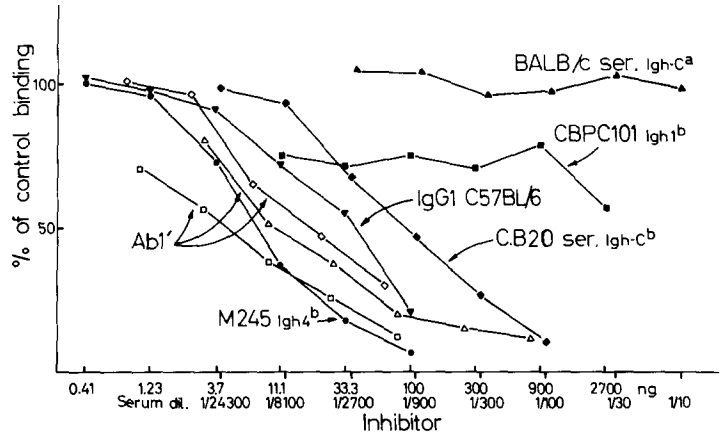

FIG. 7. Determination of the Igh4 allotype of C.B20 anti-α(1-3) Dex antibodies (Ab1'). The competitive RIA for the Igh4^b allotype is described in Materials and Methods. (◇) and (□), purified C.B20 Ab1' antibodies from two pools of five C.B20 Ab2-treated mice; (△), purified C.B20 Ab1' antibodies from a pool of six Ab2-treated and B1355-Con A-boosted mice.

expression with Igh-C^a is now well established (2, 3). It thus appeared paradoxical at first hand that this idiotype was found associated with Ab1' antibodies of C.B20 and C57Bl/6 mice known to be Igh-C^b homozygous. The Igh-C allotype of pre-immunization sera of the C.B20 mice used in this study was verified by Ouchterlony gel diffusion using polyvalent anti-Igh-C^a and anti-Igh-C^b allogeneic sera and all were typed homozygous Igh-C^b. We next investigated the allotype of purified IdX-positive IgG1 antibodies. C.B20 Ab1' antibodies purified from two pre-B1355-Con A and from four post-B1355-Con A boost pools (see Fig. 4) were tested in parallel in an assay for the Igh4^b and an assay for Igh4^a allotype determinants. The results shown in Fig. 7 indicate that the antibodies were inhibitory in the assay for the Igh4^b allotype. There was no detectable inhibition in the Igh4^a allotype assay (not shown). This indicates

that the IgG1 component of the Ab1′ comes entirely from the Igh-C^b haplotype.

A possible inhibition by contaminant normal serum IgG1 in these experiments was excluded by the following. Several Ab1′ antibody pools, chosen for high (80%) IgG1 antibody content, were divided into equal aliquots and absorbed with anti-κ, anti-λ_1, or anti-IdX 558 antibodies coupled to Sepharose. The supernatants were then retested in the allotype assay. The allotype inhibitory activity was completely removed only with the anti-λ_1 and the anti-IdX gels, but to no significant extent by the anti-κ gel (not shown). This then strongly suggested that all of the IgG1 antibodies typed as $Igh4^b$ expressed the IdX determinant.

Discussion

The essential findings reported here show that the C.B20 and C57Bl/6 mice respond with sizeable IdX-positive Ig (Ab3) and IdX-positive anti-α(1–3) Dex antibody (Ab1′) components after injections of rabbit anti-IdX 558. The antibodies were defined as Ab1′ because of their idiotypic relatedness with the responder BALB/c IdX-positive anti-α(1–3) Dex antibodies (Ab1), in analogy to a previously established nomenclature (24, 25). The anti-IdX antibodies thus acted by altering the phenotype of the response of these animals to the α(1–3) Dex glucosidic linkage, since previous studies indicated that conventional immunization with Dex B1355 leads to either an IdX-negative anti-α(1–3) Dex response (2, 7) or to no detectable response in C.B20 mice (present study).

Le Guern et al. (12), Bona et al. (11), and Hiernaux et al. (35), have similarly found that Ab2 can act by inducing an Id-positive antibody response in animals not expressing this Id upon conventional immunization. In all cases the Ab2-induced Id was shared by antibodies having the same antigen-binding activity in responder mouse strains. Furthermore, a similar approach to ours, using heterologous Ab2, was reported by Miller et al. (13). When pig Ab2 specific for an Id found on BALB/c antinuclease antibodies were injected into B10.D2 mice, the latter responded with Ig expressing this Id. The Id was absent from antinuclease antibodies induced by conventional immunization of B10.D2 mice with staphylococcal nuclease.

It was particularly important to determine the IdX-specificity of the rabbit reagent used in the idiotypic characterization of the Ab1′, as a previous work as well as the present study indicated that C57Bl/6 mice can respond with high titers of λ_1-bearing, but IdX-negative anti-α(1–3) Dex antibodies after conventional immunization with B1355-Con A (7). This was done by testing the anti-IdX reagent against a panel of reference anti-α(1–3) Dex myeloma and hybridoma antibodies (6). Only the IdX-positive antibodies were effective competitors in the IdX assay. Our reagent thus had a similar IdX-specificity than that reported by Hansburg et al. (5) and by Clevinger et al. (6). Additionally, we have purified conventional C57Bl/6 anti-α(1–3) Dex antibodies using the protocol of Geckeler et al. (7) and found these to be IdX-negative in our assay, confirming the results of a different assay (7). The conventional antibodies were thus clearly distinguishable from Ab1′ antibodies induced by Ab2 in the same strains of mice. We were thus confident that, as far as idiotypic relatedness is concerned, the Ab2-induced Ab1′ were indistinguishable from the responder BALB/c Ab1 and the reference IdX-positive myeloma and hybridoma antibodies. These results argue strongly for the existence of a nonexpressed or "silent" anti-α(1–3) Dex repertoire in Igh-C^b mice (36).

In the assay for IdX-positive Ig in whole sera it was important to determine the possible interference of any residual rabbit anti-IdX Ab2. Indeed, we and others (24) found that anti-IdX can combine with the radiolabeled proband ligand and thus mimic the effect of competitor IdX-positive molecules. For this, the sera were preabsorbed with various immunoabsorbents before being tested for IdX expression. The inhibitory activity was not eliminated by the J558 absorbent, indicating little if any residual rabbit Ab2. On the other hand, the elimation of inhibitory activity by the anti-λ_1 and anti-IdX absorbents indicated that IdX-positive Ig were being measured in the IdX assay and furthermore that most of them bore the λ_1 light chain.

In addition to Ab1′, the Ab2-treated C.B20 and C57Bl/6 mice also responded with relatively large amounts of IdX-positive Ig (Ab3), which lacked detectable binding activity to Dex B1355 or B512. In contrast to Ab1′, they appeared to lack the full Id complement of the reference J558 myeloma protein. Ab3 Ig have been reported as either anti-(anti-Id) antibodies (12, 25), i.e., directed against certain idiotopes expressed by the inducing Ab2 antibodies, or as Ig that express a part of the idiotopes present on responder Ab1 antibodies (11, 24). It is considered that Ab3 and Ab2 express complementary idiotopes (11, 37). As concerns the α(1–3) Dex system, the later definition for Ab3 may be more appropriate. This is based on the finding that Ab3 successfully competed in an IdX assay using either a rabbit or a mouse anti-J558 reagent, seen with Ab3 induced with either one of the anti-Id reagents. Similar findings were reported for Id-bearing Ig in the staphylococcal nuclease system (38) and in the *p*-azophenylarsonate (39) system. The α(1–3) Dex system also enabled us to include the light chain marker with which to define these molecules (2). With only one exception, all tested samples of Ab3 Ig were found to bear exclusively this light chain type, which additionally suggested their strong molecular similarity with the responder anti-α(1–3) Dex antibodies. Ab3 Ig have been described in several other related studies in both the rabbit and mouse (12, 24, 25, 38, 40). The true identity of the IdX-positive Ab3 Ig is still not fully understood, and studies are in progress to further characterize them.

It is revealing that Ab1′ were detectable in C.B20 and C57Bl/6 mice without prior intentional immunization with Dex. Similar observations were recently reported in which a single injection of anti-Id led to the appearance of Ig with specific antigen-binding activity (39, 41, 42) although in those studies the animals were genetic responders. It has been proposed that an Id-positive response can be induced directly by anti-Id by reacting with regulatory idiotopes (11) expressed on the membrane of B cell precursors. The hypothesis further assumes that the antigen-specific precursors constitute a subpopulation of a larger Id-positive precursor pool (11, 43). In the present study an inductive role played by endogenous flora microorganisms can not be excluded. For example, intestinal coliform saprophytes carrying cell wall Dex (44) could also have contributed to the induction of Ab1′ by further expanding the Ab1′ precursors. The delayed appearance of Ab1′ with respect to Ab3 would be consistent with this proposition. A similar view was held by Wysocki and Sato (39) in a study of the arsonate system.

On the other hand, it can not be decided to what extent an internal image (10) or a homobody (45) effect of anti-Id influenced the induction of Ab1′. In previous studies, such properties of anti-Id were indeed described (46). The immunochemical characterization of the rabbit and allogeneic anti-Id (see Materials and Methods)

indicated that for approximately equal amounts of IBC_{50} injected per animal, the IdX response of mice receiving the allogeneic anti-J558 was erratic and of low magnitude. In contrast, in all mice injected with the rabbit anti-IdX the response was more intense and rapid. To what extent this was related to the different relative anti-IdI J558 vs. anti-IdX specificities in the two anti-Id, or to their relative content of anti-binding site specificity (homobody), is not known. The response does however appear to correlate with the IdX-specificity content in the two reagents, since the rabbit compared to the mouse reagent was 100 and 25% IdX-specific, respectively.

It is significant that the Ab1′ levels of the Ab2-treated and B1355-Con A-boosted C.B20 mice did not reach the amounts found even in the primary conventional BALB/c response (~800 μg/ml). One can assume that the Igh-C^b-linked nonexpression of IdX in these mice reflects the participation of T suppressor cells as was described in other genetically linked nonresponse systems (19, 47). Thus, if specific T suppressor activity had been abolished by Ab3, as was shown in the Id 460 system (19), only some of the T cell activity may have been inactivated. In other words, only the anti-IdX-receptor-bearing T cells would be targets of Ab3, whereas other T suppressors, specific for IdX-negative precursors, would remain functional and actively suppress this arm of the anti-α(1–3) Dex response.

The early appearance of Ab3 1 wk after the second injection of Ab2, and the fact that a large fraction of this response was of the IgG1 class, suggests an involvement of T helper cells in the inductive process. The fact that IgG1 is a prominent component of many secondary responses that involve T cell help (48) and is strongly thymus dependent (49) further attests to this possibility. Ab2-presensitization of these mice appears to have changed the nature of this TI-2 type response (50) into a thymus-dependent response.

Previous studies where anti-Id (13, 51) or Id (52) was injected in vivo have implicated the participation of T helper cells. In the present system, the T helper cells may recognize regulatory idiotopes (11) shared by Igh-C^a and Igh-C^b anti-α(1–3) Dex antibodies. For example, Gleason and Köhler (53) have shown that PC-induced T helper cells recognize shared idiotopes on two PC-binding myeloma antibodies M167 and T15, each of which is specifically distinguished by anti-Id sera. Suggestive evidence that C57Bl/6 and BALB/c anti-α(1–3) Dex antibodies express regulatory idiotopes stems from recent experiments by C. Victor and C. Bona (personal communication) who achieved the total suppression of anti-α(1–3) Dex synthesis in C57Bl/6 mice injected at birth with nanogram quantities of either monoclonal CD5-3 anti-IdX 558 or monoclonal EB3-7.2 anti-Id 558 antibodies.

The finding that C.B20 mice used an Igh-C^b haplotype product for the synthesis of their IgG1 Ab1′ was not unexpected since these mice are homozygous for this heavy chain linkage group (3). In view of the tight linkage of the heavy chain locus (54), it is conceivable that the IgM and IgG3 class Ab1′ are likewise products of the same haplotype. Based on the well established linkage of allotype to idiotype of many antibody responses in the mouse (1, 3, 55), this suggests that the V_H^{Dex} gene product of these mice is coded by a different gene than that which codes for the V_H^{Dex} product in BALB/c mice. The remarkably reproducible differences in Dex-binding specificity of the Ab1′ compared with Ab1 is compatible with this hypothesis. Relevant to this is the description by Lieberman et al. (56) of PC-specific myeloma proteins from BALB/c and C57Bl/6 which displayed minor V_H framework structural differences,

and could be distinguished by appropriate anti-Id sera. The V_H markers of these antibodies behaved as allelic variants in the PC response of various mouse strains. The confirmation of the present findings at the hybridoma level should add to our understanding of regulatory processes.

Summary

The effect of IdX-specific rabbit and allogeneic antiidiotype antibodies (Ab2) was investigated in vivo in Igh-C^b mouse strains with respect to the induction of a cross-reactive idiotype (IdX)-positive anti-α(1–3) Dextran (Dex) response. These C.B20 and C57Bl/6 mice have an allotype-linked incapacity to respond with IdX-positive anti-α(1–3) Dex antibodies upon conventional immunization with Dex B1355. 7 d after the rabbit Ab2 injections, IdX-positive Ig (Ab3) and IdX-positive anti-α(1–3) Dex antibodies (Ab1′) were detected in the sera of each tested mouse. The affinity-purified Ab1′ were idiotypically indistinguishable from reference BALB/c IdX-positive myeloma proteins and BALB/c anti-α(1–3) Dex antibodies (Ab1) in a competitive inhibition radioimmunoassay, while Ab3 Ig appeared idiotypically deficient and did not bind to Dex. The response to the α(1–6) linkage of Dex was not affected in these mice. A large fraction of the Ab1′ and Ab3 responses of both mouse strains were of the IgG1 class. The Ab1′ antibodies differed from BALB/c Ab1 by lower relative binding to five of eight tested Dex, and by expressing the $Igh4^b$ allotype determinants on the IgG1 antibodies. This study identifies the products of a V_H^{Dex} gene that appears to be under regulatory control in the Igh^b mice. Its association with the b haplotype suggests that this gene may differ structurally from the BALB/c V_H^{Dex} gene.

We are grateful to all authors listed in Materials and Methods who generously supplied the hybridoma and myeloma antibody samples. We thank Dr. M. Bosma for a gift of anti-IgG1 allotype serum, Dr. E. A. Kabat for a gift of dextran samples, Dr. P. A. Cazenave for a gift of mouse anti-J558 serum, and Dr. M. Seman for BAB14 mice.

Received for publication 23 November 1982 and in revised form 25 January 1983.

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