STRAIN-SPECIFIC SILENCING OF A PREDOMINANT ANTIDEXTRAN CLONOTYPE FAMILY

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The expressed primary B cell repertoire is extremely diverse (1–3); however, certain clonotypes or clonotype families are disproportionately represented in mature B cell pools of various inbred murine strains (4–13). Numerous studies have indicated a determinative role for variable (V)[1] region gene expression in the establishment of the primary B cell repertoire. Molecular analyses have demonstrated a very large genetically encoded repertoire arising from the arrangement of the V region genes into multiple V, D (diversity), and J (joining) segments, the combinatorial rearrangements of these segments (14–17), and diversity at the junctions of rearranged segments (18, 19). Studies of bone marrow B cell precursors have demonstrated that both the diversity of the primary B cell repertoire and the unusually high representation of certain clonotypes are characteristics of the newly generated B cell population even before surface immunoglobulin (sIg) expression (20–27).

Environmental modulation of the genetically encoded repertoire in the form of tolerance (21, 28–31) and T cell or antibody-mediated idiotypic network effects (6, 10, 32–41) may significantly alter the expression of certain specificities, and thereby participate in the establishment of the primary B cell repertoire. Thus, assessing the relative contribution of genetically encoded vs. environmental influences on the differential expression of predominant clonotype families among various murine strains is essential for an understanding of the establishment of the functional mature B cell repertoire.

To further evaluate the mechanisms responsible for strain-specific differences in the expression of predominant clonotypes, we have studied T cell–dependent responses to Dextran B1355 fraction S (DEX). Antibodies specific for DEX can be divided into two broad categories based on the recognized glucose linkage conformation: antibodies specific for $\alpha(1 \rightarrow 3)$ glucose linkages, and those specific for $\alpha(1 \rightarrow 6)$ linkages (7–9). Mice of the Igh^a heavy chain haplotype are high responders to $\alpha(1 \rightarrow 3)$ linkages, with a marked dominance (80–95%) of antibodies bearing both the λ light chain and crossreactive idiotypes (IdX) (7–9, 35, 38–40, 42–44). In contrast, mice of the Igh^b haplotype express little λ-bearing

$\alpha(1 \rightarrow 3)$ linkage–specific antibodies (7–9, 35, 38–40). Historically, this phenotypic difference has been considered to represent a polymorphism in the V_H (variable heavy chain) genes encoding antibody of this specificity between these strains, and as such, has been the prototype antigen used to map recombinants within Igh between strains (7–9).

Using the splenic fragment culture system, we have examined the λ-bearing $\alpha(1 \rightarrow 3)$ DEX–specific B cell repertoire in BALB/c (Igh^a) and C.B20 (Igh^b) mice, in order to probe the mechanisms underlying the phenotypic difference in these two strains. Our studies have shown that DEX-responsive precursor cells in both splenic and environmentally naive surface sIg^- bone marrow cell populations (prereceptor B cells) obtained from BALB/c mice consist predominantly of cells that give rise to clones secreting λ-bearing $\alpha(1 \rightarrow 3)$ DEX–specific antibodies. Surprisingly, the sIg^- bone marrow cell population, in contrast to the spleen cell population of C.B20 mice also contains a predominant population of DEX-responsive cells whose antibody-forming cell clonal progeny secrete λ-bearing $\alpha(1 \rightarrow 3)$ DEX–specific antibodies. The absolute frequency of these C.B20 λ-bearing $\alpha(1 \rightarrow 3)$ DEX–specific prereceptor B cells is equivalent to the frequency in sIg^- BALB/c bone marrow cell populations. The C.B20 λ-bearing $\alpha(1 \rightarrow 3)$ DEX–specific pre-receptor B cells can be effectively stimulated in the allogeneic Igh^a (BALB/c) splenic fragment environment, but not in the Igh^b syngeneic (C.B20) environment. Since λ-bearing $\alpha(1 \rightarrow 3)$ DEX–specific splenic B cells are extremely rare in C.B20 mice, it appears that, if allowed to mature through sIg acquisition within their natural C.B20 environment, these cells are inactivated, and cannot respond in either the Igh^a or Igh^b environment. Therefore, the C.B20 λ-bearing $\alpha(1 \rightarrow 3)$ DEX–specific B cell population can be characterized as a silenced clonotype family. Thus, the phenotypic difference between BALB/c and C.B20 mice, as regards to this clonotype family, appears to be the result of C.B20 environmental clonotype–specific downregulation, and not a polymorphism in the V_H genes coding for these specificities.

Materials and Methods

Mice. BALB/c and C.B20 mice were obtained from the breeding colony at Scripps Clinic and Research Foundation. Mice of either sex, 6–12 wk of age, were used in the splenic fragment culture experiments.

Antigens and Immunizations. Limulus polyphemus hemocyanin (Hy) and bovine serum albumin (BSA) were coupled to Dextran B1355 fraction S, as previously described (35). Dextran B1355 fraction S (DEX) [35% $\alpha(1 \rightarrow 3)$ linkages] was a gift from Dr. M. E. Slodki, Northern Regional Research Laboratory, U.S. Department of Agriculture, Peoria, IL. Dextran B512 [96% $\alpha(1 \rightarrow 6)$] was obtained from Sigma Chemical Co., St. Louis, MO.

6–10-wk-old BALB/c and C.B20 mice were immunized intraperitoneally with 100 μg Hy in complete Freund's adjuvant (CFA), and boosted with 100 μg Hy in saline 4 wk later. Mice were used 4–8 wk after boosting as recipients for cell transfers.

Splenic Fragment Cultures. Monoclonal anti-DEX antibodies were produced in splenic fragment cultures as previously described (11, 25, 35, 43, 45). Briefly, 2.5 $\times$ 10^7 donor spleen cells or 5.0 $\times$ 10^7 unfractionated bone marrow cells or bone marrow cells depleted of sIg^+ B cells (see below) were transferred intravenously into Hy-primed recipient mice that had received 1,300 rad whole body irradiation from a Cs source 1–4 h earlier. Fragment cultures were prepared from recipient spleens 16–20 h after cell transfer, and were stimulated for 3 d with DEX-Hy. Cultures were incubated at 37°C in an atmosphere

of 93% O_2 and 7% CO_2. Medium was changed every 2–4 d, and culture fluids were individually collected from days 9–24 and assayed for antibody activity.

Radioimmunoassay (RIA) and Competitive Inhibition Studies. Culture supernatants collected between day 9 and 24 of culture were assayed for DEX-specific antibodies by solid-phase RIA, by a method previously described (3, 11, 25, 35, 45, 46). Murine DEX-specific antibodies bound to DEX-BSA-coated polyvinyl microtiter plates were detected after reaction with polyspecific rabbit anti–mouse Ig antisera followed by affinity-purified ^{125}I-goat anti–rabbit Ig. For Ig light chain analyses, rabbit affinity-purified anti-λ- or anti-κ-specific reagents (Litton Bionetics, Inc., Kensington, MD) were used instead of the polyspecific rabbit serum.

The linkage conformation specificity was determined by inhibition of antibody binding to DEX-BSA plates by Dextran B1355 fraction S and Dextran B512 (35). Equal volumes of Dextran B1355 fraction S or Dextran B512 and fragment culture supernatants were added to DEX-BSA-coated microtiter plates, and the inhibition of binding was assessed. Final concentrations of inhibitors were 500 µg/ml. Antibodies inhibited by only Dextran B1355 fraction S were defined as being specific for $\alpha(1 \rightarrow 3)$ linkages, whereas those inhibited by both Dextran B1355 fraction S and Dextran B512 were defined as being specific for $\alpha(1 \rightarrow 6)$ linkages. No antibodies were inhibited by only Dextran B512.

Competitive inhibition solid phase RIA for IdX determinants were performed as previously described (11, 25, 44). Briefly, 96-well microtiter plates were coated with CD3, a monoclonal anti-IdX originally produced by J. Kearney (University of Alabama, Birmingham), and generously supplied by Dr. B. Clevenger (Washington University, St. Louis, MO). Test antibodies were identified as IdX[+] if their inhibition of the binding of ^{125}I-labeled M104E (Litton Bionetics, Inc.) was greater than or equal to an equal concentration of M104E.

Depletion of sIg[+] B Cells from Bone Marrow Cell Populations. sIg^{−} cells from adult bone marrow were prepared by a modification of rosetting techniques already described (20–25, 47, 48). In brief, 10^7–10^9 washed bone marrow cells, obtained as pools from the femurs and tibias of BALB/c or C.B20 mice, were incubated with a 5–10-fold excess of tanned, glutaraldehyde-fixed sheep red blood cells coated with goat anti–mouse IgM and IgG heavy- and light chain–specific antibody (Kirkegaard and Perry Laboratories, Inc., Gaithersburg, MD). Rosetted bone marrow cells (sIg[+]) were removed by centrifugation at 4°C through Ficoll-Hypaque density gradients. 30–50% of the input bone marrow cells were generally recovered in the interface band. At least 90% of sIg[+] lymphocytes were depleted by this method, and the sIg[−] cell preparations possessed <2% sIg[+] lymphocytes, as assessed by direct fluorescence analysis employing fluorescein-labeled rabbit anti–mouse (IgM, IgG, and IgA) F(ab')₂ fragments (Zymed Laboratories, Burlingame, CA) on a Becton-Dickenson FACS IV.

Transfer Fusion. Hybridoma lines were established using the SP2/0 cell line originally described by Shulman et al. (49), in a transfer fusion procedure similar to that previously described (50, 51). Normal B cell fusion partners were obtained by the following protocol: Recipient BALB/c mice were immunized with Hy in CFA, boosted 4 wk later with Hy in saline, and used as recipients 4–8 wk thereafter. 5 d before fusion, recipient mice were irradiated at 800 rad, and 2–4 h later given 10^8 donor C.B20 unfractionated bone marrow cells intravenously. 2 h after the transfer of donor cells, recipient mice received 100 µg DEX-Hy intravenously. Recipient mice were fed water treated with puromycin.

5 d after cell transfers, the recipient mice were killed, their spleens were removed, and a cell suspension was prepared. The number of nucleated cells recovered per spleen ranged between 8×10^6 and 4×10^7, and at least two spleens were pooled per fusion. The spleen cells were mixed with SP2/0 cells at a ratio of 30:1 spleen cells to SP2/0 cells, and fusions were carried out at room temperature using 50% wt/vol polyethylene glycol (3,500 mol wt) in Dulbecco's Modified Eagle's medium containing 15% dimethylsulfoxide (52). Cells were washed after fusion, and plated out in 96-well microtiter plates in hypoxanthine, aminopterin, thymidine (HAT) selective medium. Hybrids were fed twice weekly in HAT media for 10 d, then maintained in hypoxanthine and thymidine 10 d further. Usually, 10–14 d after fusion, wells containing hybrid cells required splitting,

and at this stage the supernatants were collected and assayed for the presence of DEX-specific antibody. Positive hybrids were cloned twice at limiting dilution, and subclones were grown in large quantities. Culture fluids were saved and cells were transferred to pristane-primed BALB/c mice for the production of ascites fluid.

Results

λ-*bearing* α(*1* → *3*) *Linkage–specific DEX-responsive Cells in BALB/c Splenic and sIg⁻ Bone Marrow Cell Populations.* Results of an analysis of DEX-Hy-responsive cells in BALB/c spleen and sIg⁻ bone marrow cells in the splenic fragment culture system are summarized in Table I. The frequency (18.4 cells per 10^8 injected cells) of λ-bearing α(1 → 3) DEX–responsive cells, and the percentage (87%) of the total DEX response this clonotype family represents in the BALB/c spleen cell population were consistent with previous reports (35, 42, 43). We calculate that 1/87,000 injected B cells expressed this phenotype by assuming, as has been previously determined, that 50% of the injected spleen cells are B cells, and that the efficiency of both homing and stimulation in fragment cultures is 4% (1, 3, 53). The frequency of λ-bearing α(1 → 3) DEX–specific cells of BALB/c spleen cell populations transferred into the Igh^b allogeneic C.B20 recipients was quite similar (1/92,000 injected B cells), and also represented 87% of the total DEX-specific responses obtained. Therefore, responsiveness to DEX and the expression of the predominant clonotype family was equivalent when BALB/c spleen cells were stimulated in either Igh^a syngeneic or Igh^b allogeneic environments.

When sIg⁻ bone marrow cells were stimulated with DEX-Hy in either Igh-syngeneic or -allogeneic, carrier-primed, irradiated recipients, the frequencies of λ-bearing α(1 → 3) DEX–responsive B cells were 1.3 and 1.8 per 10^8 injected cells, respectively. The ratio of the frequencies of responsive cells of this clono-

TABLE I

λ-*bearing* α(*1* → *3*) *Linkage–specific DEX-responsive Cells in BALB/c Splenic and sIg⁻ Bone Marrow Cells*

Source of BALB/c cells*	Hy-primed recipient‡	Total cells injected (× 10^6)§	λ-bearing α(1 → 3) DEX– specific monoclonal responses per 10^8 injected cellsǀ	Percent of total anti-DEX responses producing λ-bearing α(1 → 3) linkage–specific antibodies
Spleen cells	BALB/c	500	18.4	87
	C.B20	950	17.7	87
sIg⁻ bone marrow cells	BALB/c	1,800	1.3	81
	C.B20	600	1.8	72

* BALB/c donor mice were 2–4 mo old.
‡ Recipient mice were primed with Hy in CFA, boosted with Hy in saline 4 wk later. 4–6 wk after boosting, they received 1,300 rad followed in 1–4 h by cell transfer.
§ Each recipient mouse received 2.5 × 10^7 pooled spleen cells or 5.0 × 10^7 sIg⁻ bone marrow cells intravenously.
ǀ Antibody-producing clones were detected by RIA of culture fluids collected from days 9–24 of culture.

TABLE II

λ-bearing α(1 → 3) Linkage–specific DEX-responsive Cells in
C.B20 Splenic and Bone Marrow Cell Populations

Source of C.B20 cells	Hy-primed recipient	Total cells injected ($\times 10^6$)	λ-bearing α(1 → 3) DEX–specific monoclonal responses per 10^8 injected cells	Percent of total anti-DEX responses producing λ-bearing α(1 → 3) linkage–specific antibodies
Spleen cells	C.B20	1,300	1.5	12
	BALB/c	2,600	1.0	8
sIg⁻ bone marrow cells	C.B20	1,225	0.2	6
	BALB/c	1,700	1.8	53
Unfractionated bone marrow cells	C.B20	835	0.5	15
	BALB/c	1,100	1.4	47

Procedures and cell treatments as in Table I.

type, on a per-injected-cell basis in BALB/c Ig⁻ bone marrow vs. spleen cells, was 0.07 in the Igh^a recipients, and 0.1 in Igh^b recipients. These ratios are within the range of frequency ratios of responses of sIg⁻ vs. spleen cell populations for numerous antigens studied to date, including 2,4-dinitrophenyl (DNP), phospho-rylcholine (PC), and the hemagglutinin of the PR8 influenza virus (PR8-HA) (20–25). Thus, neither the Igh^a nor Igh^b environment appears to alter the number of responsive BALB/c λ-bearing α(1 → 3) DEX–specific B cells or sIg⁻ B cell precursors.

λ-bearing α(1 → 3) Linkage–specific DEX-responsive Cells in C.B20 Splenic and Bone Marrow Cell Populations.   Previous studies have shown that, in contrast to the response of BALB/c mice, the DEX-specific response of Igh^b mice is relatively low overall, and that none, or only a very small proportion of this response is characterized by antibodies bearing the λ light chain (7–9, 40). In agreement with these reports, the observed frequency of DEX-Hy-responsive C.B20 spleen cells (12.9 per 10^8 injected cells) was lower than that for BALB/c spleen cells (21.2 per 10^8 injected cells). The frequency was the same whether C.B20 spleen cells were stimulated in either the Igh^b syngeneic C.B20 or Igh^a allogeneic BALB/c Hy-primed, irradiated recipients. Of these responding cells, only a small percentage (8–12%), representing fewer than 1/10^6 C.B20 splenic B cells, yielded clones secreting λ-bearing monoclonal antibodies specific for the α(1 → 3) linkage group (see Table II).

As anticipated from the low number of λ-bearing α(1 → 3) DEX–specific C.B20 spleen cells, sIg⁻ C.B20 bone marrow cells stimulated in the syngeneic C.B20 environment yielded only 0.2 λ-bearing α(1 → 3) DEX–specific responses per 10^8 injected cells. In marked contrast, however, when this same cell population was stimulated in the Igh^a allogeneic BALB/c environment, the frequency of λ-bearing α(1 → 3) DEX–specific responses was ninefold higher (1.8 per 10^8 injected sIg⁻ bone marrow cells), and represented 53% of all DEX-Hy-responsive cells in this population. Thus, there was an equivalent number of potential λ-

bearing $\alpha(1 \rightarrow 3)$ DEX–responsive cells in both the C.B20 and BALB/c sIg$^-$ bone marrow cell populations. However, this population of C.B20 sIg$^-$ precursor cells could only be stimulated to respond to DEX-Hy in the Igh^a allogeneic carrier-primed BALB/c environment.

These findings indicate that, although equivalent numbers of λ-bearing $\alpha(1 \rightarrow 3)$ DEX–specific precursor cells emerge from C.B20 and BALB/c bone marrow stem cells, C.B20 cells of this phenotype can neither be stimulated by DEX-Hy in their syngeneic environment, nor can they mature into splenic B cells in situ. To determine the developmental stage at which λ-bearing $\alpha(1 \rightarrow 3)$ DEX–specific C.B20 B cells are inactivated, we assessed the frequency of DEX-Hy-responsive cells in unfractionated C.B20 bone marrow cells (Table II). Again, the number of λ-bearing $\alpha(1 \rightarrow 3)$ DEX–specific cells responsive in syngeneic Igh^b recipients was substantially less than in the Igh^a environment. However, the number of λ-bearing $\alpha(1 \rightarrow 3)$ DEX–responsive cells, even in BALB/c recipients, was lower on a per-injected-cell basis (1.4 per 10^8 injected cells) than was the frequency for sIg$^-$ cells (1.8 per 10^8 injected cells). Thus, it appears that there are few, if any, λ-bearing $\alpha(1 \rightarrow 3)$ DEX–specific sIg$^+$ bone marrow B cells in C.B20 mice. This implies that potentially responsive C.B20 precursor cells of this clonotype are apparently rendered nonresponsive to DEX-Hy (silenced) by an environmental regulatory mechanism functioning in temporal association with the acquisition, in the bone marrow, of their sIg receptors.

λ-bearing $\alpha(1 \rightarrow 3)$ Linkage–specific Hybridoma Monoclonal Antibodies Derived from C.B20 Bone Marrow Cells by Transfer Fusion. To qualitatively study the DEX-specific antibodies derived from C.B20 bone marrow cells, we have obtained hybridomas by the transfer of C.B20 bone marrow cells to permissive Hy-primed, irradiated BALB/c recipients for stimulation and subsequent transfer fusion (50, 51). From two separate fusions, a total of 15 DEX-specific hybridomas were obtained. Of these, nine (60%) were κ-bearing and $\alpha(1 \rightarrow 6)$ linkage–specific, and six (40%) were λ-bearing and $\alpha(1 \rightarrow 3)$ linkage–specific. This represents a distribution of specificities similar to that found using C.B20 bone marrow cells in the splenic fragment culture system. All six λ-bearing $\alpha(1 \rightarrow 3)$ DEX–specific hybridoma antibodies of C.B20 origin bore the IdX idiotypic determinant, as defined by their inhibition of binding of M104E to the monoclonal anti-IdX antibody, CD3.

Discussion

The composition of the expressed primary B cell repertoire is the product of both the utilization of V region gene elements in cells emerging in the adult bone marrow, and receptor-specific environmental influences acting on maturing cells of each unique clonotype. Previous studies in this laboratory (23) have shown that, at least for the immune response to one antigen (PR8-HA), there is substantial repertoire diversity before V region–specific environmental regulatory effects are encountered. However, the extent to which environmental influences, in the form of tolerance induction and/or idiotypic network interactions, may influence the expression of the mature B cell repertoire is still not known. As clone-specific environmental influences on the B cell repertoire are presumably mediated by the clonally distributed V regions of the sIg receptors, bone marrow cells depleted of sIg$^+$ cells should contain only sIg$^-$ pre-B cells that have not been subjected to environmental regulatory mechanisms. In previous

studies (20–25) we have shown that cells within this sIg⁻ pool apparently acquire sIg receptors and respond to antigenic stimulation in fragment cultures. Additionally, these cells are relatively immature by the criteria of tolerance susceptibility and potential for isotype expression (20–25). Thus, by analyzing responses of these prereceptor B cells, stimulated in different environments, the selective effects of genetic and of postgenetic environmental influences on the repertoire can be evaluated.

Antigens that induce immune responses unique to various murine strains have been useful tools for examining mechanisms affecting B cell repertoire expression (4–13). In these systems, the phenotypic differences of the antibody responses may be the result of either polymorphism in the relevant V region gene pools of various strains, or differences in the postgenetic environmental molding of the B cell repertoire in each strain. For several responses, including murine responses to azophenylarsonate (ARS), PC, and DEX, strain-specific differences have already been attributable to polymorphisms in inherited heavy or light chain V region genes (54–56). Polymorphism in genes other than those encoding Ig V regions may also affect repertoire expression. The control of the frequency of mature PC responsive B cells in various murine strains appears to be under multigenic control (57), and in mice with the CBA/N sex-linked immunologic defect, PC-responsive cells are generated normally, but are eliminated by environmental mechanisms once they express their sIg receptors (25).

The studies in this report have used the splenic fragment culture system to evaluate the origin of the well-established phenotypic difference in the λ-bearing $\alpha(1 \rightarrow 3)$ DEX–specific response in BALB/c (Igh^a) (high responder) and C.B20 (Igh^b) (low responder) mouse strains, by evaluating the response to DEX-Hy of spleen cells and sIg⁻ bone marrow cells from these two strains in Hy-primed BALB/c and C.B20 recipients. The frequency of λ-bearing $\alpha(1 \rightarrow 3)$ DEX–responsive BALB/c splenic B cells was ~1:5 × 10⁶ injected cells, and represented 87% of the total response to Dextran B1355S in either Igh^a or Igh^b carrier-primed irradiated recipients. This frequency is consistent with that reported by other laboratories (35, 40, 42, 43) using the splenic fragment culture technique to assess precursor cell populations responsive to DEX in BALB/c mice, and thus confirms the conclusion of these laboratories that the λ-bearing DEX-specific antibody clonotype family dominates the DEX-responsive splenic B cell population even before overt immunization. The frequency of sIg⁻ bone marrow cells of this clonotype that are responsive in Igh^a and Igh^b recipients was 1.3 and 1.8 per 10⁸ injected cells, respectively. Again, this represented the vast majority of cells responsive to DEX. The ratio of splenic B cells vs. sIg⁻ precursor cells of this clonotype in BALB/c mice was consistent with the ratio of splenic to sIg⁻ responsive cells observed for several antigens, including influenza virus PR8 antigens, PC, and DNP (20–25). Thus, as was the case for PC (25), the high frequency of precursor cells of the predominant clonotype family appears to reflect a similarly high rate of expression of the relevant V regions in the bone marrow stem cell pool, rather than environmental up-regulation. These findings are consistent with the interpretation of findings of other investigators concerning the generation of Igh^b cells bearing the NP^b idiotype (26) and BALB/c cells bearing the MOPC 460 idiotype (27). Thus, in all instances reported to date, the expression of a high frequency of a given clonotype or clonotype family in the

mature primary B cell pool can be attributed to a high level of expression of the V regions necessary for that clonotype in B cell precursors in the bone marrow generative cell pool.

Recently, Near et al. (54), have reported that the expression of the V region genetic elements responsible for the p-azophenylarsonate (ARS) IdX in A/J mice is consistent with the extent of use of each of these elements per se within the entire preimmune B cell population. These investigators concluded that the level of expression of a given clonotype is the product of the stochastic probability of rearrangements of the appropriate gene elements from within the V region gene pool. This occurrence would be relatively rare for the ARS IdX, which is dependent on the use of the single appropriate V_H, V_L (variable region light chain), and J_L, with little leeway with respect to permisable D, J_H, and junctional diversity (58). Thus, it is not surprising that Sigal (59) demonstrated that the representation of B cells expressing ARS IdX in A/J primary splenic B cells is at most only 1 in 2.8×10^6 B cells. In this context, the much higher expression of the λ-bearing $\alpha(1 \rightarrow 3)$ DEX–specific clonotype family in both splenic and sIg^- BALB/c precursor cells would require a higher probability of the expression of involved V region genetic elements for it to be the product of stochastic mechanisms. The λ_1 light chain, which is required for the expression of this specificity, is present on 2–4% of all BALB/c serum antibodies and B cells (26, 56, 60). Since there is only one V region and one J region included in the λ_1 complex (14), as opposed to hundreds of V_κ genes, which recombine with four J_κ genes (17), this one V_λ, J_λ sequence is apparently used at a higher overall frequency than the average use of given V_κ, J_κ combinations would be. Therefore, we anticipate that clonotypes that use the λ_1 gene would have a substantially higher probability of expression than clonotypes using most κ genes. Additionally, an analysis of the λ-bearing $\alpha(1 \rightarrow 3)$ DEX–specific clonotype family in BALB/c mice showed that, whereas apparently only one V_H gene was used in this family, rearrangement with any of several D segments, and at least two J_H segments, could yield an appropriate H chain (44, 61). Thus, it is perhaps not surprising that B cells expressing appropriate light and heavy chains for the expression of the λ-bearing $\alpha(1 \rightarrow 3)$ DEX–specific clonotype family could be many times higher, as demonstrated herein, than those expressing the ARS IdX in A/J mice. However, the postulated stochastic use of V gene segments may be an oversimplified explanation for clonotype expression, since it could not account for the high frequency of expression of the TEPC-15 idiotype–bearing PC-responsive B cells in the sIg^- precursor pool of BALB/c mice (25). TEPC-15 expression can use only a single V_H, D, J_H, V_L, and J_L (62). Additionally, it has been shown (25) that the high frequency of TEPC-15 expression is due to a high frequency of occurrence of this clonotype from within the bone marrow pool, rather than the expression of unusually large clones of this clonotype. This would indicate that, while the stochastic probability of the rearrangement of V region gene elements from within the entire gene pool may be one of the factors that determine the level of expression of certain clonotypes, the expression of at least some specificities may reflect a higher than random probability of gene rearrangement of various V region gene elements.

The frequency of C.B20 splenic B cells that yield λ-bearing $\alpha(1 \rightarrow 3)$ DEX–specific antibodies is less than a tenth the frequency found in splenic B cells of

BALB/c mice, regardless of the Igh haplotype of the carrier-primed recipients used. This finding is consistent with the well-established phenotypic difference in both serum antibodies and precursor cells in these two strains (7–9, 40, 43). Remarkably, the frequency of λ-bearing $\alpha(1 \rightarrow 3)$ DEX–specific antibody responses obtained from sIg⁻ C.B20 bone marrow cells transferred to carrier-primed BALB/c (Igh^a) recipients is higher than the frequency of responses of this specificity found in C.B20 splenic B cells on a per-injected-cell basis, and is at least as high as the frequency observed in sIg⁻ bone marrow cells of the responder BALB/c strain. This finding indicates that there is no deficiency in the capacity of stem cells of Igh^b mice to generate precursor cells expressing the appropriate rearranged V genes necessary for the λ-bearing $\alpha(1 \rightarrow 3)$ DEX–specific cells. The finding that cells of this specificity are apparently inactivated or silenced during their maturation implies that the classical phenotypic polymorphism in the expression of λ-bearing $\alpha(1 \rightarrow 3)$ DEX–specific antibodies between Igh^a and Igh^b mice is the product of environmental regulatory mechanisms, as opposed to differences in V region gene expression.

To verify this surprising finding by an additional methodologic approach, and to obtain sufficient antibodies and cells for qualitative analyses, we have established λ-bearing $\alpha(1 \rightarrow 3)$ DEX–specific hybridomas using C.B20 bone marrow cells. The proportion of λ-bearing $\alpha(1 \rightarrow 3)$ DEX–specific hybridomas obtained from transfer fusions using C.B20 bone marrow cells and BALB/c recipients was consistent with the proportional representation of monoclonal fragment culture responses of this specificity among all responses to DEX-Hy obtained using the same donor cell–recipient combination. Characterization of these hybridomas indicates that the antibodies bear the IdX idiotypic determinant common to most BALB/c antibodies of this specificity (7–9, 43, 44). This finding represents the first demonstration, in the absence of any idiotype-selective manipulation, of the natural potential of C.B20 (Igh^b) mice to generate antibody-producing cells that secrete λ-bearing $\alpha(1 \rightarrow 3)$ DEX–specific antibodies expressing the IdX idiotypic determinants.

Recent studies from several laboratories (34–39, 63, 64) have demonstrated the induction of silent clonotypes in several responses. For example, the ARS IdX⁺ antibodies, normally a characteristic of responses of A/J mice, have been induced in BALB/c mice (63, 64). Additionally, the bacterial levan A481d clonotype (34), and the DEX IdX⁺ clonotypes normally characteristic of BALB/c mice (38, 43), have been induced in Igh^b mice. In all of these cases, expression of the silent clonotype required selective idiotypic manipulations (administration or induction of the antiidiotype, followed, after a considerable rest period, by antigenic stimulation), and thereby differ significantly from the findings reported herein. By exerting a selective manipulation, a rare clone bearing the marker in question may be expanded, resulting in a large population of cells of the normally silent clonotype being present at the time of antigenic stimulation. The presence of this silent clonotype therefore may bear little direct relationship to the animal's naturally expressed repertoire. In the one system wherein V region sequence data is available, the ARS IdX⁺ response of BALB/c mice, the newly selected clonotype in BALB/c mice uses a totally different V$_H$ gene segment than that used by A/J B cells (64). In the studies described in this report, C.B20 sIg⁻ bone marrow cells of the λ-bearing

$\alpha(1 \rightarrow 3)$ DEX–specific clonotype respond to antigen at the same frequency as BALB/c sIg⁻ bone marrow cells of this clonotype with no prior selective idiotypic manipulation. Therefore, these cells presumably represent the direct expression of the genetically encoded repertoire of C.B20 mice.

Since dextran is a naturally occuring environmental antigen, several mechanisms could potentially account for the low expression of λ-bearing $\alpha(1 \rightarrow 3)$ DEX–specific responses in Igh^b mice. These include tolerance, clonal dominance of κ-bearing B cells, antiidiotypic suppression and clonotype-specific differences in T cell help. In the latter two instances, stimulation of B cells in Igh allogeneic rather than syngeneic recipients could facilitate their activation. Specifically, previous experiments (45) have shown that both immature and mature primary B cells are highly susceptible in fragment culture to inactivation by antibody-specific immunoregulatory mechanisms which can, for example, reside in recipients primed with both the hapten and carrier rather than the carrier alone. Importantly, this suppression is totally specific for primary B cells that share the Igh haplotype of the recipient. The finding that sIg⁻ C.B20 λ-bearing $\alpha(1 \rightarrow 3)$ DEX–specific precursor cells respond in the carrier-primed Igh^a allogeneic but not the Igh^b syngeneic environment suggests the presence of a preexisting Igh-linked suppressive network in the C.B20 recipients similar to that induced by hapten-carrier (45) or viral (46) immunization. In this context, these findings are compatible with the conclusion that natural exposure of C.B20 but not BALB/c mice to dextran induces an environmental milieu that downregulates C.B20 λ-bearing $\alpha(1 \rightarrow 3)$ DEX–specific B cells. The C.B20 suppressive environment does not affect the expression of BALB/c splenic or sIg⁻ precursor cells of this clonotype. This suggests that the C.B20 environment supplies sufficient antigen processing and T cell help for the stimulation of λ-bearing $\alpha(1 \rightarrow 3)$ DEX–specific clones, and may imply that the V regions used by these two strains for this response differ in amino acid sequence, or that the suppressive mechanism is allotype-dependent, as has been previously reported (46) for responses to influenza. The demonstration that fragment cultures derived from C.B20 recipients contain a naturally acquired suppressive mechanism may also implicate the same mechanism in the suppression of λ-bearing $\alpha(1 \rightarrow 3)$ DEX–specific C.B20 B cells during their development in situ. If this is the case, then the demonstration of the presence of DEX-responsive λ-bearing $\alpha(1 \rightarrow 3)$–specific cells in sIg⁻ bone marrow cells, and their absence in the mature bone marrow and splenic B cells in C.B20 mice may represent the first evidence of a role for naturally acquired network-type regulation in fashioning the primary B cell repertoire.

Summary

The immune response to dextran is characterized by marked phenotypic differences among murine strains. In particular, Igh^a strains, as opposed to strains of other Igh haplotypes, respond relatively vigorously to dextran B1355 fraction S (DEX), producing predominantly antibodies bearing the λ light chain, and specific for the $\alpha(1 \rightarrow 3)$ glucose linkage. We have investigated this disparity in BALB/c (Igh^a) vs. C.B20 (Igh^b) mice at the individual precursor cell level. Consistent with previous findings (7–9, 35, 40, 42, 43), there was a 10-fold higher frequency of λ-bearing splenic B cells specific for the $\alpha(1 \rightarrow 3)$ linkage in Igh^a mice. As with previously studied (25–27) predominant specificities, the

origin of this high frequency of λ-bearing $\alpha(1 \rightarrow 3)$ DEX–specific B cells appears to be a reflection of a high expression of this specificity in surface Ig (sIg)–negative cells emerging from the bone marrow generative cell pool. Surprisingly, although C.B20 mice (Igh^b) have a low frequency of λ-bearing $\alpha(1 \rightarrow 3)$ DEX–specific B cells in their mature primary splenic population, the frequency of precursor cells of this clonotype in their sIg$^-$ bone marrow cell population is equivalent to that of BALB/c sIg$^-$ cells. These cells could only be stimulated in allotype allogeneic (Igh^a), as opposed to allotype syngeneic (Igh^b), carrier-primed irradiated recipients. This finding was confirmed by the finding that a high proportion of antidextran hybridoma cell lines derived from C.B20 bone marrow cells produced λ-bearing $\alpha(1 \rightarrow 3)$ DEX–specific antibodies that were IdX$^+$. These findings have led us to conclude that the well-established phenotypic difference between Igh^a and Igh^b mice with respect to the expression of λ-bearing $\alpha(1 \rightarrow 3)$ DEX–specific antibody responses is not, as previously assumed (7–9), the result of an inability of Igh^b mice to generate B cells of this clonotype, but rather, is the product of environmental, possibly antiidiotypic, silencing of cells of this clonotype as they mature in Igh^b mice.

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