IDIOTYPE PROFILE OF AN IMMUNE RESPONSE

II. Reversal of the Relative Dominance of Major and Minor Cross-Reactive Idiotypes in Arsonate-Specific T-Independent Responses*

BY JOHN D. CONGER, EDMUNDO LAMOYI,‡ GEORGE K. LEWIS, ALFRED NISONOFF,‡ AND JOEL W. GOODMAN§

From the Department of Microbiology and Immunology, University of California at San Francisco, San Francisco, California 94143; and the ‡Rosenstiel Research Center, Department of Biology, Brandeis University, Waltham, Massachusetts 02254

The antibody (Ab)[1] response of A/J mice to the *p*-azobenzenearsonate (ABA) hapten includes a substantial cross-reactive idiotypic (CRI or IdX) component (1). Serological (2) and amino acid sequence (3–5) studies on ABA-specific monoclonal Abs (mAb or HP) produced by hybridomas (derived using A/J ABA-hyperimmune splenocytes) have indicated that all the HP examined are unique. However, CRI^+ HP can be shown to share one or more conserved IdX determinant(s) (6). Despite the microheterogeneity of the family of molecules bearing the major CRI, it appears that the heavy chain variable (V_H), and probably the light chain variable (V_L) regions are each encoded by a single germline gene that undergoes somatic variation (7, 8). Recently, Milner and Capra (9) showed that the available N-terminal V_H sequences could be classed into three distinct V_H homology families, which might be termed: (*a*) the CRI_A group (prototypes HP: R16.7, 93G7 [2], and 36–65 [4]); (*b*) the $CRI_{c'}$ family (prototypes: HP 92D5 [9] and 36–60 [10]); and, (*c*) the 96B8 family, which has not previously been serologically detected as a distinct IdX entity in the Ab response. It seems likely that these homology families result from the expression of at least three different V_H genes present in the strain A germline.

A comparable complexity was recently demonstrated by Brown et al. (11) to exist at the serological level among induced anti-ABA Abs of A/J mice. Utilization of an anti-Id serum, prepared against HP R16.7, which had previously been shown to bear virtually all of the major CRI determinants found among induced

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§ To whom correspondence should be addressed at the Dept. of Microbiology and Immunology, University of California at San Francisco, San Francisco, CA 94143.

[1] *Abbreviations used in this paper:* Ab, antibody; ABA, *p*-azobenzenearsonate; AD8, a rat monoclonal anti-CRI antibody that defines Id-AD8; Ag, antigen; Bru, killed *Brucella abortus*; CGG, chicken gamma globulin; CRI_A, a major cross-reactive idiotype related to that of HP R16.7; $CRI_{c'}$, A/J anti-ABA Ab idiotype related to CRI_c of BALB/c; CRI_m, minor cross-reactive idiotype(s); HB, hydroxybenzimidate; HP, hybridoma product (antibody); IdX, cross-reactive idiotype; KLH, keyhole limpet hemocyanin; LPS, lipopolysaccharide; mAb, monoclonal antibody; PFC, plaque-forming cell(s); RC, erythrocytes; TD, T cell dependent; TI-1, type 1 T-independent (Ag); V_H, heavy chain variable region; V_L, light chain variable region.

Abs (2), permitted the further dissection of the Id nature of the response. It showed that the following IdX relationships existed among A/J serum anti-ABA Abs: (*a*) ~30–50% of these were bound by rabbit (Rbt) anti-CRI sera, so these typed as CRI^+; (*b*) ~80% of the CRI^+ Abs could also be bound by Rbt anti-Id R16.7, and, thus, these were (by definition) CRI_A^+; (*c*) the fraction of CRI^+, CRI_A^- Abs was referred to as CRI_m^+, i.e., positive for minor CRI (12); and (*d*) about one-third of CRI_m^+ Abs were Id-related to CRI_c, the predominant IdX of BALB/c anti-ABA Abs (10, 13). We here refer to the last-mentioned group as $CRI_{c'}^+$. In addition, a substantial fraction lacks any demonstrable intrastrain cross-reactive idiotype. These relations are illustrated in Fig. 1.

Recently, we, as well as others (14), have become interested in the Id character of the Ab response induced by T cell-independent (TI) antigenic forms of the ABA hapten. In the present studies, we used (killed) *Brucella abortus* (Bru) organisms and *S. typhimurium* lipopolysaccharide (LPS) as the directly mitogenic carriers. These were haptenated with ABA and employed in sub-polyclonal-activating doses, i.e., as TI-1 Ags. The results indicate that these Ags induce a different Id profile than that seen in responses to typical T-dependent (TD) Ags, such as ABA-KLH.

Materials and Methods

Mice. Most (~75%) A/J mice were obtained from the Jackson Laboratory, Bar Harbor, ME. Male and female mice were used at 6–16 wk of age, except for adoptive transfer recipients, which were female "retired breeders."

A smaller number of mice was obtained from the U.C. Berkeley breeding facility. Breeders from the Jackson Laboratory are introduced there at every third generation to

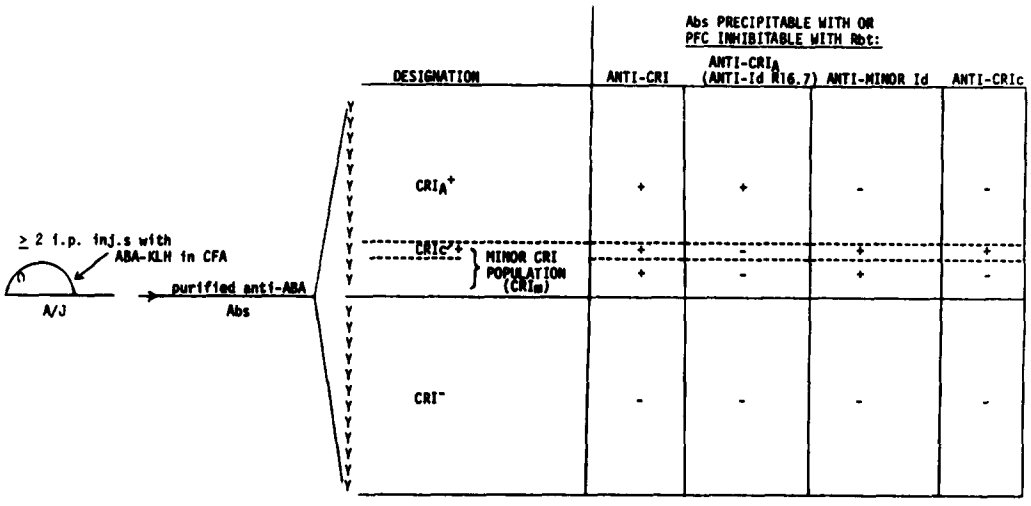

FIGURE 1. Relationships and relative dominance levels of IdX expressed in the A/J hyperimmune Ab response to ABA-KLH. Based on data of Brown et al. (11). The proportions shown should be considered as averages for a large pool of mice and not typical of all individual animals. Anti-CRI_m is a Rbt anti-CRI adsorbed on an HP R16.7-Sepharose 4B column. Anti-CRI_c is Rbt anti-CRI prepared against BALB/c hyperimmune anti-ABA Abs elicited by ABA-KLH.

maintain uniformity.

Breeding pairs of CBA/N mice were obtained from the National Institutes of Health. F_1 progeny of CBA/N and A/J mice were bred at U.C., San Francisco.

Antigens and Immunization. ABA-KLH and ABA-CGG were prepared as described (15). ABA-*Brucella* was prepared according to Lucas and Henry (14), ie., by direct diazotization. Purified *Salmonella typhimurium* lipopolysaccharide (LPS) was purchased from the List Biological Laboratories, Inc., Campbell, CA, and reacted with an equal mass quantity (10 mg) of the *N*-hydroxysuccinimide conjugate of *p*-hydroxyphenylpropionic acid (the latter derivative being prepared according to Pohlit et al. [16]) in 2 ml dimethyl sulfoxide at room temperature. The product was extensively dialyzed against borate buffer (pH 9.0) and then phosphate-buffered saline (pH 7.3), before conjugation with 1 ml 1 M ABA-diazonium salt and further dialysis.

The TD Ags were emulsified in complete Difco adjuvant H37Ra (Difco Laboratories, Detroit, MI) and injected i.p., ABA-LPS was injected i.p. in PBS only, while ABA-Bru (in PBS) was injected i.v. into a lateral tail vein.

Antisera. The standard Rbt anti-CRI was prepared according to Ju et al. (17). Preparation of anti-Id (R16.7) and anti-CRI_m (anti-CRI adsorbed on an HP R16.7-Sepharose 4B column) has been described elsewhere (11, 13).

Monoclonal Abs to Thy-1.2 and Lyt-2.2 were produced by the murine hybridoma cells, HO-13 and HO-2.2 (American Type Culture Collection, Bethesda, MD). For Ab-mediated cytotoxic elimination of lymphoid cell subsets, optimal dilutions of the appropriate supernate were employed along with agarose-adsorbed guinea pig complement (C; Gibco Laboratories, Grand Island, NY).

A preparation of the rat anti-CRI mAb AD8 was provided by P. Hornbeck, U.C. San Francisco (18). For plaque inhibition 10 μl of a 0.5 mg/ml solution in PBS was used.

Cultures. Splenocytes were adjusted to either 5×10^6/ml (for ABA-Bru or ABA-LPS cultures) or 10^7/ml (for SRC cultures) in culture medium (RPMI-1640 + 10% fetal calf serum; Gibco Laboratories), plated in 0.5-ml cultures, and left for 3 d at 37°C, without rocking (SRC cultures were rocked), in an atmosphere of 7% O_2, 10% CO_2, and 83% N_2. Ag concentrations used were: 1/200 final dilution ABA-Bru, 1 μg/ml ABA-LPS, or 15 μl per culture of a 0.1% suspension of SRC (Colorado Serum Co., Denver, CO). Triplicate cultures were pooled and assayed. Results from at least four separate experiments are given for each in vitro data point.

Hemolytic Plaque Assay. The procedure for the plaque assay (15) was modified so that all antisera (anti-IdX, anti-IgM, or anti-IgG) and C, were added directly to the agar. The conjugation of target erythrocytes with the ABA-HB-imidoester was altered from the protocol of Isakson et al. (19) as follows: (*a*) the concentration of RC in the haptenating mixture was 1/2.5 rather than 1/5; (*b*) the haptenation mixture was rocked for no more than 13 h; and (*c*) horse- or goat-RC were substituted for sheep-RC. (HB refers to the *p*-hydroxybenzimidate group.)

Serological Assays. Anti-ABA Abs were isolated by affinity chromatography on an ABA-BGG-Sepharose 4B column (13). Radiolabeling of Abs was done in the solid phase as described by Herzenberg and Herzenberg (20). Briefly, 10 μg of a pool of anti-ABA Abs was passed over an ABA-BGG-Sepharose 4B column, washed, iodinated with ^{125}I by the chloramine-T method (21) and washed again. Labeled Abs were eluted with 0.5 M *p*-arsanilic acid plus 1 mg/ml BSA, pH 8.0. Eluted Abs were exhaustively dialyzed. Anti-ABA titers were determined as given elsewhere (1).

Adoptive Transfers. In the adoptive transfer protocol (22) recipient mice were irradiated (600 rad) ~6 h before reconstitution with splenocytes; and were challenged with Ag (0.2 ml 1.25% ABA-Bru solution i.v. or 0.2 ml 10% SRC, i.p.) 12 h after reconstitution. Mice were maintained on acidified water.

Id Calculations and Statistics. Nonspecific plaque numbers were subtracted from all PFC numbers before calculation of percent IdX^+ PFC by the formula given previously (15). The statistical significance of differences of values in two distinct groups was

determined using the nonparametric (Wilcoxon) rank sum test or Student's *t* test for independent groups (23).

Results

Disparities Between Rbt Anti-CRI and a Monoclonal Anti-V Region Ab in Inhibition of ABA-Specific PFC Induced by TI or TD Ags. Initial indications that the Id character of the Ab response to ABA, induced by TI Ags differed from that induced by TD ABA Ags came from PFC inhibition using a monoclonal rat Ab (AD8 kindly supplied by P. Hornbeck, UCSF) that defines Id-AD8 (18). It reacts with an idiotope present on several CRI_A^+ HP.

A/J mice were immunized with one or more injections of either the TI-1 Ags ABA-*Brucella* or ABA-LPS or the TD Ags ABA-KLH or ABA-CGG (the latter two in adjuvant). Percentages of primary or hyperimmune ABA-specific PFC inhibitable with Rbt anti-CRI or with AD8 are given in Table I. The data show that CRI expression correlates well with the expression of Id-AD8 in TD, but not TI, responses. The ratio values obtained indicate that >80% of CRI^+ PFC (defined by Rbt anti-CRI) induced by hyperimmunization with ABA TD Ags, and >90% of those in the early primary response to ABA-KLH are also Id-$AD8^+$. In contrast, only slightly greater than half the CRI^+ PFC in the in vivo TI responses were also Id-$AD8^+$. Incorporation of both AD8 and Rbt anti-CRI into the plaquing medium did not result in additive inhibition (data not shown). Individual inhibition ratio values are given for several mice (Table I, lines *1* and *4*). They show that, in contrast to the primary ABA-KLH response, where the correlation is very close for all animals, the individual ratio values vary widely in the primary ABA-Bru response, from 0.21 to 0.82. The mean ratio values between these primary responses (TI vs. TD) differ significantly (probability of

TABLE I

Idiotype and Idiotope Expression in ABA-Specific TD and TI PFC Responses

Group	Antigen*	Type	Response‡	% PFC Inhibition by:				Inhibition ratio§ AD8/RbtαCRI	
				RbtαCRI		AD8		IgM	IgG
				IgM	IgG	IgM	IgG		
1	A-K	TD	1°	84 ± 10	92 ± 6	79 ± 12	91 ± 6	0.94 (0.93, 0.97, 0.90, 0.97)	0.99 (0.97, 1.01, 1.00, 0.98, 0.99)
2	A-K	TD	Hyp	—‖	40 ± 20	—	36 ± 18	—	0.89
3	A-CGG	TD	Hyp	—	34 ± 14	—	26 ± 18	—	0.83
4	A-Bru	TI	1°	87 ± 9	—	48 ± 18	—	0.56 (0.54, 0.41, 0.82, 0.53, 0.38, 0.71, 0.21, 0.70, 0.54, 0.71)	—
5	A-Bru	TI	Hyp	—	60 ± 17	—	33 ± 25	—	0.53
6	A-Bru	TI	1° in vitro	72 ± 2	—	1 ± 19	—	0.01	—
7	A-LPS	TI	1°	77 ± 19	—	45 ± 20	—	0.55	—
8	A-LPS	TI	1° in vitro	58 ± 6	—	6 ± 4	—	0.13	—

* A-K is ABA-KLH; A-Bru is ABA-*Brucella*; A-CGG is ABA-Chicken gamma globulin. A-LPS and TD Ags (in adjuvant) were injected i.p. while A-Bru (0.2 ml of a 1.25% solution) was injected i.v.

‡ 1° = primary response assayed on day 9 or 10 for TD Ags and either day 3 or 4 for TI responses. *Hyp*, hyperimmune, at least three injections total. Geometric Mean PFC per spleen or per culture (in vitro responses) were for groups 1–8: 27,447 (μ) 18,215 (γ); 433,653 ($\mu + \gamma$); 82,454 ($\mu + \gamma$); 9799 (μ); 219,696 ($\mu + \gamma$); 633 (μ); 6248 (μ); 518 (μ), respectively.

§ Arithmetic mean of ratios for each individual animal. Since in a few cases all mice were not tested with both anti-Id reagents, this value may differ slightly from the ratio of mean values given in the RbtαCRI and AD8 columns.

‖ In Hyp responses to A-K or A-Bru, IgM PFC per spleen were relatively low and so were usually not determined separately from IgG PFC. Primary TI responses consisted solely of IgM PFC. Values given are arithmetic means ± 1 SD.

equality, $P < 0.001$). The most striking disparity is seen in the primary in vitro responses to the TI Ags. Here Id-AD8 expression is practically nil even though most PFC are CRI^+.

Further Evidence that CRI_A Does not Dominate the CRI^+ Component of ABA-Specific TI Ab Responses. In the next assays, a polyspecific rabbit antiserum directed to the Id of HP R16.7 (i.e., the defining antiserum for CRI_A positivity) was used to examine antibody responses. A/J mice hyperimmunized with ABA-Bru produced sufficiently high titers of anti-AAB Abs (mostly IgG) for serological analysis. Sera were obtained from A/J mice hyperimmunized (>3 injections) with either ABA-Bru (5 mice) or ABA-KLH (4 mice) and tested for total content of anti-ABA Abs and the proportion that was CRI_A^+. The data in Table II indicate, first, that the anti-ABA titers of the ABA-Bru sera averaged ~20 times lower than those of the ABA-KLH sera. This result is different than that seen in the plaque assay, in which hyperimmune responses to these two Ags were often comparable (Table I). The discrepancy may reflect the fact that the serum Ab assay detects Ab accumulation, whereas the PFC analysis provides an "instantaneous" picture only.

Anti-ABA Abs from serum samples were affinity-purified and tested for their capacity to inhibit the binding of ^{125}I-labeled HP R16.7 to Rbt anti-CRI (Table II, column *3*). An average of 39 ng of Abs from the ABA-KLH sera was required to cause 50% inhibition, whereas ~7 times as much—282 ng—was required from the ABA-Bru sera. This demonstrates that, on a proportional basis, ABA-specific Abs induced by ABA-KLH contained seven times as much CRI_A as those induced by ABA-Bru. However, a substantial fraction of the Abs in the anti-ABA-Bru sera was nonetheless CRI^+, as shown by the data in the last four columns of Table II. A pool of ABA-specific Abs from the ABA-Bru sera was prepared by combining equal weight quantities from each of the samples, and labeled with ^{125}I. A total of 42% of the Ab (which is 60% of the maximum precipitable by anti-Fab) could be precipitated with the anti-Id (R16.7) and anti-CRI_m. (Anti-CRI_m is a Rbt anti-CRI serum preadsorbed on an HP R16.7-

TABLE II

CRI_A and CRI_m Expression in Serum Abs Induced by ABA-KLH or ABA-Bru

Ag*	Anti-ABA titer‡	Anti-ABA Ab required for 50% inhibition§	% Labeled anti-ABA Ab bound by anti:‖			
			Mouse Fab	CRI	Id R16.7	CRI_m
	μg/ml	*ng*				
ABA-KLH	3,675 ± 826	39 ± 19 (28, 49, 60, 18)	NT	72	55	NT
ABA-Bru	181 ± 136	282 ± 136 (115, 358, 450, 312, 176)	70	NT	12	30

* Sera were from mice immunized three times with ABA-KLH (i.p. in adjuvant) or four times with ABA-Bru (i.v.).

‡ Arithmetic means ± 1 SD, column 2 and 3.

§ Each test utilized 10 ng of ^{125}I-labeled HP R16.7 and slightly less than an equivalent amount of Rbt anti-CRI.

‖ Values given represent plateau levels of precipitation for each reagent. Excess goat anti-Rbt Fc was used to precipitate complexes. *NT*, not tested.

Sepharose 4B column.) Interestingly, more than twice as much material was precipitable with the anti-CRI_m serum as with the anti-Id R16.7 serum (30% vs. 12%, respectively). This result has never been seen with anti-ABA Abs induced by ABA-KLH. For comparison, results obtained using pooled anti-ABA Abs from ABA-KLH sera (different from those used in the inhibition assay) are also presented in Table II. 72% of these Abs were precipitable with Rbt anti-CRI and 55% with Rbt anti-Id R16.7, indicating that 76% of the CRI^+ Abs were CRI_A^+, while only ~24% were CRI_m^+. Except for the somewhat high percentage of CRI^+ Abs (30–50% is more typical), this result is similar to those reported previously by Brown et al. (11).

The same question was explored by plaque analysis, using anti-Id R16.7 as an inhibitor. The results in Table III are similar to those obtained using AD8 as an inhibitor of plaques. The ratio values (anti-Id R16.7/anti-CRI) of 0.92 and 0.96 for ABA-CGG and ABA-KLH, respectively, indicate that >90% of the CRI^+ PFC induced by these TD Ags were CRI_A^+. In contrast, only ~50–60% of CRI^+ PFC induced in vivo by ABA-Bru or ABA-LPS, respectively, were CRI_A^+. Once again, these lower average values reflect the presence of some individual animals with a high, and some with a low relative proportion of CRI_A. Note also that CRI_A expression was again very weak in the primary in vitro response to ABA-Bru. <30% of the CRI^+ PFC were CRI_A^+ and this figure may actually be somewhat artifactually inflated due to some nonspecific inhibitory activity (up to 15%) of the particular Rbt anti-Id R16.7 used.

$CRI_{c'}$ Is Only Weakly Expressed in the TI Responses. The preceding data indicate that one or more members of CRI_m is at least equi-dominant with CRI_A in in vivo responses to ABA TI-1 Ags, and considerably more preponderant in in vitro responses to the same Ags. The possibility was considered that most of the CRI_m^+ PFC induced by the TI Ags were $CRI_{c'}^+$. The data in Table IV indicate that this is probably not the case. PFC inhibition by a Rbt anti-$CRI_{c'}$ antiserum prepared against the $CRI_{c'}^+$ HP 36–60 (anti-Id 36–60 kindly provided by Dr. A. Marshak-Rothstein, Massachusetts Institute of Technology) was practically negligible for the anti-ABA responses induced by the TI Ags. In contrast, this same antiserum completely inhibited PFC produced by the CRI_c^+ (i.e., BALB/c origin) hybridoma cells, CH.BALB (kindly provided by Dr. C. Henry and M. Slomich,

TABLE III

Comparative Expression of CRI and Id R16.7 in Primary IgM anti-ABA PFC Responses

Group	Ag*	% PFC inhibition by:		Inhibition ratio
		RbtαCRI	RbtαId R16.7	αIdR16.7/αCRI‡
1	A-K	86 ± 6	83 ± 7	0.96
2	A-CGG	64 ± 15	60 ± 18	0.92
3	A-Bru	91 ± 7	45 ± 22	0.49
4	A-Bru (in vitro)	57 ± 13	14 ± 11	0.29
5	A-LPS	92 ± 7	56 ± 27	0.61

* See Footnotes to Table I. Day-6 response for A-K, day 9 for A-CGG, and day 3 or 4 for A-Bru and A-LPS. Geometric mean PFC per spleen or per culture ($\mu + \gamma$ for A-CGG, all others μ only) were for groups 1 thru 5: 16,482; 18,583; 11,731; 578; 6,311, respectively.

‡ Ratio values for TI responses differ significantly from those for TD responses, $P<.01$.

TABLE IV

CRI_c' (Id^{36-60}) Expression in Primary PFC Responses to ABA-TI Ags

Group	Ag*	Response	% PFC inhibition by:	
			RbtαCRI	RbtαId^{36-60}
1	—	CH.BALB cells‡	100	100
2	A-Bru	In vivo§	87 ± 12	14 ± 15
3	A-Bru	In vitro	72 ± 3	3 ± 11
4	A-LPS	In vivo	91 ± 9	7 ± 9
5	A-LPS	In vitro	54 ± 5	12 ± 8

* See Footnotes to Table I.

‡ CH.BALB cells are hybridomas made by fusing ABA hyperimmune spleen cells from a BALB/c mouse with cells of the NS-1 line. They secrete IgM anti-ABA Ab which is CRI_c^+ by PFC inhibition (communicated by Dr. C. Henry).

§ Geometric mean PFC response numbers (all μ) per spleen or per culture for groups 2–5 were: 12,708; 330; 6,063; 321, respectively.

UC Berkeley).

Weak Expression of CRI_A in TI Responses Is Probably Not Due to Ts Cell Activity. One possibility to explain the weak expression of CRI_A in the TI responses, compared with TD responses, would be that CRI_A-specific Ts cells are more active in the former than in the latter. On this basis, if T cells are eliminated it would be expected that the CRI_A/CRI ratio would increase in responses to ABA-Bru. To test this, normal or anti-Thy-1.2 plus C-treated spleen cells (2.5×10^6 per 0.5-ml culture) from unprimed or SRC-primed (day −3) A/J mice were cultured in the presence of ABA-Bru or SRC, respectively. Cultures were assayed on day 3. The data (Table V) show that anti-Thy-1.2 treatment did not decrease the number of PFC induced by ABA-Bru, but it did reduce the (secondary) anti-SRC PFC by 85%. (The anti-SRC response has been reported by others to contain a small TI component [24].) However, the CRI_A/CRI ratio was not increased in the cultures containing cells treated with anti-Thy-1.2 plus C; if anything, it decreased somewhat although the difference is not statistically significant. Similar experiments were done using adoptive transfer methodology (lower half of Table V). Irradiated (600 rad) recipients received $4–5 \times 10^7$ normal or anti-Thy-1.2 plus C-treated splenocytes, followed by either ABA-Bru or SRC (control). The results were similar—the CRI_A/CRI ratio did not increase. Furthermore, in a few experiments anti-Thy-1.2 treatment was replaced by an elimination step using anti-Lyt-2.2 plus C. Again, comparable results were obtained (data not shown).

Influence of the xid Defect on CRI_A Expression. One of the models that we considered for explaining the preceding data (see Discussion) was that CRI_A and CRI_m might be asymmetrically expressed by functionally distinct B cell subpopulations. It is known (25) that mice expressing the CBA/N *xid* gene(s)—CBA/N mice or male F_1 progeny of CBA/N females and normal males—lack a major B cell subpopulation (Lyb-5^+). For this reason, we bred F_1 progeny of CBA/N female and A/J male (=NAF_1), as well as offspring of A/J female and CBA/N male (=ANF_1), and examined their anti-ABA PFC responses induced by ABA-Bru. The results in Table VI demonstrate that the normal F_1 mice express CRI_A

TABLE V
Effect of T Cell Removal on CRI_A Expression

Response	Ag*	Treatment	PFC/culture	Reduction	CRI^+	$CRI_A^+/CRI^{+‡}$
				%	%	
In vitro	A-Bru	C	528	−4	55	0.36
	A-Bru	Anti-Thy-1.2 +C	550		64	0.18
	SRC	C	12,333	85		
	SRC	Anti-Thy-1.2 +C	1,845			
In vivo adoptive transfer	A-Bru	C	2,922§	18	83	0.54
	A-Bru	Anti-Thy-1.2 +C	2,392§		71	0.53
	SRC	C	25,084§	87		
	SRC	Anti-Thy-1.2 +C	3,294§			

* See footnotes to Table I. SRC is sheep erythrocytes. Culture conditions and adoptive transfer details are given in the text.
‡ % CRI_A^+ is an average figure based on inhibition obtained with both AD8 and Rbt anti-Id R16.7.
§ Geometric mean specific PFC per recipient spleen. Each figure based on results from at least seven recipients.

TABLE VI
Relative Magnitude and CRI_A Representation in Anti-ABA-Bru Responses of F_1 Progeny of CBA/N and A/J Mice

Mice*	PFC/Spleen‡	$\%CRI_A^{+§}$
NAF_1 ♀	12,210	36
NAF_1 ♂ (*xid*)	3,395	16
ANF_1 ♀	3,429	39
ANF_1 ♂	7,555	38

* Mice received one i.v. injection of 0.2 ml of 1.25% ABA-Bru in PBS and were plaqued on day 4 (peak of the response for all animals). Results for 7 or 8 mice of each of the indicated types are given.
‡ Geometric mean IgM PFC/spleen.
§ Arithmetic mean of %Id-$AD8^+$ and % Id-$R16.7^+$ PFC.

at slightly lower levels than do A/J mice; however, responses by the NAF_1 male *xid* animals were particularly depleted of CRI_A. In the response to ABA-Bru by the three normal types of F_1 mice, CRI_A^+ PFC varied from 2 to 68% of anti-ABA PFC, with means of 35–40% in each case. In contrast, NAF_1 male responses ranged from 0–29% CRI_A^+ with a mean of 16%, which is significantly lower than the means of all three of the normal groups ($P < 0.05$).

Discussion

The present data show that the Id profiles of A/J ABA-specific Ab responses induced by TD and TI forms of ABA are distinct. Two different CRI populations, CRI_A and CRI_m, show differential relative representation in the TI and TD responses. CRI_A overwhelmingly dominates (>90%) the CRI^+ component of the primary in vivo PFC response induced by the TD Ags ABA-KLH and ABA-

CGG, at least around the peak of the response (day 9 or 10). Secondary responses to TD Ags also show predominance of CRI_A among the CRI^+ PFC, i.e., >80% of CRI^+ PFC are CRI_A^+. This result is in accord with the serologic studies of Brown et al. (11). In contrast, a significantly lower percentage of CRI^+ PFC—slightly more on average than 50%—were CRI_A^+ in primary and hyperimmune in vivo responses to ABA-*Brucella* or ABA-LPS. When individual animals were examined for their CRI_A/CRI ratios among primary PFC activated by ABA-KLH or ABA-Bru, values of >0.9 were seen for all animals in the case of the former Ag, whereas, in responses to the TI-1 Ag, ratio values of well below 0.5 were seen in some mice, indicating CRI_m predominance. Serologic comparison of the in vivo hyperimmune Abs induced by ABA-Bru and ABA-KLH demonstrated that the CRI_A/CRI ratios in the two responses were at least as discrepant, if not more so, than the PFC analysis indicated. However, the weakest expression of CRI_A was noted to occur in primary in vitro PFC responses to ABA-Bru and ABA-LPS. Thus, the magnitude of the CRI_A/CRI ratio shows the order: 1° TD in vivo > 2° TD in vivo > 1°, 2° TI in vivo > 1° TI in vitro. The CRI_m/CRI ratio follows (by definition) exactly the reverse order. The ABA hapten, presented in TI-1 form, appears to activate a more heterogeneous idiotypic response than it does when presented in TD form. The major fraction of the PFC (or Abs) induced by the TI-1 Ags are CRI^+, but not CRI_A^+.

One or more members of CRI_m is at least equi-dominant with CRI_A in ABA-specific TI-1-type Ab responses in A/J mice, in contrast to responses to the potent TD Ags, ABA-KLH or ABA-CGG, where CRI_A predominates. But what member(s) of this heterogeneous group (26) is expressed? (Here it might be noted that two nonequivalent definitions for CRI_m were previously offered: one based on the presence of direct binding of anti-ABA Abs to Rbt anti-CRI, along with very weak inhibition of the standard CRI-anti-CRI reaction (12), while the second definition (26) required direct binding by Rbt anti-CRI with concurrent lack of same by Rbt anti-Id R16.7. The criterion used herein is the second one; PFC inhibition by anti-Id is clearly a direct binding assay.) Several types of CRI_m components have been defined. One of these is $CRI_{c'}$ (10, 11). Data given in this report, however, indicate that $CRI_{c'}$ is only a very minor component in the TI responses, including the in vitro ones. Another class of CRI_m^+ molecules have L chains (but not H chains) corresponding serologically to those present in CRI_A^+ molecules (27). Another possibility would be that most of the CRI_m^+ Abs are Id-related to the 96B8 family (9). Finally, many of the CRI_m^+ Abs might be CRI_A-related but represent extensively altered somatic variants that have lost the most immunodominant CRI_A-IdX determinants. Further serological and structural analyses of the CRI_m^+ Abs elicited by the TI Ags will be required to pinpoint the exact nature of these.

The most extreme example of Id profile discrepancy from the TD pattern involves the in vitro primary TI responses. The expression of CRI_A was considerably weaker in these responses than in in vivo responses to the same Ags. Our data concurs with those presented in a communication of Robertson et al. (28). These workers reported that none of their ABA-specific B cell hybridomas, generated using A/J lymphocytes polyclonally stimulated in vitro with LPS and dextran sulfate, secreted CRI^+ Abs. Presumably, CRI positivity was examined

using the competition RIA which detects only CRI_A^+ Abs. The unusual Id profile was elicited irrespective of hapten-receptor interaction, since the mitogens used were not ABA-substituted. Thus, the lack of activation of CRI_A^+ clones cannot be attributed to the preferential stimulation of B cells bearing high affinity receptors for ABA. It seems that virgin, ABA-specific, CRI_A^+ B cells may be refractory to in vitro activation. This seems to us to be strongly suggestive evidence that CRI_A determinants may be selectively expressed by B cells belonging to a particular subset. It is also likely that this property of CRI_A^+ precursors accounts for our total inability to induce anti-ABA responses in vitro to TD ABA Ags using unprimed A/J B cells, even in the presence of carrier-primed T cells.

The likeliest theories for explaining the present data would invoke the differential activation, in different types of responses, of either B cell subsets or T cell subsets. In the latter case, one could propose, in accordance with the network hypothesis (29), that particular Id-specific Th or Ts cells play quantitatively different roles in TI vs. TD responses. The data obtained in the experiments involving anti-Thy-1- (or anti-Lyt-2-) treated A/J splenocytes would, however, tend to rule out enhanced activity of CRI_A-specific Ts cells as being responsible for the low CRI_A expression observed in the TI responses. This leaves the possibility that CRI_A-specific Th cells are more active in in vivo responses to TD ABA Ags than to TI ABA Ags. While it is logically difficult to see why this would be the case, this theory cannot be formally ruled out.

In fact, the hypothesis that seems most consistent with the data is one based on the differential expression of IdX dominance by functionally distinct B cell subpopulations, with the selective activation of these subsets under different conditions. The differential expression of Ig V region elements by different B cell subsets is not a novel idea: Slack et al. (30) originally suggested this possibility and the data of Ward et al. (31) and Primi et al. (32) supports this hypothesis. Furthermore, Wicker et al. (33) have recently presented evidence that BALB/c PC-specific B cell precursors which belong to the $Lyb\text{-}5^+$ subset are largely T15 IdX^+, while those in the $Lyb\text{-}5^-$ subset are almost entirely T15 IdX^-. A similar situation may exist among A/J ABA-specific B cell precursors, and we propose the following model. ABA-specific B cell precursors belonging to subset 1 are predominantly (75–100%) CRI_A^+, while those in subset 2 are at least 50% CRI_m^+ with a lower, and variable, proportion being CRI_A^+ (0–30%). In vitro challenge of A/J splenocytes with TI-1 ABA Ags results in the almost exclusive activation of B cells in subset 2, possibly due to a higher density of cell surface receptors for the polyclonal activating structures on these Ags. In contrast, in vivo challenge of A/J mice with ABA conjugates of TD carriers, such as KLH, apparently results in the almost exclusive activation of ABA-specific B cells belonging to subset 1. In vivo challenge with TI-1-type ABA Ags results in the stimulation of both subpopulations, although one or the other subset may tend to predominate in different individuals; this would explain the IdX profile variation seen in mice responding to ABA-Bru or ABA-LPS.

The preceding functional properties attributed to subsets 1 and 2 are not purely speculative but, rather, are based on reported properties of B cells belonging, respectively, to the $Lyb\text{-}5^+$ and $Lyb\text{-}5^-$ subsets (34, 35). Furthermore, (CBA/N × BALB/c)F_1 (NBF_1) male mice gave almost no primary anti-PC Ab

response to PC-KLH in CFA (average 1/10 the female NBF_1 response) (36). This result would suggest that the primary response to this Ag in normal mice is almost entirely attributable to the Lyb-5^+ B cell subset, and, as would be expected, is strongly T15-IdX dominant in BALB/c mice (37).

At first, Sigal's recent evidence (38) that only 2.6% of unprimed ABA-specific B cells from A/J mice are CRI_A^+ would seem to contradict our model. It is possible, however, that there was some bias in the splenic focus assay which resulted in under-representation of CRI_A^+ precursors. In particular, B cells belonging to our hypothetical subset 2 may have been preferentially stimulated.

Finally, we would point out that the two types of theories—the IdX partitioning among different B cell subsets vs. differential Id-specific regulation by T cells—are not totally exclusive. Thus, Lyb-5^- B cells may be refractory to helper factors produced by IdX-specific Th cells. Moreover, Lyb-5^+ B cells may derive from an Lyb-5^- pool (39). If that is the case, then CRI_A-specific Th cells might be envisioned as playing a key role in promoting the selective differentiation of immature CRI_A^+ ABA-specific precursors to the Lyb-5^+ subset, which could result in a skewed IdX representation between the two B cell subsets. To test these ideas it would be highly informative if the anti-ABA responses of A/J *nu/nu* mice (or B cells therefrom) could be examined.

Summary

Two different cross-reactive idiotype (CRI) groups are distinguishable in the Ab response of A/J mice to the *p*-azobenzenearsonate (ABA) hapten: CRI_A and CRI_m. These two groups showed distinct patterns of relative dominance in the ensuing response depending on whether the inducing Ag was a T cell-dependent (TD) form of ABA, such as ABA-KLH or ABA-CGG, or a T-independent type 1 (TI-1) form, such as ABA-*Brucella abortus* or ABA-lipopolysaccharide (LPS), *and* on whether the response was elicited in vivo or in vitro. The CRI^+ component of primary in vivo plaque-forming cell (PFC) responses to TD ABA Ags was largely (>90%) CRI_A^+ as was, to a slightly lesser extent (>75%) the CRI^+ portion of secondary or hyperimmune serum Ab or PFC responses to the same Ags. In contrast, in vivo primary and hyperimmune PFC responses to ABA-Bru or ABA-LPS showed a significantly lower CRI_A/CRI ratio, averaging 0.5–0.6, with some individual mice giving figures as low as 0.2, indicating predominance of CRI_m over CRI_A. Serological analysis of hyperimmune anti-ABA Abs from a group of 5 A/J mice immunized with ABA-Bru gave a figure of <0.5 for the CRI_A/CRI ratio. The most striking disparity from the TD pattern was seen in primary in vitro PFC responses to the TI ABA Ags; here ratios of <0.2 were generally seen. Since T cell removal did not alter the Id pattern in the TI responses, CRI_A-specific Ts cells do not account for the weak expression of CRI_A in such responses. We propose a model that explains these results on the basis of differential expression of IdX dominance by two distinct B cell subpopulations—equatable to the Lyb-5^+ and Lyb-5^- B cell subsets—along with differential relative activation of these subsets in different types of responses. Examination of anti-ABA PFC responses of F_1 progeny of CBA/N and A/J mice to ABA-Bru lends support to this hypothesis since CRI_A expression was significantly lower in mice with the

xid defect.

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