Brief Definitive Report

B CELL REPERTOIRE DIVERSITY IN ATHYMIC MICE*

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The expression of the B cell specificity repertoire is a highly complex process. Although it is clear that the inheritance of appropriate variable region genes play an integral role in repertoire determination (1–4), recent findings have indicated that the control of phenotypic repertoire expression is multigenic (5) and may be greatly affected by the microenvironment of B cells during clonal maturation (6–8). Studies from several laboratories indicate that the thymus may play an important role in the determination of the T cell specificity repertoire (9, 10), and data have been presented that suggest that the presence of mature T cells may also be crucial to the acquisition of the B cell repertoire (11, 12). The availability of conventional inbred murine strains congenic for the nu/nu locus renders this latter postulate amenable to experimental investigation.

Previous studies have demonstrated that athymic mice have an apparently normal B cell compartment in terms of the frequency of B cells responsive to sheep erythrocytes (13). Consistent with these findings are several unpublished observations, from this and other laboratories, that have demonstrated similar frequencies of responsive B cells in athymic and conventional BALB/c mice for 2,4-dinitrophenyl, 2,4,6-trinitrophenyl, and phosphorylcholine, (S. K. Pierce, A. F. Schrater, P. J. Gearhart, and N. R. Klinman. Unpublished observations.). It remains of interest, however, to determine whether the B cell repertoire of athymic mice is as diverse as that of conventional mice.

Considerable repertoire definition may be obtained through the use of monoclonal antibodies specific for the hemagglutinin (HA) of the influenza virus, PR8 (A/PR/8/34 [H0N1]), by analyses of the reactivity patterns (RP) displayed by these antibodies in their binding to the HA molecules of heterologous influenza virus strains (14, 15). It has been possible to delineate over 40 distinct PR8-HA-specific clonotypes, and infer the existence of at least 100 such clonotypes, among splenic B cells of immune or nonimmune conventional BALB/c mice. In addition, the efficacy of this approach in discriminating a less-diverse repertoire has been demonstrated by the analysis of the PR8-HA-specific repertoire in neonatal BALB/c mice (16), where a more restricted array of clonotypes is observed than among adult individuals.

Our studies use the primary HA-specific response of athymic BALB/c mice to assess the role of the thymus in the generation of antibody diversity at the B cell level. The results indicate that the degree of diversity among splenic B cells of athymic mice is

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indistinguishable from that of conventional BALB/c mice.

Additionally, because these responses were obtained in histocompatible, but allotype-distinct recipient mice, and primary stimulation in this system is exquisitely dependent upon antigen-primed recipient T cells (15, 17–19), these results also indicate that the stimulation of a diverse array of B cell clonotypes may be obtained in the absence of allotype-identical T cells.

Materials and Methods

Mice. DBA/2 mice were purchased from The Jackson Laboratory, Bar Harbor, Maine. BALB/c mice were obtained from our own breeding colony (University of Pennsylvania, Philadelphia, Pa.). Athymic nu/nu BALB/c mice were purchased from ARS Sprague-Dawley, Madison, Wis.

Viruses and Immunizations. The influenza virus strains PR8 (A/PR/8/34 [H0N1]); WSE (A/WSE/33 [H0N1]); MEL (A/Mel./35 [H0N1]); BEL (A/Bel./42 [H0N1]); CAM (A/Cam./46 [HIN1]); Eq-PR8 (A/equine/Miami/1/63 [HEq]2-A/PR/8/34[NI]); BH (A/BH/35 [H0N1]); WEISS (A/Weiss/43 [H0N1]); and the influenza B virus, LEE, were obtained from W. Gerhard, The Wistar Institute of Anatomy and Biology, Philadelphia, Pa. The purification and quantitation of viral preparations by hemagglutination was accomplished as described previously (14, 15). DBA/2 mice used as recipients in adoptive transfer experiments were immunized intraperitoneally with 1,250 hemagglutinating units of purified PR8 virus 6–8 wk before use. The use of irradiated, primed recipients that are syngeneic to the donor B cells at the H-2 locus but allogeneic at the immunoglobulin allotype locus has been shown to maximize T cell-B cell collaboration while minimizing an antibody-specific suppression (17).

Splenic Fragment Culturing and Radioimmunoassay. The methods used for obtaining monoclonal anti-PR8-HA responses and the radioimmunoassay of monoclonal antibodies have been described previously (14, 15, 20).

Results

Frequency of PR8-HA-specific B Cells in Spleens of Conventional Adult and nu/nu BALB/c Mice. The results of a series of experiments in which each recipient DBA/2 mouse received either 2×10^7 spleen cells from conventional 8- to 12-wk-old BALB/c mice, or 1×10^7 spleen cells from 8- to 12-wk-old nu/nu BALB/c mice are presented in Table I. The frequency of PR8-HA-specific B cells in nu/nu BALB/c mice ranged from 3.3 to 5.6 per 10^6 donor splenic B cells. This frequency is approximately one-third to one-half the frequency seen among the B cells of conventional BALB/c mice.

RP Analysis of Monoclonal PR8-HA-specific Antibodies Derived from nu/nu BALB/c Mice. The PR8-HA-specific antibodies obtained from spleen cells derived from nu/nu BALB/c mice were tested for reactivity toward a panel of six heterologous influenza viruses. Only antibodies from cultures that had produced at least 80 ng of antibody could be examined in this fashion. In our analysis of 86 PR8-HA-specific antibodies from nu/nu BALB/c mice, 33 distinct RP were observed.

Examination of Table II, where the present data is compared with the RP observed among 128 antibodies from conventional adult BALB/c mice (15), suggests that little difference exists between the two populations with respect to repertoire heterogeneity. Statistical treatment of the data by two previously employed techniques supports this interpretation. First, estimation of the total HA-specific repertoire from these samples by the technique of Wybrow and Berryman (21, 22), results in figures of similar magnitude for both the normal (15) and nu/nu repertoires. Secondly, application of a recently developed test (16, 23), designed to compare the degree of heterogeneity between two populations based upon this type of sample, indicates that the nu/nu

TABLE I

Frequency of HA-specific B Cells in Normal and nu/nu BALB/c Mice

Donor	Experiment	Total spleen cells transferred ($\times 10^{-7}$*)	HA-specific foci (per 10^6 cells injected‡)	HA-specific cells (per 10^6 splenic B cells§)
Normal BALB/c	1	4	0.25	15.0
	2	8	0.20	12.0
	3	8	0.23	13.5
	4	8	0.16	9.6
	5	ND‖	ND	ND
nu/nu BALB/c	1	10	0.13	3.9
	2	9	0.11	3.3
	3	11	0.15	4.4
	4	11	0.15	4.4
	5	15	0.19	5.6

Mean ± SD HA-specific precursors per 10^6 B cells in normal BALB/c and in nu/nu BALB/c = 12.5 ± 2.3 and 4.32 ± 0.85, respectively.

* In each experiment, either 2×10^7 normal or 1×10^7 nu/nu spleen cells were transferred to each irradiated, antigen-primed recipient.

‡ Specificity of the monoclonal viral-specific antibodies for the viral HA molecule was determined by radioimmunoassay as described previously (15).

§ Frequencies are calculated after correction for homing and cloning efficiencies, as well as the proportion of B cells in each donor cell inoculum (20).

‖ Not done.

and adult repertoires are indistinguishable in this regard. Based on these findings, it is likely that the nu/nu HA-specific repertoire is comparable in diversity to the normal BALB/c.

Discussion

Previous studies from this laboratory have demonstrated that the B cell population of adult conventional BALB/c mice expresses an extraordinarily diverse repertoire. This is evident in the response to the PR8-HA antigenic moiety, where 1 in $1\text{–}2 \times 10^5$ adult splenic B cells respond to this antigen when stimulation is maximized (15). Statistical treatment of the RP analysis of these antibodies indicated that over 100 distinct HA-specific antibodies were probably present within the adult BALB/c population. It must be assumed that the RP analysis probably underestimates the full extent of repertoire diversity, because antibodies that recognize identical determinants may differ from one another; or distinct determinants might be shared by the same combination of virus strains in the panel used. These findings, therefore, imply that the diversity of the conventional adult BALB/c repertoire approaches 10^7 distinct clonotypes.

The studies presented here ask whether this degree of diversity is dependent upon the development of B cells in the presence of a normal T cell population. The results indicate that the degree of diversity observed among splenic B cells of athymic nu/nu BALB/c mice is not demonstrably less than that of conventional BALB/c mice. It is not yet possible to either fully assess the extent of diversity within the conventional splenic B cell repertoire or to unambiguously demonstrate identities between the repertoires of conventional and nu/nu mice. Because the repertoire of neonates has been shown to be demonstrably restricted by the same experimental system, the

TABLE II

*Relative Frequencies of RP in Normal and nu/nu BALB/c Primary Anti-PR8-HA Responses**

WEISS	CAM	BEL	BH	+	+	+	−	+	−	−	−
			WSE	+	+	−	+	−	+	−	−
			MEL	+	−	+	+	−	−	+	−
+	+	+		9.3‡	2.3	x§	3.4	x	x	x	x
				7.0	1.6	x	1.6	x	x	x	x
−	+	+		3.4	x	x	x	x	1.2	x	2.3
				3.1	x	x	0.8	x	0.8	x	2.3
+	−	+		4.6	2.3	2.3	4.6	x	x	1.2	2.3
				1.6	x	0.8	x	0.8	x	x	1.6
+	+	−		x	x	1.2	2.3	x	1.2	x	x
				2.3	x	2.3	x	x	x	x	x
−	−	+		x	x	x	x	x	x	2.3	2.3
				x	x	0.8	x	x	2.3	0.8	2.3
−	+	−		3.4	x	1.2	x	x	x	1.2	2.3
				0.8	0.8	0.8	x	x	2.3	4.7	1.6
+	−	−		4.6	x	x	x	x	1.2	1.2	1.2
				1.6	x	x	x	0.8	5.5	1.6	1.6
−	−	−		4.6	2.3	3.4	2.3	4.6	4.6	3.4	10.5
				5.5	2.3	1.6	7.8	3.1	6.2	5.5	14.0

* The ability of each monoclonal antibody specific for PR8-HA to bind the HA of six heterologous viruses was determined by solid-phase radioimmunoassay. The majority of reactions with a heterologous virus was either close to 100% or nil, but the lower limit for a positive reaction was set at 10% of the binding observed with PR8 because previous work has shown that when binding to a heterologous virus exceeds this figure, all anti-HA activity present may be adsorbed by that virus. Each RP describes the spectrum of heterologous viruses that share the determinant recognized by a particular antibody, and thus defines a single or small group of clonotypes that is distinct from antibodies that exhibit a different RP. Relative frequencies are given as percentage of total responses and are based upon 129 normal BALB/c (15) and 86 nu/nu BALB/c monoclonal HA-specific antibodies.

‡ Upper number refers to the nu/nu BALB/c response; lower number refers to the normal BALB/c response.

§ x, RP not observed.

comparable diversity of the adult nu/nu and normal splenic B cell repertoires clearly indicates that considerable diversification can occur independent of normal thymic influences. Because nu/nu mice display no functional T cell compartment except perhaps in very early development, the demonstration that nu/nu mice develop and maintain a highly diverse B cell repertoire is in basic disagreement with findings of other investigators that indicate that extensive B cell diversification may be dependent upon the presence of a thymus or mature T cells (11). Because the latter experiments analyze only the early development of B cells in adoptive hosts, it is possible that B cell repertoire development is only somewhat retarded in the absence of T cells, or that the adoptive transfer system does not permit the normal diversification process to occur.

A second finding presented here is evidence that the stimulation of an extremely diverse specificity repertoire in an adoptive host requires neither T cells of donor origin nor T cells that are syngeneic to the responding B cells at the immunoglobulin allotype locus. Because the stimulation of primary PR8-HA-specific B cells in this as

well as other systems is entirely dependent upon the presence of antigen-primed recipient T cells (18, 19) it is unlikely that B cell stimulation in this case was a result of allogeneic effects. This finding puts considerable constraints on theories that imply the necessity of T cells that recognize B cell allotypes or idiotypes in the process of B cell stimulation (24, 25). Because strains differing at allotype generally also differ greatly in their B cell clonotype expression (1–3, 5, 17), it would appear that either T cells recognize and help clonotypes that are not normally expressed within the syngeneic B cell compartment, or that many specificities can be stimulated in the absence of clonotype-specific T cell interactions.

Because the frequency of PR8-responsive splenic B cells from nu/nu mice in this system is lower than that of conventional mice, it is possible that there is some degree of dependence upon T cells for the expansion of B cell clones specific for these viral determinants. Because this is apparently not the case for responses to other antigenic determinants, it might, instead, reflect differences between the normal and athymic murine B cell compartments created by proliferative influences of T cell-independent environmental antigens. Thus, clonotypes recognizing exclusively T cell-dependent determinants may be relatively disadvantaged in an athymic host and appear at a lower relative frequency than among B cells of conventional individuals. It will therefore be of interest to compare responses of germ-free mice to assess the role of environmental stimulation on repertoire establishment in conventional and athymic mice.

Summary

The extent of B cell repertoire diversity among nu/nu BALB/c mice has been assessed and compared with that of normal BALB/c mice. This was accomplished through the characterization of monoclonal, influenza hemagglutinin-specific antibodies by reactivity pattern analysis. The results indicate that the repertoire of athymic mice is equivalent in diversity to that of normal mice. Moreover, because these responses were obtained in recipients that were histocompatible but distinct at immunoglobulin allotype loci, these findings indicate that a very diverse array of B cell clonotypes may be stimulated in the absence of allotype-identical T cells.

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