IMMUNE RESPONSE TO A THYMUS-DEPENDENT FORM OF B512 DEXTRAN REQUIRES THE PRESENCE OF Lyb-5$^+$ LYMPHOCYTES

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Isomaltohexaose (IM6)[1] is a six-sugar hapten whose structure is homologous with antigenic determinants in $\alpha(1\rightarrow6)$-linked dextrans, such as B512 dextran (Dex). IM6-keyhole limpet hemocyanin (KLH) conjugates induce thymus-dependent (TD) antibody responses in adult mice in which almost all the anti-hapten antibody binds to Dex (1, 2). Thus, IM6-KLH can be used as a TD antigenic analogue of Dex to examine the immune responsiveness of neonatal mice and of mice expressing the *xid* determined immune defect (*xid* mice) to Dex and to other polysaccharide antigens. These soluble polysaccharides and their hapten derivatives are members of a class of antigens which we have designated as type 2 antigens (3, 4) (Mond, J. J., J. Ferrar, W. E. Paul, M. Schaefer, and M. Howard, manuscript in preparation).

xid mice lack the subset of B lymphocytes characterized by the Lyb-3 (5) and Lyb-5 (6) cell surface markers. These cells (designated Lyb-5$^+$ B cells) are present at low frequencies in 2-wk-old normal mice and do not reach adult levels until ~3–4 wk of age (4). In both neonatal mice and mice with the *xid* defect, the failure to respond to polysaccharide antigens correlates with an absence or diminished number of this mature subset of B cells (4). A requirement for Lyb-5$^+$ B cells in the in vitro antibody response to the type 2 antigen, trinitrophenylated-Ficoll (TNP-Ficoll) has been demonstrated (7).

We have examined the ability of IM6-KLH to stimulate production of anti-Dex antibodies in neonatal mice and in mice with the *xid* defect. We report here that the production of anti-Dex antibodies in response to this TD analogue of Dex is deficient in both groups of mice and that in normal adult mice the production of an anti-Dex antibody response to IM6-KLH and to IM6 *Brucella abortus* (IM6-BA) requires the presence of Lyb-5$^+$ B cells. These results, together with previous studies (8) of unresponsiveness to phosphocholine (PC) conjugated to BA, suggest that the expresssed B cell repertoire is skewed such that the vast majority, if not all, of the B cells

[1] *Abbreviations used in this paper:* BA, *Brucella abortus*; BSA, bovine serum albumin; C, complement; CFA, complete Freund's adjuvant; Dex, dextran; IM6, Isomaltohexaose; KLH, keyhole limpet hemocyanin; PC, phosphocholine; PFC, plaque-forming cell; RIA, radioimmunoassay; TD, thymus-dependent; TNP, trinitrophenyl.

capable of responding to carbohydrate determinants, whether presented as type 1, type 2, or TD antigens, are in the Lyb-5$^+$ subset.

Materials and Methods

Mice. All mice were obtained from the Small Animal Section, Division of Research Services, National Institutes of Health, or The Jackson Laboratory, Bar Harbor, ME, and were immunized at 12–16 wk of age unless otherwise indicated. (CBA/N × DBA/2)F_1 male mice used as recipients in the adoptive transfer experiments were 6 mo old.

Immunizations. CBA/N, CBA/CaHN, and various CBA/N F_1 mice were given primary immunizations of 50 μg IM6-KLH in complete Freund's adjuvant (CFA) (H37Ra; Difco Laboratories, Detroit, MI) administered in the hind footpads (25 μg) and subcutaneously behind the neck (25 μg). They were bled 3 wk after the primary immunization. Mice that were boosted were given 20 μg IM6-KLH in incomplete Freund's adjuvant 4–8 wk after the previous immunization and were bled 1 wk later. C57BL6/J mice used in the ontogeny experiment were immunized with 50 μg IM6-KLH in CFA given intraperitoneally, and were bled 4 wk after immunization. Mice used in the adoptive transfer experiments were immunized with 50 μg of IM6-KLH or dinitrophenylated (DNP)-KLH in CFA given in the footpads and subcutaneously, and 1–2 mo later they were boosted with 20 μg of IM6-KLH or DNP-KLH in phosphate-buffered saline given intraperitoneally. 1 wk after boosting, their spleens were removed and processed for donor cells.

Antigens. IM6-KLH was prepared as described previously (1). IM6-BA was prepared by the reaction of 30 μmol of the isothiocyanate derivative of IM6-phenethylamine and 0.2 ml of packed BA as described by Smith et al. (9). TNP-BA was prepared by the method described by Mond et al. (10). DNP_{10}-KLH was prepared by a modification of the method of Benacerraf and Levine (11).

Anti-Lyb-5 Treatment. Anti-Lyb-5 serum was prepared by immunization of C57BL/6 mice with DBA/2 spleen cells; the serum was rendered specific by absorption with thymus and spleen cells from male (CBA/N × DBA/2)F_1 mice, as described by Ahmed et al. (6). Spleen cells were treated with anti-Lyb-5 antiserum and complement (C) or with a control serum (DBA/2 anti-C57BL/6 spleen cells) and C as described by Ahmed et al. (6).

Plaque-Forming Cell (PFC) Assays. Anti-Dex PFC were measured as described by Stein et al. (1). Anti-TNP PFC were measured by the method of Rittenberg and Pratt (12).

Radioimmunoassay (RIA). RIA analysis of anti-IM6 (assayed on IM6-bovine serum albumin [BSA]-coated plates) and anti-Dex (assayed on Dex-coated plates) antibodies was performed as described by Stein et al. (1). Titrations consisting of serial threefold dilutions of sera were performed. Analyses for total IgG antibody and for antibody of IgG subclasses were carried out in the presence of 0.1 M 2-mercaptoethanol.

Results

Anti-Dex Response in xid Mice Immunized with IM6-KLH. (CBA/N × DBA/2)F_1 male mice, which express the *xid*-determined immune defect, were immunized and boosted with IM6-KLH. In Table I IgG anti-IM6 and anti-Dex titers of primary, secondary, and tertiary sera are compared to the primary response of normal F_1 males derived from the reciprocal cross [i.e., (DBA/2 × CBA/N)F_1 males]. Even after three immunizations, sera from the defective animals contained little if any IgG antibody capable of binding Dex, whereas substantial IgG1 anti-Dex titers and measurable IgG3 and IgG2b anti-Dex antibodies were observed in the sera of normal mice after a single immunization with IM6-KLH. The *xid* mice did produce some IgG anti-hapten antibody to IM6-KLH; this was mainly of the IgG1 class and bound IM6-BSA, but only a small fraction of it cross-reacted with Dex. *xid* mice derived from another cross (CBA/N × C57BL/6N) did produce anti-Dex antibody after secondary immunization (Table II). The anti-Dex titers of sera of F_1 males from this cross were less than those

TABLE I

Response of (CBA/N × DBA/2)F$_1$ Mice to IM6-KLH

		Isotype of antibody							
		IgG3		IgG1		IgG2b		IgG2a	
		IM6-BSA*	Dex	IM6-BSA	Dex	IM6-BSA	Dex	IM6-BSA	Dex
(C × D)F$_1$ (*xid*)	1°‡	40	15	630	356	27	27	10	30
	2°	40	0	5,700	350	210	160	500	50
	3°	20	0	2,500	100	60	10	130	15
(D × C)F$_1$ (normal)	1°	3,000	1,500	40,000	14,000	729	1,000	110	115

* Sera from (CBA/N × DBA/2N)F$_1$ [(C × D)F$_1$] and (DBA/2N × CBA/N)F$_1$ [(D × C)F$_1$] male mice (10 mice/group) were pooled and titrated on IM6-BSA or Dex-coated microtiter plates and tested with ^{3}H-labeled anti-subclass reagents. Results here and in subsequent tables are reported as the inverse of the dilution of serum that binds 1% of the added ligand. Mice were bled 3 wk after the primary immunization and 1 wk after the secondary and tertiary immunizations.

‡ 1°, primary; 2°, secondary; 3°, tertiary.

TABLE II

Response of (CBA/N × C57BL/10)F$_1$ Mice to IM6-KLH

	Isotype of antibody									
	IgG3		IgG1		IgG2b		IgG2a^a‡		IgG2a^b	
	IM6-BSA*	Dex	IM6-BSA	Dex	IM6-BSA	Dex	IM6-BSA	Dex	IM6-BSA	Dex
(C × B)F$_1$ male										
Preimmune	0	0	0	0	200	200	0	0	0	0
2° anti-IM6-KLH	729	1,400	20,000	6,500	1,600	700	600	175	1,200	1,200
(C × B)F$_1$ female										
Preimmune	0	100	100	18	100	100	0	37	0	0
2° anti-IM6-KLH	729	3,000	125,000	35,000	3,200	3,200	800	600	1,200	1,400

* Sera from (CBA/N × C57BL/10)F$_1$ [(C × B)F$_1$] male and female mice were pooled and titrated on IM6-BSA or Dex-coated plates and tested with ^{3}H-anti-subclass reagents. (See *, Table I).

‡ IgG2a^a and IgG2a^b allotypes were analyzed separately using allotype-specific reagents.

TABLE III

Response of CBA/N Mice to IM6-KLH

	Isotype of antibody									
	IgM		IgG3		IgG1		IgG2b		IgG2a	
	IM6-BSA*	Dex	IM6-BSA	Dex	IM6-BSA	Dex	IM6-BSA	Dex	IM6-BSA	Dex
CBA/N (*xid*)										
Preimmune	25	25	10	0	12	0	100	100	38	20
2° anti-IM6-KLH	600	550	2,200	2,200	6,500	3,000	6,600	2,200	24,000	6,600
CBA/CaHN (normal)										
Preimmune	12,000	4,000	125	425	20	25	200	81	80	30
2° anti-IM6-KLH	120,000	180,000	120,000	100,000	59,000	59,000	30,000	10,000	48,000	6,600
Ratio $\frac{\text{CBA/CaHN}}{\text{CbA/N}}$	200	327	55	45	9	20	5	5	2	1

* Sera from 10 mice/group were pooled and titrated on IM6-BSA or Dex-coated plates and assayed with ^{3}H-labeled anti-isotype sera (see *, Table I.)

of phenotypically normal females by a factor of five for IgG1, the most prevalent IgG isotype in this response, and showed variable, and often smaller, differences in the other IgG isotypes. However, the titers of IgG3, IgG2b, and IgG2a anti-Dex antibodies in the sera of IM6-immunized normal F$_1$ female mice of this cross were quite low. We

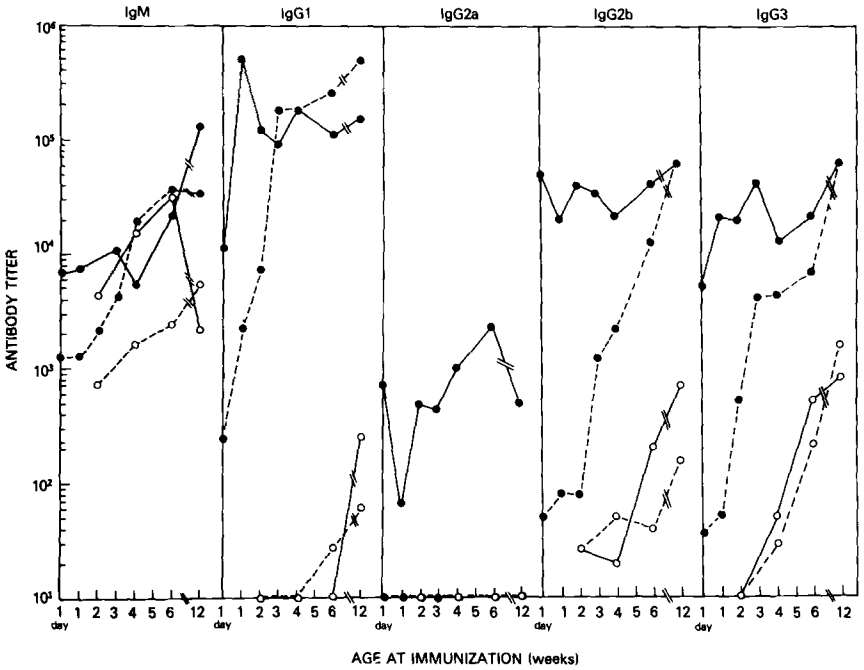

FIG. 1. Antibody titers of pools of sera from 10–30 mice per group immunized at various times after birth with either IM6-KLH (●) or Dex (○) and bled 1 mo later. Titrations were performed on either IM6-BSA (——) or Dex (- - -) and isotypes were measured using ^{3}H-labeled anti-subclass reagents.

examined responsiveness to IM6-KLH of a third set of *xid* mice, CBA/N mice, using as a control the closely related, phenotypically normal, CBA/CaHN strain. The results of analysis of a secondary response to IM6-KLH are shown in Table III. The CBA/N mice developed measurable titers of anti-Dex but these were lower than those of CBA/CaHN by as much as 300-fold, depending on the isotype. The degree of difference was greatest for those isotypes that were the most highly represented in the normal response, IgM, IgG3, and IgG1. We conclude from these experiments that a major defect exists in the response of *xid* mice to IM6-KLH. This defect is a markedly reduced capacity to produce antibodies that bind Dex and is most pronounced in those isotypes that are most highly represented among the anti-Dex antibodies produced by the normal, control strain in response to IM6-KLH.

Response to Dex and to IM6-KLH in Neonatal Mice. C57BL/6J mice were immunized with Dex or with IM6-KLH at various ages from 1 d to 12 wk after birth. Sera from mice immunized at each time were pooled and tested for their ability to bind to IM6-BSA and to Dex-coated plates. As shown in Fig. 1, maximum anti-Dex antibody titers after immunization with Dex were not achieved until the mice were 12 wk old and the titers of IgG antibody raised against Dex were similar whether assayed on IM6-BSA or Dex. IgM antibodies raised against Dex in mice immunized at 6 wk of age or younger bound IM6-BSA substantially better than they bound Dex. The major IgG subclass in the anti-Dex response to Dex was IgG3; IgG1 and IgG2b titers were lower and no IgG2a was detected.

Immunization with IM6-KLH at 1 d of age produced substantial anti-IM6 titers

of all isotypes. However, the anti-IM6 antibodies of young mice bound to IM6-BSA but not to Dex. The anti-Dex titers among IgM and IgG antibodies produced in response to IM6-KLH rose steadily with age. The IgM anti-Dex titers achieved a maximum at 6 wk of age. IgG1 and IgG3 anti-Dex titers reached plateaus at 3–4 wk of age, although the IgG3 anti-Dex titers of animals immunized at 12 wk of age with IM6-KLH were considerably higher than those immunized at 6 wk of age. In contrast,

TABLE IV

Ratio of Anti-IM6 to Anti-Dex Titers in Sera of Mice Immunized with IM6-KLH

Isotype	Age at immunization*			
	1 d	1 wk	4 wk	12 wk
IgG3	143	400	3	1.0
IgG1	45	229	1	0.3
IgG2b	1,000	247	9	1.0
IgG2a	—‡	—	—	—

* Data taken from experiment in C57BL/6J mice shown in Fig. 1. The numbers represent the ratio of the antibody titer obtained on IM6-BSA to that obtained on Dex.

‡ —, Undefined, because the titers on Dex were all <10, the lowest dilution of serum tested.

TABLE V

Effect of Anti-Lyb5 + C on the Adoptive IM6-KLH Response

	Immunization		Anti-Dex or TNP PFC*/spleen	
			Antibody treatment	
	Immunizing antigen	Antigen at transfer	Control serum + C	Anti-Lyb-5 + C
Experiment 1	IM6-KLH	IM6-KLH	6,227 ×/÷ 1.28 (5)‡	<20 (5)
Experiment 2	IM6-KLH	IM6-KLH	5,178 ×/÷ 1.33 (4)	<20 (5)
	DNP-KLH	DNP-KLH	2,412 ×/÷ 1.83 (5)	590 ×/÷ 2.33 (4)
Experiment 3	IM6-KLH	IM6-KLH	6,660 ×/÷ 1.18 (2)	200 ×/÷ 1.93 (3)
	IM6-KLH	IM6-BA§	1,800 ×/÷ 1.81 (4)	120 ×/÷ 1.07 (4)
	IM6-KLH	TNP-BA	480 ×/÷ 2.30 (6)	980 ×/÷ 2.34 (6)
	No cells transferred‖	TNP-BA	140 ×/÷ 1.31 (5)	
	DNP-KLH	DNP-KLH	4,680 ×/÷ 1.72 (6)	4,200 ×/÷ 2.00 (5)
Experiment 4	IM6-KLH	IM6-KLH	1,840 ×/÷ 1.27 (4)	<20 (6)
	IM6-KLH	IM6-BA	680 ×/÷ 1.60 (4)	<20 (6)
	No cells transferred	IM6-BA	<20 (4)	
	IM6-KLH	TNP-BA	2,340 ×/÷ 1.17 (7)	1,460 ×/÷ 1.08 (2)
	No cells transferred	TNP-BA	<20 (4)	
	DNP-KLH	DNP-KLH	3,120 ×/÷ 1.26 (5)	3,480 ×/÷ 1.10 (5)

* Indirect PFC, day 7 after transfer of 1×10^7 cells. Geometric mean ×/÷ relative SE.

‡ Parentheses indicate number of mice per group.

§ Mice received 10^9 IM6-BA or TNP-BA.

‖ Response of irradiated recipients that received antigen and no cells.

TABLE VI

Antibody Titers of Adoptive Transfer Recipients

Serum source	Antibody titers assayed on:			
	IM6-BSA		Dextran	
	IgM	Total IgG	IgM	Total IgG
Nonimmunized (D × C)F_1 males*	1,800	55	800	729
Immunized (D × C)F_1 males	130,000	22,000	770,000	$>10^6$
Recipients of control serum + C-treated cells‡	19,680	7,200	65,000	177,000
Recipients of Anti-Lyb-5 + C-treated cells	0	30	0	500

* All sera were pooled from four or five mice per group. (DBA/2 × CBA/N)F_1 [(D × C)F_1] male normal serum was obtained from unimmunized mice. (D × C)F_1 male immune serum was obtained from the primed donor mice used in experiment 2, Table V at the time of killing.

‡ Animals were bled 7 d after adoptive transfer and challenge with IM6-KLH. Sera used in this experiment were pooled from the recipient mice from experiment 2, Table V. These pooled sera were titrated on IM6-BSA or Dex-coated plates.

the IgG2b anti-Dex titers continued to rise among animals immunized at different times over the first 12 wk of life, and no IgG2a anti-Dex was detected in the sera of such mice. The changing pattern of specificity among the IgG subclasses of anti-IM6 antibodies from mice immunized at different times during the first 12 wk of life can be appreciated by comparing the ratios of antibody titers measured on IM6-BSA to those on Dex (Table IV). The ratios vary between 45 (IgG1) and 1,000 (IgG2b) in sera from mice immunized on the first day of life but approach a value of 1 as the age at immunization reaches 12 wk.

Effect of Anti-Lyb-5 and C Treatment on the Response to IM6-KLH. To assess the role of Lyb-5^+ lymphocytes in the production of anti-IM6 antibodies that bind Dex, adoptive transfer experiments were performed in which spleen cells from phenotypically normal (DBA/2N × CBA/N)F_1 male mice, primed and boosted with IM6-KLH, were treated with anti-Lyb-5 plus C or a control serum plus C and then transferred with antigen to previously irradiated (CBA/N × DBA/2N)F_1 male mice. This treatment should eliminate Lyb-5^+ B cells and thus allow a determination of the role of Lyb-5^- cells in the production of anti-Dex antibodies. As a control for the response of the Lyb-5^- cells to IM6-KLH, DNP-KLH, TNP-BA, and IM6-BA were used as antigens. The results of four experiments (Table V) indicate that anti-Lyb-5 plus C treatment completely eliminated the ability of IM6-KLH-primed cells to transfer an anti-Dex PFC response to recipients challenged with IM6-KLH or IM6-BA. When cells from donors primed to DNP-KLH were similarly treated and tested for the ability to transfer an anti-DNP PFC response to DNP-KLH challenge, anti-Lyb-5 plus C treatment had no effect (experiments 3 and 4) or diminished but did not eliminate the response (experiment 2). To demonstrate that Lyb-5^- cells from IM6-KLH-primed animals could respond to some antigen, recipients of such cells received a primary immunization with TNP-BA (experiments 3 and 4). In both experiments a substantial number of TNP PFC were observed in recipients. The anti-TNP PFC are less than maximal because the response was measured on day 7 after transfer, which is optimal for IM6-KLH or DNP-KLH but not for TNP-BA and because only indirect

PFC were measured. However, the responses obtained are considerably greater than those of irradiated (CBA/N × DBA/2)F_1 males, which received no cells.

These experiments demonstrate that the production of anti-Dex antibodies in response to IM6-KLH is dependent on the presence of Lyb-5^+ lymphocytes. As virtually all of the anti-IM6 antibodies stimulated by IM6-KLH in adult mice cross-react with Dex (1), it is expected that both the anti-IM6 and the anti-Dex responses to IM6-KLH would be eliminated by anti-Lyb-5 plus C, i.e., that there would be no residual antibodies that bound IM6-BSA but not Dex. The prediction is confirmed in an experiment in which recipients of IM6-KLH primed cells treated with anti-Lyb-5 plus C produced no anti-IM6 antibodies upon challenge with IM6-KLH (Table VI). This experiment also demonstrates that the production of both IgM and IgG anti-Dex antibodies are dependent upon Lyb-5^+ cells in adult mice.

Discussion

We have examined the anti-Dex response in *xid* mice and in neonatal normal mice using the thymus-dependent analogue of B512 Dex, IM6-KLH. Mice with the *xid* defect produce very low levels of anti-Dex antibodies in response to IM6-KLH, even after boosting. This is in marked contrast to immunization of *xid* mice with DNP-KLH, which stimulates a very good anti-DNP response (13). These results suggest that the response to IM6-KLH, like the response to Dex itself, requires the presence of Lyb-5^+ lymphocytes, which are lacking in *xid* mice. To examine this more carefully, we studied the ontogeny of the anti-Dex response using IM6-KLH as an immunogen. Although the total response to the IM6-hapten reaches adult levels in animals 1 wk old, anti-IM6 antibodies that cross-react with Dex are produced in substantial amounts only in animals immunized after the age of 3–4 wk. The kinetics of the development of the anti-Dex component of the response are very similar to those reported for the development of the response to TNP-Ficoll (4) and to the capacity of normal B cells to proliferate in response to anti-μ stimulation (14), both of which have been shown to require Lyb-5^+ lymphocytes. It has been reported that few cells bearing the Lyb-3 (5) and Lyb-5 (6) markers are present before 2 wk of age, and that adult frequencies are reached at 4 wk of age. The data reported here show that the development of the cross-reactive anti-Dex response to IM6-KLH coincides with the reported development of Lyb-5^+ lymphocytes. Once the Lyb-5^+ cells reach mature levels, at 4 wk of age, IM6-KLH stimulates anti-Dex antibodies at levels equivalent to adult responses. That IM6-KLH stimulates a maximum anti-Dex response earlier in ontogeny than Dex itself is an interesting, but unexplained, observation.

The dependence of the IM6-KLH response on the presence of Lyb-5^+ cells was tested directly in adoptive transfer experiments using cells from adult animals that had been primed with IM6-KLH and treated with anti-Lyb-5 plus C before transfer. The experiments demonstrate that the adult antibody response to IM6-KLH is eliminated by anti-Lyb-5 plus C. Moreover, the cells remaining after immune elimination with anti-Lyb-5 were not capable of responding to IM6-BA but were capable of responding to DNP-KLH and TNP-BA. Our interpretation of these results is that the expressed repertoire of adult B cells is skewed such that the great majority of the activatable precursors of anti-Dex antibody-secreting cells are in the Lyb-5^+ subpopulation. There are several possible explanations for this skewing. The first of these is that Dex (and other polysaccharide)-specific B cells do not appear among

Lyb-5$^-$ cells because the genes for V regions capable of binding such polysaccharides are not expressed in these cells. This would imply that unresponsiveness in *xid* mice to polysaccharides and to their nitrophenyl conjugates (e.g., TNP-Ficoll) are independent defects. We think this unlikely. A second possibility is that among Lyb-5$^-$ cells, precursors with receptors for polysaccharides do appear just as they do among Lyb-5$^+$ B cells. Environmental polysaccharides fail, however, to activate these cells, whereas cells with receptors for nonpolysaccharide antigens (e.g., proteins) are expanded due to environmental immunization. Thus, polysaccharide-specific B cells would become a diminishing fraction of Lyb-5$^-$ cells. A third possibility is that Lyb-5$^-$ cells with receptors for carbohydrates are tolerized after encounter with environmental polysaccharides, thereby actively deleting precursors of anti-polysaccharide antibody-secreting cells from the Lyb-5$^-$ subset. In contrast, Lyb-5$^+$ cells, when they appear in development, may be expanded after exposure to environmental polysaccharides. This would lead to the overwhelming majority of polysaccharide-reactive cells being in the Lyb-5$^+$ subset.

The question of whether or not neonatal Lyb-5$^-$ cells are susceptible to tolerance induction by soluble polysaccharides has not been conclusively answered. Two reports of attempts to tolerize neonatal cells to type 2 antigens indicate that Lyb-5$^-$ cells, soon after birth, could not be tolerized to Dex or bacterial levan (15) or to TNP-Ficoll (16). If Lyb-5$^-$ cells are indeed not tolerized by environmental antigens, then our data suggest that they are not capable of responding to them. Lyb-5$^-$ cells from *xid* mice, on the other hand, have been reported to be more easily subject to antigen blockade by TNP-Ficoll (17) than normal adult B cells.

The ability of some *xid* mice to make small amounts of anti-Dex antibodies would suggest either that *xid* mice may have a small number of Lyb-5$^+$ B cells or that some Lyb-5$^-$ B cells can respond to Dex determinants. Similarly, *xid* mice have been reported to make anti-PC antibodies to some PC-containing antigens (18–21). In view of the finding that young adult *xid* mice make no anti-TNP antibody in response to TNP-Ficoll, we believe that the "leakiness" in the anti-Dex response is more likely to represent a meager response by Lyb-5$^-$ cells rather than the presence of small numbers of normal Lyb-5$^+$ cells. The presence of small numbers of defective Lyb-5$^+$ cells, capable of responding to TD forms of polysaccharides but incapable of being triggered by environmental polysaccharides, would also be compatible with our findings.

Summary

Studies of the ontogeny of the immune response to B512 dextran (Dex) show that antibody responses equal to those of adult mice are not attained until 12 wk of age. We have examined the anti-Dex response after immunization with a thymus-dependent antigen isomaltohexaosyl-keyhole limpet hemocyanin (IM6-KLH) and have shown that the development of the cross-reacting anti-Dex response parallels the development of Lyb-5$^+$ B cells. Adult levels of anti-Dex antibody after immunization with IM6-KLH are achieved in mice between 3 and 12 wk of age, a time when Lyb-5$^+$ cells have reached adult levels. Neonatal mice, immunized at 1 d or 1 wk after birth, failed to produce a significant amount of anti-Dex antibodies, although they did produce IM6-specific antibodies after immunization with IM6-KLH. Data, which support the conclusion from these experiments that Lyb-5$^+$ cells are required for an anti-polysaccharide response even when the immunizing antigen is thymus-dependent,

include the failure of IM6-KLH to stimulate a normal anti-Dex response in mice with the *xid* defect and the direct demonstration in normal adult mice that elimination of Lyb-5$^+$ cells from spleens of mice primed with IM6-KLH abolishes the ability of these cells to transfer an anti-Dex response. The data imply that the expressed B cell repertoire in adult animals is skewed such that the vast majority of B cells capable of responding to polysaccharide determinants are in the Lyb-5$^+$ subset.

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