ROLE OF ACCESSORY CELLS IN B CELL ACTIVATION

III. Cellular Analysis of Primary Immune Response Deficits in CBA/N Mice: Presence of an Accessory Cell-B Cell Interaction Defect*

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Mice that express the X-linked CBA/N genetic defect lack a subpopulation of B cells that appears late in normal murine B cell ontogeny and that expresses on its surface Lyb3, Lyb5, and possibly Lyb7 B cell determinants (1–3). These mice are functionally abnormal in that as adults they display an immune response pattern to virtually all classes of antigens normally characteristic of neonatal mice (4, 5). The most profoundly affected immune responses in adult CBA/N mice are those to thymic-independent type-2 (TI-2)[1] antigens such as trinitrophenyl (TNP)-conjugated Ficoll (TNP-Ficoll) and pneumococcal polysaccharide, which are entirely absent (6, 7), except perhaps in very old mice (8). Less affected, but still considered to be abnormal, are responses to thymic-independent type-1 (TI-1) antigens such as TNP-lipopolysaccharide (LPS) and TNP-*Brucella abortus* (BA) (9, 10). In contrast, the effect, if any, of the CBA/N genetic defect on responses to thymic-dependent (TD) antigens such as TNP-keyhole limpet hemocyanin (KLH) and sheep erythrocytes (SRBC) is controversial (2, 11, 12). One explanation for abnormal immune responsiveness in adult CBA/N mice is that those B cells these mice do possess are not themselves abnormal, but, rather, that CBA/N mice lack that B cell subpopulation characterized by the expression of the Lyb5 determinant that is present in normal mice and that responds to all classes of antigens.

Left unexplained by this hypothesis is the failure of the B cells that adult CBA/N mice do possess to respond to TI-2 antigens, low doses of TI-1 antigens, and possibly

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[1] *Abbreviations used in this paper:* aecm-Ficoll, aminoethylcarboxymethyl, Ficoll; BA, *Brucella abortus*; C′, complement; CBD2F$_1$, F$_1$ male offspring from crosses between CBA/N ♀ and DBA/2 ♂; D2CBF$_1$, F$_1$ male offspring from crosses between DBA/2 ♀ and CBA/N ♂; KLH, keyhole limpet hemocyanin; LPS, lipopolysaccharide; PFC, plaque-forming cells; SAC, spleen adherent cells; RAMB, rabbit anti-mouse brain serum; SRBC, sheep erythrocytes, TD, thymic dependent; TI-1, thymic independent type 1; TI-2, thymic independent type 2; TNP, 2,4,6-trinitrophenyl, TNP-Ficoll, TNP-conjugated Ficoll.

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to low doses of TD antigens. Because most antibody responses, especially those to TD antigens, result from the activation of B cells by their interaction with the products of helper T cells and/or antigen-presenting accessory cells, rather than from the activation of B cells by antigen alone, it seemed possible that the abnormal response phenotype of CBA/N mice might be the result of a nonproductive cellular interaction between CBA/N B cells and another required cell type. Indeed, it has recently been observed that in normal B cell populations depleted of $Lyb5^+$ cells, the remaining $Lyb5^-$ B cells failed to be activated by their interaction with antigen-presenting accessory cells (13).

To determine the cellular basis for the abnormal primary immune responses observed in CBA/N mice, the present report has assessed the ability of each of the interacting cell subpopulations from these mice to function in TD responses to TNP-KLH as well as in thymic-independent responses to TNP-Ficoll, TNP-LPS, and TNP-BA. The results of these experiments suggest that the abnormal responses observed in mice that express the CBA/N genetic defect could be the consequence of a single cell interaction defect between antigen-presenting accessory cells and B cells.

Materials and Methods

Animals. F_1 mice derived from crosses between DBA/2 and CBA/N mice were obtained from the small animal section, National Institutes of Health, Bethesda, Md., or from Flow Laboratories, Inc., Rockville, Md. Because the immune defect of CBA/N mice is X-linked, F_1 male offspring from crosses between CBA/N ♀ and DBA/2 ♂, (CBA/N × CBA/2)F_1 ♂ (CBD2F_1), are hemizygous for the defective gene xid and are immunologically abnormal, whereas the F_1 male offspring from crosses between DBA/2 ♀ and CBA/N ♂, (DBA/2 × CBA/N)F_1 ♂ (D2CBF_1), are immunologically normal. All mice were used at 6–12 wk of age, unless otherwise stated.

Antigens. Aminoethylcarboxymethyl$_{70}$Ficoll (aecm-Ficoll) was obtained from Biosearch Labs, San Rafael, Calif.; KLH (lot 530195) was obtained from Calbiochem Behring Corp., American Hoechst, San Diego, Calif.; LPS was obtained from the phenol extract of *Salmonella minnesota* R595 hexose-less mutant and was a gift from Dr. John Ryan, Yale University, New Haven, Conn.; BA was obtained from the U. S. Department of Agriculture, Ames, Iowa. All four antigens were conjugated with 2,4,6-trinitrobenzene sulfonate (Pierce Chemical Co., Rockford, Ill.) as previously described (13, 14) to yield TNP-Ficoll (28 TNP groups/400,000 dalton aecm-Ficoll), TNP-KLH (20 groups/100,000 dalton KLH), TNP-LPS, and TNP-BA. Fresh SRBC were obtained weekly from a single sheep, No. 1245.

In Vivo Immunization. In those experiments in which in vivo primary responses were assayed, mice were immunized intravenously with antigen in saline on day 0 and assayed for the number of TNP-specific plaque-forming cells (PFC) in their spleens on day 4. In vivo responses to TNP-KLH over the antigen dose-response range examined were shown to be T cell dependent in adoptive transfer experiments (data not shown).

Preparation of Cell Populations

DEPLETION OF T CELLS. Spleen cells were depleted of T cells to yield (B + accessory) cell populations by pretreatment with a rabbit anti-mouse brain serum (RAMB) + complement (C′). The specificity of the RAMB reagent employed has been described previously and, under the treatment conditions used, is specifically cytotoxic for T cells (15).

DEPLETION OF ADHERENT ACCESSORY CELLS. Spleen cells were depleted of adherent accessory cells to yield (T + B) cell populations by passage over G-10 Sephadex (lot 5164, Pharmacia Fine Chemicals, Div. of Pharmacia Inc., Piscataway, N. J.) as previously described (15). Viable cell recovery after G-10 Sephadex passage ranged between 50 and 60%. Such treatment significantly reduces the number of phagocytic cells but does not significantly affect the percentage of T or B cells (16).

PREPARATION OF T CELLS. Spleen cells were passed over nylon fiber columns and the

nonadherent eluate collected. Such nylon nonadherent spleen-cell populations are markedly depleted of surface immunoglobulin positive (sIg^+) cells and enriched in theta-bearing cells (15).

PREPARATION OF SPLEEN ADHERENT CELLS. Glass-adherent spleen cells (SAC) were prepared as previously described (15). All SAC populations were irradiated with 1,000 rad, in most cases after prior treatment with RAMB + C′, and precultured at 10^7 cells/ml overnight on a rotating drum at 37°C before addition to the antibody cultures. Such populations have been characterized and consist of 50–80% latex-ingesting cells, 8–15% nonphagocytic surface Ig^+ cells, <0.3% Thy 1.2^+ cells, and 15–25% cells negative for these markers (16). The accessory cell activity of the SAC population in antibody responses has previously been shown to reside in a non-T, non-B, glass adherent, radiation-resistant, Ia^+, phagocytic cell that probably can be considered a macrophage or macrophage-like cell (17, 18).

PULSING OF SAC WITH SOLUBLE ANTIGENS. SAC were prepared as above except that they were precultured overnight in the presence of TNP-Ficoll (1 μg/ml) or TNP-KLH (100 μg/ml). At the end of the overnight preculture, the pulsed SAC were washed at least three times and resuspended for addition to the antibody cultures. To control for the possibility of soluble antigen carry-over in the pulsed SAC suspensions, supernates from the final SAC were always assayed for the presence of soluble antigen by its ability to stimulate responses in competent spleen cell populations.

PULSING OF SAC WITH TNP-BA. SAC were prepared as above except that they were precultured overnight in the presence of ~10^9 TNP-BA organisms/ml. Because TNP-BA organisms are in suspension and not in solution, an additional control to that of assaying the final SAC wash for antigen was performed. To control for the possibility that free TNP-BA organisms not associated with SAC had pelleted to the bottom of the tube during the SAC washes, a mock pulsing was performed in parallel by culturing an equivalent amount of TNP-BA in a tube that contained no SAC. At the end of the overnight preculture, both the pulsed SAC and the control were washed at least three times and resuspended for addition to the antibody cultures.

CULTURE CONDITIONS. 0.5–1 × 10^6 unprimed responding spleen cells were placed into culture in a medium that contained 5 × 10^{-5} M 2-mercaptoethanol in 0.2-ml final vol flat-bottomed microtiter plates for 4 d at 37°C in a 5% CO_2-humidified air atmosphere as previously described (14). Unless otherwise stated, antigens were added so that their final concentrations in culture were: TNP-KLH, 5 μg/ml; TNP-Ficoll, 10^{-2} μg/ml; TNP-BA, 0.05% of stock suspension that contained ~10^{11} organisms/ml; TNP-LPS, 2 μg/ml; SRBC, 0.01% vol:vol. Exclusive of the use of TNP-LPS that induced proliferation, viable cell recovery was independent of the presence or absence of antigen.

There was also no difference in the viable cell recovery of abnormal $CBD2F_1$ spleen cells and normal $D2CBF_1$ spleen cells.

PFC ASSAY. SRBC were conjugated with TNP (TNP-SRBC) by the method of Rittenberg and Pratt (19). Direct (IgM) PFC to TNP-SRBC and unmodified SRBC were assayed by the slide modification of the Jerne hemolytic plaque technique (20). Anti-TNP PFC responses have been shown to be TNP-specific by two criteria: (*a*) >90% inhibition by 5 × 10^{-5} M TNP-bovine serum albumin (14, 15), and (*b*) the absence of virtually any PFC on unhaptenated SRBC (Unpublished results.).

Results

In Vivo Primary Anti-TNP PFC Responses to TNP-Ficoll and TNP-KLH in Mice that Express the CBA/N Immune Defect. The in vivo responses of unprimed abnormal $CBD2F_1$ male mice to optimal doses of soluble TNP-Ficoll or TNP-KLH were compared with those of their normal reciprocal F_1 male counterparts ($D2CBF_1$). As expected, normal $D2CBF_1$ mice responded to both antigens, whereas abnormal $CBD2F_1$ mice responded only to TNP-KLH (Table I). The lower response observed in abnormal F_1 mice to TNP-KLH was consistent with their having approximately one-half the number of spleen B cells of their normal counterparts as has been suggested (11, 21). Indeed, the in vivo responses of abnormal $CBD2F_1$ male mice to

TABLE I

In Vivo Primary IgM PFC Responses to Soluble TNP-Ficoll and TNP-KLH

Strain	TNP-KLH*	TNP-Ficoll‡	No antigen
		PFC/10^6 spleen cells§	
Abnormal CBD2F$_1$ ♂	327 (1.23)	3 (1.48)	3 (1.41)
Normal D2CBF$_1$ ♂	861 (1.18)	>1,000	17 (1.02)

* 100 μg intravenous.

‡ 50 μg intravenous.

§ Geometric mean with SE in parentheses of responses from at least three individual mice assayed 4 d after intravenous immunization.

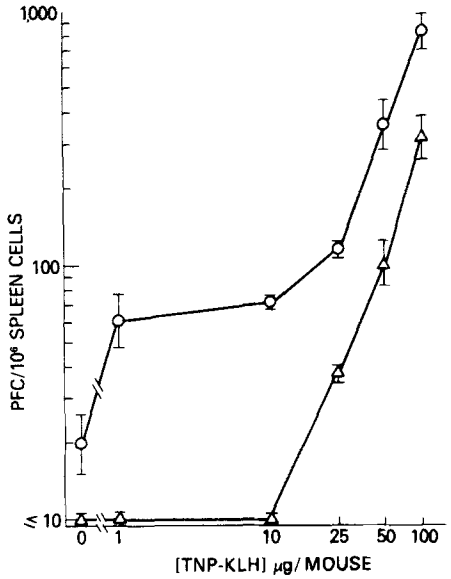

FIG. 1. Comparison of the in vivo primary immune responses to soluble TNP-KLH of normal and abnormal F_1 male mice. Normal D2CBF$_1$ male mice (○) and abnormal CBD2F$_1$ male mice (△) were immunized intravenously with graded doses of TNP-KLH in saline. Primary IgM anti-TNP PFC responses were measured 4 d after immunization. Each point represents the geometric mean of the responses of three to five mice.

TNP-KLH was consistently approximately one-half those of their reciprocal normal F_1 counterparts to high doses (25–100 μg) of TNP-KLH (Fig. 1). However, this ratio of responsiveness did not hold at lower doses of antigen because normal D2CBF$_1$ mice generated detectable responses even at the lowest dose (1 μg) of TNP-KLH tested, whereas abnormal CBD2F$_1$ mice did not respond to even 10 times that amount (Fig. 1). These results suggest that, whereas differences in number of B cells might be a sufficient explanation for the lower responses of abnormal mice to high doses of TNP-KLH, it is not a satisfactory explanation for the failure of abnormal mice to generate any response to low doses of TNP-KLH. Rather, they suggest the possibility that the cellular interactions stimulated by low doses of TNP-KLH might be insufficient to trigger the B cell populations in abnormal mice, even though they are apparently sufficient to activate the B cell populations in normal mice.

In Vitro Analysis of the Ability of Abnormal CBD2F$_1$ Male Spleen Cells to Generate Primary

Responses to TNP-KLH. To further examine the possibility that the cellular activation requirements of B cells from abnormal F_1 mice might differ from those from normal F_1 mice for the helper T cell-dependent response to TNP-KLH, the ability of unprimed spleen cells from these mice to generate primary PFC responses was assessed in vitro. As noted in earlier reports, spleen cells derived from abnormal F_1 mice responded in vitro to optimal concentrations of the T cell-independent antigens TNP-LPS and TNP-BA, but were absolutely unresponsive to optimal concentrations of the T cell-independent antigen TNP-Ficoll (Table II, experiment 1). However, quite unexpected was the profound failure of spleen cells from abnormal F_1 mice to generate any primary helper T cell-dependent response in vitro to TNP-KLH (Table II, experiment 1). In fact, spleen cells from abnormal F_1 mice were unable to generate in vitro responses to even 50 times the amount of TNP-KLH immunogenic for spleen cells from normal F_1 mice (Table II, experiment 2). Identical results were observed with spleen cells from abnormal $CBD2F_1$ mice that ranged in age from 2–5 mo, even though under the primary culture conditions used: (*a*) spleen cell populations from normal mice were competent to respond to TNP-KLH, (*b*) spleen cell populations from abnormal F_1 mice generated anti-TNP responses to TNP-LPS and TNP-BA, and (*c*) the viable cell recovery of cells from unresponsive abnormal $CBD2F_1$ cultures were equivalent to those from unstimulated normal $D2CBF_1$ cultures. Because the spleen cells from these mice were also unable to generate T cell-dependent responses under these culture conditions to SRBC (Table II, experiment 3), an antigen unrelated to TNP-KLH, it can be concluded that mice that express the X-linked CBA/N genetic immune abnormality are profoundly deficient in their ability to generate helper T cell-dependent primary antibody responses under limiting in vitro micro-

TABLE II

In Vitro Primary IgM PFC Responses of Normal and Abnormal F_1 Male Spleen Cells to TD and Thymic-independent Antigens

Experiment	Antigen	Antigen class	Normal $D2CBF_1$ ♂ spleen cells	Abnormal $CBD2F_1$ ♂ spleen cells
			*PFC/10^6 cultured cells**	
1	TNP-LPS	TI-1	264 (1.11)	366 (1.14)
	TNP-BA	TI-1	172 (1.10)	253 (1.17)
	TNP-Ficoll	TI-2	114 (1.05)	0
	TNP-KLH	TD	154 (1.10)	6 (2.71)
	No antigen		3 (1.82)	0
2	TNP-KLH 1 μg/ml		86 (1.19)	3 (1.76)
	5 μg/ml		276 (1.40)	2 (1.54)
	10 μg/ml		392 (1.04)	4 (1.16)
	25 μg/ml		250 (1.18)	5 (1.10)
	50 μg/ml		242 (1.09)	5 (1.27)
	No antigen		3 (2.05)	2 (1.49)
3	TNP-LPS	TI-1	386 (1.07)	332 (1.31)
	TNP-KLH	TD	330 (1.11)	0
	SRBC	TD	107 (1.25)	2 (1.60)
	No antigen		2 (1.25)	2 (1.25)

* Geometric mean with SE in parentheses of triplicate cultures.

culture conditions. An understanding of the cellular basis of this genetic defect requires that the cell subpopulation(s) be identified in which the genetic defect is expressed.

Assessment of Helper T Cell Function and Antigen-presenting Accessory Cell Function for Responses to TNP-KLH in Abnormal CBD2F$_1$ Spleen Cell Populations. The ability of T cells from abnormal F_1 mice to provide helper function for responses to TNP-KLH was assessed by the addition of graded numbers of spleen T cells from either normal or abnormal F_1 mice to cultures that had been depleted of T cells by treatment with RAMB + C′ (Fig. 2). T cells derived from abnormal spleen cell populations were essentially as competent as those from normal spleen cell populations in providing help to T cell-depleted populations derived from normal D2CBF$_1$ mice (Fig. 2A). In contrast, neither helper T cell population was able to stimulate T cell-depleted populations from abnormal F_1 mice to respond to TNP-KLH (Fig. 2B). Thus, the unresponsiveness of abnormal CBD2F$_1$ spleen cells to TNP-KLH was not the result of defective helper T cell function, but rather the result of a defect in the ability of their non-T cell population to function in responses to TNP-KLH.

To determine whether the failure of CBD2F$_1$ T cell-depleted populations to function in responses to TNP-KLH was the result of the inability of the accessory cells in these populations to present TNP-KLH, SAC from both normal and abnormal F_1 spleens were prepared as accessory cell populations, pulsed overnight with TNP-KLH, and assayed for their ability to function as TNP-KLH-presenting cells. Graded numbers of these antigen-presenting cells were added to cultures of spleen cells depleted of adherent accessory cells by G-10 Sephadex passage (Fig. 3). SAC from both normal and abnormal F_1 mice were equally competent in their ability to present TNP-KLH to normal F_1 lymphocytes (Fig. 3A), demonstrating that accessory cells from abnormal mice were not defective in their ability to present T cell-dependent antigens. However, the addition of these same competent TNP-KLH-presenting accessory cell populations to lymphocytes from abnormal CBD2F$_1$ mice did not result in the generation of any response (Fig. 3B).

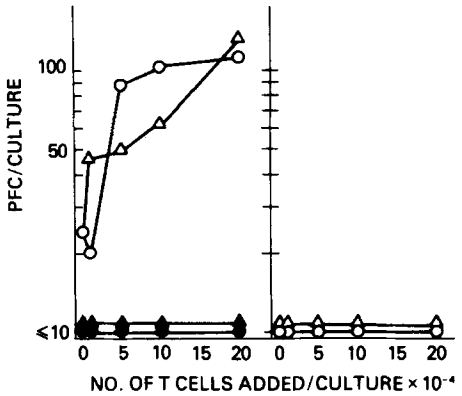

FIG. 2. Helper cell function of T cells from normal and abnormal F_1 male mice for responses to TNP-KLH. Graded numbers of T cells from normal D2CBF$_1$ male spleens (○) or abnormal CBD2F$_1$ male spleens (△) were added to cultures that contained 4×10^5 RAMB + C′-treated spleen cells from either normal (A) or abnormal (B) mice. Cultures were stimulated by the addition of either TNP-KLH (open figures) or medium alone (closed figures). No PFC were generated in cultures containing only T cells and antigen.

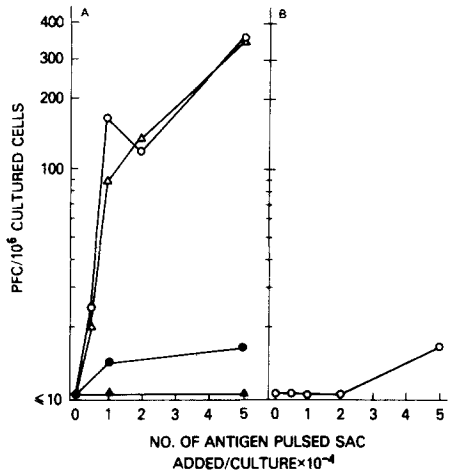

FIG. 3. Antigen-presenting accessory cell function of SAC from normal and abnormal F_1 male mice for responses to TNP-KLH. Graded numbers of SAC from either normal $D2CBF_1$ male mice (○) or abnormal $CBD2F_1$ male mice (△) that had been pulsed with TNP-KLH (open figures) or medium (closed figures) were added to cultures that contained 5×10^5 G-10-passed spleen cells from either normal mice (A) or abnormal mice (B).

These experiments demonstrate that the inability of abnormal $CBD2F_1$ spleen cells to generate primary in vitro responses to TNP-KLH is not the result of either defective helper T cell function or defective antigen-presenting accessory cell function. Rather, it is the result of an inability of competent accessory cells and competent helper T cells to activate B lymphocytes from abnormal mice to respond to TNP-KLH.

Cellular Analysis of the CBA/N Genetic Defect in the Generation of T-independent Responses to TNP-Ficoll. Because the profound failure of abnormal $CBD2F_1$ spleen cells to generate helper T cell-dependent responses in vitro to TNP-KLH did not result either from defective helper T cell function or from defective antigen-presenting accessory cell function, it seemed reasonable to hypothesize that the B cell defect responsible for unresponsiveness to TNP-KLH might also be responsible for the failure of abnormal spleen cells to generate T cell-independent responses to TNP-Ficoll. Although the inability of CBA/N mice to respond to TNP-Ficoll has generally been assumed to derive from a defect in their B cell compartment, TNP-Ficoll responses do require accessory cells (14), and the inability of abnormal CBA/N mice to generate TNP-Ficoll responses might be the result of defective accessory cell function for TNP-Ficoll in genetically defective mice. This possibility was examined by depleting normal $D2CBF_1$ spleen cells of adherent accessory cells by G-10 Sephadex passage (Table III). Such treatment did not influence the ability of spleen cells to respond to either TNP-LPS or TNP-BA (Table III), which confirms previous observations that responses to these two antigens did not require the presence or function of adherent accessory cells (13). In contrast, the ability of normal $D2CBF_1$ spleen cells to respond to TNP-Ficoll was abrogated by G-10 passage and the attendant removal of adherent accessory cells (Table III). SAC from both normal $D2CBF_1$ and abnormal $CBD2F_1$ mice were equally competent in reconstituting the ability of G-10-passed normal $D2CBF_1$ lymphocytes to respond to TNP-Ficoll, and so were indistinguishable in their ability to provide accessory cell function for this response (Table III). In addition to

TABLE III

Accessory Cell Function for T Cell Independent Responses to TNP-Ficoll

Spleen cells 5×10^5/culture	Treatment of spleen cells	Strain of SAC (1×10^4/culture)	TNP-Ficoll	TNP-LPS	TNP-BA	No antigen
			*PFC/10^6 cultured cells**			
Normal $D2CBF_1$ ♂	—	—	126 (1.28)	332 (1.08)	220 (1.05)	2 (1.38)
Normal $D2CBF_1$	G-10 Passage	—	16 (1.38)	572 (1.29)	228 (1.07)	2 (1.52)
Normal $D2CBF_1$	G-10 Passage	$D2CBF_1$	154 (1.07)	ND‡	ND	2 (1.52)
Normal $D2CBF_1$	G-10 Passage	$CBD2F_1$	182 (1.10)	ND	ND	2 (1.00)

* Geometric mean with SE in parentheses of triplicate cultures.

‡ ND, not determined.

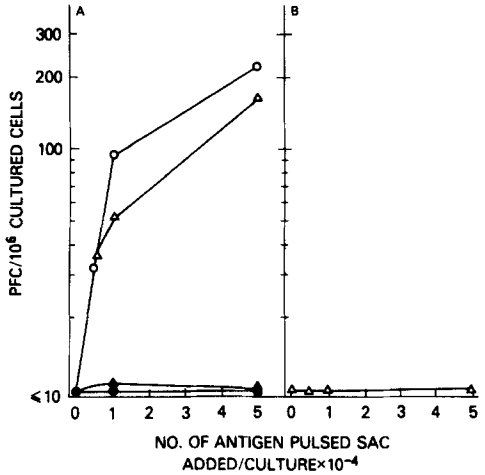

FIG. 4. Antigen-presenting accessory cell function of SAC from normal and abnormal F_1 male mice for responses to TNP-Ficoll. Graded numbers of SAC from either normal $D2CBF_1$ male mice (○) or abnormal $CBD2F_1$ male mice (△) that had been pulsed with TNP-Ficoll (open figures) or medium (closed figures) were added to cultures that contained 5×10^5 G-10-passed spleen cells from either normal mice (A) or abnormal mice (B).

functioning equally well as TNP-Ficoll accessory cells, SAC from both normal $D2CBF_1$ and abnormal $CBD2F_1$ mice that had been pulsed with TNP-Ficoll were equally competent in functioning as TNP-Ficoll-presenting cells in triggering normal B cells to respond to TNP-Ficoll (Fig. 4). All the TNP-Ficoll present in these cultures was cell-associated because assays of the SAC washes were always negative for the presence of soluble TNP-Ficoll along with the pulsed SAC. In addition, the ability of pulsed SAC to present TNP-Ficoll to B cells was not merely a result of their having trapped antigen, because it has been previously shown that TNP-Ficoll-pulsed SAC must be viable to function as TNP-Ficoll-presenting cells (14). However, in contrast to the ability of normal F_1 B cells to be activated by both TNP-Ficoll-presenting cell populations, B cells from abnormal F_1 mice could not be triggered by either presenting cell population to respond to TNP-Ficoll (Fig. 4). Thus, these findings suggest that expression of the CBA/N genetic defect in $CBD2F_1$ mice results in an inability, or at least a deficiency, in the B cells from these mice to be activated by antigen-presenting accessory cells.

Failure of Abnormal CBD2F$_1$ B Cells to Respond to TNP-BA Presented by Accessory Cells. The data presented thus far demonstrate that abnormal CBD2F$_1$ B cells are not activated by their interaction with either TNP-Ficoll- or TNP-KLH-presenting accessory cells. However, there still exist two alternative explanations for these observations. As already suggested, the genetic defect might result in a triggering deficit such that abnormal B cells cannot be activated by their interaction with antigen-presenting accessory cells. Alternatively, it is possible that the genetic defect results in a recognition deficit such that the abnormal B cells cannot recognize certain antigens, and that it is only coincidental that responses to those antigens all required presentation by accessory cells. This latter possibility seemed unlikely because the B cell responses examined in this study were all TNP specific. Nevertheless, it was possible that the TNP-specific responses to the different antigens examined in this study required recognition of carrier determinants by B cells, and that the TNP-specific B cells from abnormal CBD2F$_1$ mice were incapable of recognizing TNP-Ficoll or TNP-KLH but could recognize TNP-LPS and TNP-BA. Consequently, a carrier-recognition defect in abnormal B cells could only be ruled out by experiments in which the failure to trigger responses in abnormal B cells could not be the result of a failure of these B cells to recognize the antigen per se. These experiments were carried out using TNP-BA that, although capable of activating B cells directly in the absence of either T cells (4, 13) or accessory cells (13) (Table III), might also be capable of activating B cells when presented by accessory cells. To observe such presentation of TNP-BA by accessory cells, the accessory cells were first pulsed with TNP-BA in the absence of responding B cells and then added to culture as antigen-presenting accessory cells.

Indeed, SAC that had been pulsed with TNP-BA were capable of activating TNP-specific B cells from normal F$_1$ mice (Fig. 5A, 13). These responses were caused by TNP-BA presented by SAC and not by free TNP-BA inadvertently transferred along with the pulsed SAC, because neither the supernate from the final SAC wash nor the

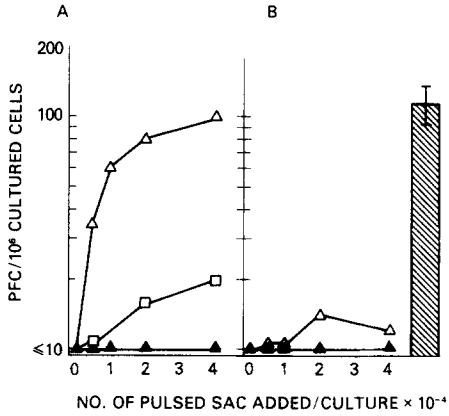

FIG. 5. TNP-BA pulsed SAC activate B cells from only normal mice. Graded numbers of CBD2F$_1$ SAC that had been pulsed with TNP-BA (△) or medium (▲) were added to cultures containing 5 × 10^5 G-10-passed spleen cells from either normal mice (A) or abnormal mice (B). In addition, a mock pulsing was performed by treating TNP-BA suspension containing no SAC (□) precisely as the pulsed SAC as a control for the possible pelleting of free TNP-BA organisms with SAC in the pulsed SAC groups. The response to TNP-BA suspension added directly to culture is also indicated (▨).

control TNP-BA pellet from the mock TNP-BA pulsing performed in parallel contained sufficient antigen to stimulate significant responses in these same F_1 B cells (Fig. 5 A). In contrast to the ability of B cells from normal F_1 mice to be activated by TNP-BA-pulsed SAC, B cells from abnormal F_1 mice were not activated by these TNP-BA-presenting accessory cells, even though these B cells were activated by TNP-BA free in suspension (Fig. 5 B).

Thus, these results demonstrate that TNP-specific B cells from abnormal F_1 mice are not responsive to antigen-presenting accessory cells, even when the antigen being presented was one to which these B cells could otherwise respond. These results suggest that the cellular basis of the abnormal immune responses observed in mice that inherit the CBA/N genetic defect is that the B cells in these mice fail to productively interact with antigen-presenting accessory cells.

Discussion

Because the B lymphocyte population in mice that express the X-linked CBA/N genetic defect is either entirely devoid or extremely depleted of an $Lyb5^+$ subpopulation, it is not surprising that B cell responses in these mice are not identical to those in normal mice. However, responses to all types of antigens are not equally affected by the absence of the $Lyb5^+$ B cell subpopulation. Indeed, expression of the CBA/N genetic defect results in responses to TI-2 antigens being entirely absent, whereas responses to TI-1 antigens and TD antigens are affected less dramatically. The present report has approached this issue by an analysis of the responses of CBA/N mice to TI-1, TI-2, and TD antigens from the perspective that the response to each antigen class represented an independent assessment of the cellular activation requirements of B cell populations genetically deprived of $Lyb5^+$ cells.

Analysis of the TD response to TNP-KLH revealed that genetically defective $CBD2F_1$ male mice can generate primary helper T cell-dependent responses in vivo, but only to high doses of antigen. The failure of these mice to generate primary responses to low doses of TNP-KLH in vivo was observed for all concentrations of TNP-KLH under in vitro microculture conditions in which cell numbers and the potential number of cellular interactions were probably limiting. The profound unresponsiveness of these mice in vitro was the result of neither incompetent helper T cell function nor incompetent antigen-presenting accessory cell function. Indeed, within the limits of the in vitro assay to assess such functions, there were no discernible differences in the ability of the appropriate cell populations from normal or abnormal mice to perform as helper T cells or antigen-presenting accessory cells. Similar analysis of thymic-independent responses to TNP-Ficoll similarly revealed no flaw in the ability of accessory cells from abnormal mice to present TNP-Ficoll. Thus, the failure to generate in vitro either primary TD responses to TNP-KLH or thymic-independent responses to TNP-Ficoll resulted from the inability of B cells from genetically defective mice to be activated by their interaction with competent helper T cells and/or competent antigen-presenting accessory cells.

The inability of B cells from abnormal mice to be activated by antigen-presenting accessory cells was not limited to accessory cell presentation of TD and TI-2 antigens, but included accessory cell presentation of TI-1 antigens as well. In the experiments presented in this report, even though abnormal B cells were triggered by TNP-BA in suspension, these same B cells were not triggered by TNP-BA presented by antigen-

pulsed accessory cells. Taken together, the results of these experiments strongly suggest that the B cells from genetically defective mice are not activated by their interaction with antigen-presenting accessory cells, regardless of the antigen being presented. These conclusions are consistent with and extend the recent observation that CBA/N B cell populations fail to physically bind and form clusters around macrophages (22).

Because at least one nonproductive cellular interaction in mice that express the CBA/N genetic defect appeared to be between B cells and antigen-presenting accessory cells, is it possible that all of the abnormal response patterns observed in CBA/N mice resulted from this single interaction defect? Indeed, it is possible to construct a cell interaction model that does account for many of the response abnormalities observed in genetically defective mice. One model of the cell interactions possible in normal and genetically defective mice is schematically illustrated in Fig. 6. In this model, only Lyb5$^+$ B cells can be triggered by accessory cell activation signals (13), and it is the Lyb5$^+$ B cell subpopulation that is absent in mice that express the CBA/N genetic defect (1, 23). The profound defect in mice that express the CBA/N genetic defect in response to TI-2 antigens results from the fact that TI-

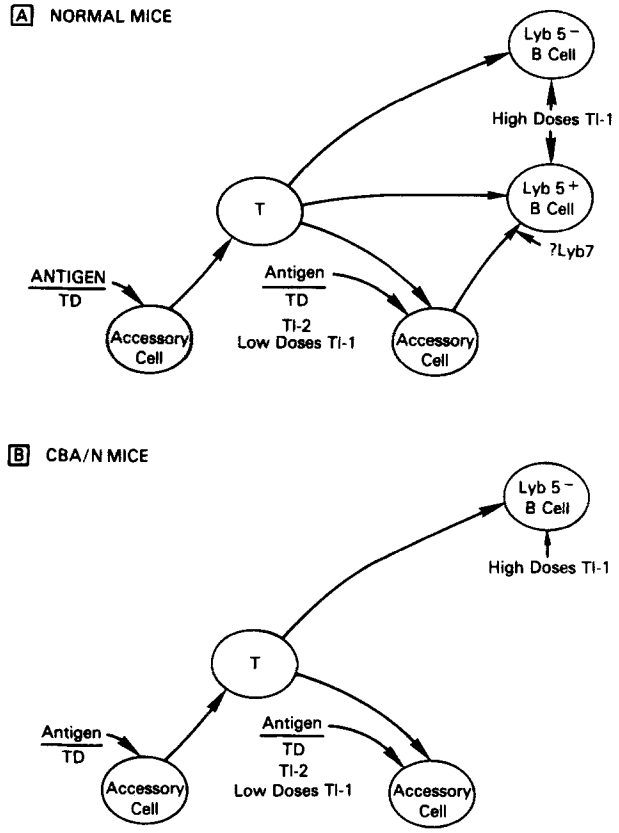

FIG. 6. One model of the cell interactions possible in normal and abnormal mice. This model presumes that the cell populations present in CBA/N mice are intrinsically normal and that the abnormal primary immune responses observed in CBA/N mice result entirely from the absence of Lyb5$^+$ B cells and the inability of antigen-presenting accessory cells to activate Lyb5$^-$ B cell populations in either normal or abnormal mice.

2 antigens can only activate B cells via an accessory cell-B cell interaction pathway that is nonfunctional in these mice. In contrast, there exist other pathways by which TI-1 and TD antigens can activate B cells. Specifically, TI-1 antigens at high doses activate both $Lyb5^+$ and $Lyb5^-$ B cell subpopulations directly; at low doses, however, these antigens behave like TI-2 antigens in that they are taken up by accessory cells and subsequently presented only to $Lyb5^+$ B cells. TD antigen responses are even more complex in that there exist several different interaction pathways by which B cells can be activated. For example, after the triggering of helper T cells by antigen-presenting accessory cells, activated helper T cells can directly interact with both $Lyb5^+$ and $Lyb5^-$ B cell subpopulations, as well as with accessory cells. Under conditions in which T cell help is transmitted to B cells indirectly via accessory cells, the result is the activation of only $Lyb5^+$ B cells, and it is under these particular conditions that B cells from abnormal mice ($Lyb5^-$) would be unresponsive to TD antigens. However, under other conditions, T cells could interact directly with B cells and so activate both $Lyb5^+$ and $Lyb5^-$ cell subsets, and it is under these conditions that B cells from abnormal mice would be responsive to TD antigens.

The specific conditions under which direct rather than indirect T cell-B cell interactions would occur might reasonably be expected to be those in which a great deal of T cell help is generated (e.g., in vivo or with antigen-primed cell populations in vitro) because the chance encounter of a specific helper T cell with a specific B cell must be infrequent. In contrast, because accessory cells are not antigen specific, they potentially represent a more efficient, if indirect, means of transmitting limited amounts of specific T cell signals to specific B cells, as has been suggested by others (24). Thus, under conditions in which the number of specifically activated T cells is limiting (e.g., in vivo with low concentrations of antigen or in vitro microcultures using unprimed cells), the T cell-accessory cell-$Lyb5^+$ B cell activation pathway would predominate, and it is under such conditions that abnormal mice would be unresponsive to TD antigens.

This model of the cell interactions leading to B cell activation in both normal and genetically defective mice offers several potentially useful insights. (*a*) The rather confusing and sometimes conflicting patterns of responsiveness and unresponsiveness observed in mice that express the CBA/N genetic defect can be the result of a single nonproductive cell interaction that derives both from the absence of $Lyb5^+$ B cells in these mice and the inability of $Lyb5^-$ B cells to be triggered by accessory cell activation signals. (*b*) Because the activation of B cells by accessory cells may be the only defective cell interaction in these mice, the absence of a specific response in mice that expresses the CBA/N genetic defect is suggestive evidence that that response requires an accessory cell-B cell activation step. (*c*) Because the responses to TI-2 antigens, which this model proposes as proceeding only via an accessory cell-B cell activation step, have been reported to be inhibited by anti-Lyb7 specific serum (3), it is possible that anti-Lyb7 serum blocks the interaction of B cells with accessory cells, possibly by interfering with reception of accessory cell activation signals by the B cell. (*d*) The feature that primarily distinguishes TD antigens from TI-2 antigens according to this model is the ability of TI-2 antigens to directly stimulate accessory cells to activate B cells. Thus, it is possible that the structural features that characterize TI-2 antigens result in their being able to directly activate accessory cells, rather than B cells. (*e*) Finally, in terms of the current controversy regarding the importance of H-2-

restricted helper T cell activation of B cells, this model proposes that activation of B cells in helper T cell-dependent responses might not always occur via direct interactions between helper T cells and B cells but, rather, could also proceed via an accessory cell intermediary. In this case, it would not be surprising if T cell recognition of B cell Ia determinants did not occur, as indeed has been observed in this in vitro primary response system (25). In contrast, it is also possible that under conditions in which direct T cell B cell interaction can occur, T cell recognition of B cell Ia determinants might be required for direct activation of B cells by helper T cells. Thus, depending upon the B cell activation pathways permitted by the experimental conditions, one or both B cell subpopulations would be activated and H-2-restricted T-B interactions would or would not be observed.

Whether or not this particular cell interaction model is correct, the present data do suggest that mice that express the CBA/N genetic defect are unresponsive to antigens that require accessory cell presentation. It is suggested that such unresponsiveness results from the absence in these mice of that B cell subpopulation ($Lyb5^+$) that can be activated by antigen-presenting accessory cells. Thus, these mice are always unresponsive to TI-2 antigens but fail to respond to TI-1 and TD antigens only under those experimental conditions that discourage direct activation of $Lyb5^-$ B cells. Finally, it should be emphasized that even though a single interaction defect between accessory cells and B cells is sufficient to account for many of the observed response abnormalities in mice that express the CBA/N genetic defect, these studies do not preclude the possibility that other cellular interaction deficits may also exist in these mice.

Summary

The effect of the X-linked CBA/N genetic defect on the ability of mice to generate primary responses to thymic-dependent and thymic-independent antigens was assessed by comparing the ability of abnormal (CBA/N × DBA/2)F_1 male mice and normal (D8A/2 × CBA/N)F_1 male mice to generate 2,4,6-trinitrophenyl (TNP)-specific plaque-forming cell responses to TNP-keyhole limpet hemocyanin (KLH), TNP-conjugated Ficoll (TNP-Ficoll), TNP-*Brucella abortus* (BA), and TNP-lipopolysaccharide (LPS). The reciprocal F_1 combinations used in this study differ genetically only in the origin of their X chromosome, but differ immunologically in that (CBA/N × DBA/2)F_1 male mice express all the CBA/N immune abnormalities, whereas (DBA/2 × CBA/N)F_1 male mice are immunologically normal.

Analysis of thymic-dependent responses to TNP-KLH revealed that abnormal F_1 mice were capable of generating primary responses in vivo to high doses of TNP-KLH, but failed to generate responses to suboptimal doses of TNP-KLH that were still immunogenic for normal F_1 mice. Furthermore, under limiting in vitro microculture conditions, the abnormal F_1 mice failed to generate primary thymic-dependent responses to any dose of TNP-KLH, even though under the identical conditions normal F_1 mice consistently responded to a wide antigen dose range. The cellular basis of the failure of abnormal F_1 mice to respond in vitro to TNP-KLH was investigated by assaying the ability of purified populations of accessory cells, T cells, and B cells from these mice to function in responses to TNP-KLH. The results of these experiments demonstrated that helper T cells and antigen-presenting accessory cells from abnormal F_1 mice were competent and functioned as well as the equivalent

cell populations from normal F_1 mice. Instead, the failure of CBA/N mice to generate primary in vitro responses to TNP-KLH was solely the result of a defect in their B cell population such that B cells from these mice failed to be triggered by competent helper T cells and/or competent accessory cells.

Similarly, the failure of abnormal F_1 mice to respond either in vivo or in vitro to TNP-Ficoll was not the result of defective accessory cell presentation of TNP-Ficoll, but was the result of the failure of B cells from these mice to be activated by competent TNP-Ficoll-presenting accessory cells.

In contrast to the failure of B cells from abnormal F_1 mice to be activated in vitro in response to either TNP-KLH or TNP-Ficoll, B cells from abnormal F_1 mice were triggered to respond to TNP-BA and TNP-LPS, antigens that did not require accessory cell presentation. The specific failure of B cells from abnormal F_1 mice to be activated in responses that required antigen-presentation by accessory cells suggested the possibility that the X-linked CBA/N genetic defect resulted in B cell populations that might be deficient in their ability to interact with antigen-presenting accessory cells. To further explore this possibility, accessory cells were pulsed with TNP-BA (an antigen to which B cells from abnormal F_1 mice responded) and added to culture as TNP-BA-presenting cells. Although B cells from abnormal F_1 mice were triggered and responded to TNP-BA free in suspension, the same abnormal B cell populations failed to be triggered by TNP-BA presented by pulsed accessory cells.

The results of these experiments suggest that the B cell populations that abnormal CBA/N mice possess fail to be triggered by their interaction with antigen-presenting accessory cells, regardless of the antigen being presented. Indeed, it is possible to explain many of the immune abnormalities observed in CBA/N mice as the result of a single interaction defect between antigen-presenting accessory cells and CBA/N B cells.

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