X-LINKED B-LYMPHOCYTE IMMUNE DEFECT IN CBA/N MICE

II. Studies of the Mechanisms Underlying the Immune Defect*

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CBA/N (CN)[1] mice are a subline of CBA mice with a marked defect in the function and a diminution in the number of thymus-independent (B) lymphocytes. These animals are unable to form specific antibody to Type III pneumococcal polysaccharide (SIII), bacterial lipopolysaccharide (LPS) (1), or polyriboinosinic-polyribocytidylic acid (poly I·C) (2). Each of these antigens is known to evoke an antibody response in all conventional mouse strains studied, even if these mice are deprived of thymus-dependent (T) lymphocytes (3-5). The inability of the CN mice to respond to these "T-independent" antigens is inherited as an X-linked recessive trait (1, 2). We have shown that CN mice and F_1 male mice of the CN × DBA/2N (DN) cross (CN × DN F_1 males) have a diminished number of immunoglobulin (Ig)-bearing spleen cells, an impaired response to agents mitogenic for B lymphocytes (B mitogens) and a diminished ability to participate in antibody-dependent cell-mediated cytotoxicity (6).

Although the X-linked immune defect in the CN mice is expressed as a functional abnormality of B lymphocytes, the mechanism underlying the defect has not been studied. In particular, it is not known whether the abnormality of CN B-lymphocyte function represents a defect intrinsic to the B-lymphocyte line or results either from an abnormality in the microenvironment in which CN B lymphocytes differentiate or from abnormal function of CN T lymphocytes. Because of the X-linked nature of the immune defect of CN mice, lymphoid cell

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[1] *Abbreviations used in this paper:* anti-θ, anti-Thy 1.2; B, thymus independent; BRBC, burro erythrocytes; Con A, concanavalin A; CN, CBA/N mice; DN, DBA/2N mice; DNP-lys-Ficoll, 2,4-dinitrophenyl-lysyl-derivative of Ficoll; LPS, bacterial lipopolysaccharide; PFC, plaque-forming cell; poly I·C, polyriboinosinic-polyribocytidylic acid; SIII, type III pneumococcal polysaccharide; T, thymus dependent; TNP, trinitrophenyl.

transfers between phenotypically normal F_1 female mice and their abnormal male littermates can be performed. By immunization of recipients of transferred cells with either of two T-independent antigens, poly I·C and the 2,4-dinitrophenyl-lysyl-derivative of Ficoll (DNP-lys-Ficoll) (7, 8), we have studied the cellular basis of the immune defect of CN mice. Our data indicate that the failure of these mice to respond to T-independent antigens is a result of a deficiency or an intrinsic abnormality of B lymphocytes and/or their progenitors rather than a microenvironmental or T-lymphocyte abnormality.

Materials and Methods

Animals. CN,[2] DN mice, and F_1 animals derived from these strains, (CN × DN) F_1, were obtained from the Rodent and Rabbit Production Section of the National Institutes of Health, Bethesda, Md. All mice were 6–12wk of age at the time of study. The CN mice are a distinct subline of CBA mice and the history of their establishment has been described elsewhere (1, 2). F_1 mice of both sexes were produced by breeding CN females with DN males (CN × DN F_1 males or females).

Lethally irradiated mice (1,000 rads, ^{60}cobalt source, dose rate 40 rads/min) were reconstituted on the day after irradiation by the administration of spleen or bone marrow cells intravenously. CN × DN F_1 males or females were thymectomized at 6 wk of age by aspiration through a sternum-splitting incision. These mice were lethally irradiated 14 days after surgery and reconstituted with 10×10^6 F_1 male or female bone marrow cells which had been treated with AKR anti-Thy 1.2 (anti-θ) plus rabbit complement (C) as has been previously described (9).

Cell Suspensions. Mice were killed by cervical dislocation and their thymuses and/or spleens removed. Spleen and thymus cells were obtained by gentle teasing with a rubber policeman and forceps into RPMI 1640 (Grand Island Biological Co, Grand Island, N. Y.). Bone marrow cells were flushed from the femur and tibial bones using a 25 gauge needle. Cell aggregates were disrupted by passing the cell suspensions through a 26 gauge needle. The single cell suspensions were then washed twice with RPMI 1640 and the number of cells counted.

Antigens. Poly I·C, prepared by annealing the homopolymers polyriboinosinic acid and polyribocytidylic acid, (P-L. Biochemicals, Inc., Milwaukee, Wis.) was dissolved in borate buffer, pH 8, and emulsified with an equal volume of complete Freund's adjuvant (Difco Laboratories, Detroit, Mich.) so that 0.3 ml of the final emulsion contained 100 μg of antigen. Mice were immunized with 0.3 ml of this material by intraperitoneal injection.

DNP-lys-Ficoll was prepared according to the method of Sharon et al. (8). Briefly, Ficoll (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.) was reacted with cyanuric chloride at 4°C, followed by the addition of ϵ-DNP-L-lysine and subsequent reaction at room temperature. The DNP-lys-Ficoll used in these studies had a molar ratio of DNP to Ficoll of 32:1.

Sheep erythrocytes (SRBC) and burro erythrocytes (BRBC) were obtained from a single sheep or burro and were collected sterilely in citric acid-dextrose solution (ACD solution; Abbott Laboratories, Chemical Marketing Div., North Chicago, Ill.). These were washed three times in Hanks' balanced salt solution before use. SRBC were heavily conjugated with the trinitrophenyl (TNP) hapten by reacting sodium 2,4,6-trinitrobenzenesulfonate with SRBC according to the method of Kettman and Dutton (10). Mice were immunized by the intravenous injection of 2×10^8 of these heavily conjugated TNP-SRBC immediately after their preparation.

Antibody and Plaque-Forming Cell Assays. Antibody directed against poly I·C was assayed in sera obtained by orbital sinus puncture of immunized mice by an ammonium sulfate precipitation assay using [^{14}C]poly I·C (Miles Laboratories Inc., Miles Research Div., Elkhart, Ind.) as ligand (11). Data are expressed as the percent binding of 80 ng of [^{14}C]poly I·C (4,000 dpm/μg) by 25 μl of mouse serum. Binding of greater than 20% was considered a positive response; in previous studies we have shown that serum from unimmunized mice do not exceed this degree of binding (2) as is the case for mice immunized with noncross-reacting antigens or adjuvant alone.

Spleen cells from mice immunized with SRBC, heavily substituted TNP-SRBC or DNP-lys-

[2] CBA/N mice were formerly designated CBA/HN.

Ficoll were assayed for cells releasing specific antibody by a modification of the Jerne hemolytic plaque technique (12), using SRBC or lightly conjugated TNP-BRBC or TNP-SRBC as indicator cells, respectively. TNP-SRBC and TNP-BRBC were prepared by the method of Rittenberg and Pratt (13). There was less than 1% cross-reactivity between SRBC and BRBC in our system. Plaque-forming cells (PFC) releasing IgG antibodies were detected according to the method of Pierce et al. (14) by inhibition of IgM PFC with goat antimouse μ-chain antibody incorporated in the agar and development of IgG PFC with rabbit polyvalent antimouse γ-chain antibody (both antisera were the gift of Dr. R. Asofsky, NIAID, Bethesda, Md.). Data are expressed as the mean PFC per spleen or the PFC per 10^6 cells ± SE unless otherwise indicated.

Results

Response of CN × DN F_1 Male and Female Mice to SRBC and TNP-SRBC. As a basis for comparison with subsequent studies of T-independent antibody responses of male and female CN × DN F_1 mice, we initially evaluated the response of these animals to two highly thymus-dependent antigens, SRBC and TNP-SRBC. In each of these experiments, the IgM response of F_1 male mice at 4 days after immunization with SRBC or TNP-SRBC was less than those of female mice when considered on the basis of PFC per spleen (Table I). However, since male mice have substantially fewer nucleated cells per spleen (6), it is more instructive to consider the antibody response on the basis of the number of PFC per 10^6 spleen cells. In the first two experiments shown in Table I, the male response, considered on this basis, was approximately half that of the female response and in the third experiment, only slightly less than that of the female. These results are particularly significant in view of our recent finding that the percent of B lymphocytes in the spleen of F_1 male mice is substantially less than that of F_1 female mice (6), and they indicate that F_1 male mice make considerable responses to thymus-dependent antigens. Or on other hand, the IgG anti-SRBC response of F_1 male mice was substanially less than that of F_1 females

TABLE I
IgM and IgG Responses of CN × DN F_1 Male and Female Mice to TNP-SRBC

Exp.	Sex (no. of mice)	Immunoglobulin	TNP-BRBC*		SRBC*	
			PFC/spleen	PFC/10^6 cells	PFC/spleen	PFC/10^6 cells
1	Male (6)	IgM	—	—	2,000 ± 488	44 ± 6.1
	Female (6)		—	—	7,583 ± 1,450	77 ± 10.6
2	Male (3)	IgM	4,037 ± 678	83.4 ± 10.7	4,175 ± 724	86 ± 11.4
	Female (3)		17,443 ± 2,362	208.2 ± 23.4	15,400 ± 1,584	166 ± 30.0
3	Male (3)	IgM	28,900 ± 8,636	586.0 ± 158.0	9,925 ± 1,615	204 ± 22.4
	Female (3)		59,417 ± 17,774	700.0 ± 76.0	29,933 ± 11,855	287 ± 95.4
3	Male (4)	IgG	—	—	2,858 ± 414	36 ± 8.2
	Female (4)		—	—	18,427 ± 3,080	216 ± 19.2

* The mean number of IgM or IgG PFC measured at 4 or 11 days, respectively, in response to 2 × 10^8 heavily conjugated TNP-SRBC. Data are expressed as the net mean number of PFC ± SE.

even when considered on a per B-cell basis (Table I, exp. 3).

Response of CN and CN × DN F_1 Mice to DNP-lys-Ficoll. DNP-lys-Ficoll has been recently demonstrated to be a potent T-independent antigen which elicits both IgM and IgG anti-DNP antibody in many strains of mice (8). CN × DN F_1 male and female mice were immunized with varying doses of DNP-lys-Ficoll and their direct (IgM) anti-DNP PFC response was measured at 4 days (Table II). All of the F_1 female mice formed large numbers of PFC after immunization with from 0.1–500 μg of DNP-lys-Ficoll. In contrast to the vigorous responses of the F_1 females, 10 of 12 F_1 males immunized with DNP-lys-Ficoll had fewer PFC than the number seen in unimmunized F_1 males. Two of the F_1 male mice had responses of 800 and 1,600 PFC/spleen after immunization with 0.1 and 1.0 μg of DNP-lys-Ficoll, respectively. These values were the highest responses seen with CN × DN F_1 male mice in our experience and are in

TABLE II

Response of CN × DN F_1 Male and Female Mice to Varying Doses of DNP-Lys-Ficoll

DNP-lys-Ficoll	TNP-SRBC at day 4*			
	Male		Female	
	PFC/spleen	PFC/10^6 cells	PFC/spleen	PFC/10^6 cells
μg				
0	250 ± 72	6.9 ± 1.4	955 ± 325	12 ± 3.6
0.1	330 ± 235	9.4 ± 7.6	13,600 ± 3,372	156 ± 24.0
1.0	641 ± 480	11.8 ± 9.0	22,108 ± 9,125	357 ± 113.1
20.0	150 ± 68	3.3 ± 0.5	37,000 ± 4,485	340 ± 60.4
500.0	100 ± 14	2.1 ± 0.6	13,616 ± 3,156	169 ± 28.4

* The mean number (three animals per group) of IgM PFC (±SE) measured on day 4 in response to varying doses of DNP-lys-Ficoll.

the same range as the number of background TNP-SRBC plaques seen in CN × DN F_1 females.

Studies of the 4- and 8-day IgM and IgG anti-DNP PFC responses of CN and CN × DN F_1 mice to DNP-lys-Ficoll are shown in Table III. The CN males and females and CN × DN F_1 males had no response (less than background) while the CN × DN F_1 females made large numbers of PFC at both 4 and 8 days. These experiments demonstrate the CBA/N mice have an X-linked defect in responsiveness to another T-independent antigen, DNP-lys-Ficoll. Moreover, since CN × DN F_1 male mice are able to form antibody to the highly cross-reactive hapten (TNP) coupled to erythrocytes, their X-linked immune defect is not a simple absence of the genetic information required to synthesize anti-DNP antibody.

Reconstitution of poly I·C Response in Lethally Irradiated CN × DN F_1 Mice by CN × DN Spleen Cells. In order to study the influence of the CN × DN F_1 male and female environment on the ability of splenic B lymphocytes to respond to T-independent antigens, lethally irradiated F_1 male and female mice were reconstituted with either F_1 male or F_1 female spleen cells and immunized immediately with poly I·C. At 14 days, antibody to poly I·C was found in 9 of 11

TABLE III
IgM and IgG Responses of Male and Female CN and CN × DN F_1 Mice to DNP-Lys-Ficoll (100 μg)

Strain	Sex	TNP-SRBC*			
		IgM (day 4)		IgG (day 7)	
		PFC/spleen	PFC/10^6 cells	PFC/spleen	PFC/10^6 cells
CN	Male	325 ± 160	5.8 ± 3.1	125 ± 11.1	2.6 ± 0.2
	Female	125 ± 32	2.9 ± 1.6	13 ± 2.0	0.3 ± 0.1
CN × DN	Male	150 ± 25	3.0 ± 0.2	25 ± 5.0	0.8 ± 0.3
	Female	11,775 ± 1,041	201.0 ± 24.1	3,695 ± 150.0	53.0 ± 10.2

* The mean number (four animals per group) of IgM or IgG PFC ± SE measured at 4 or 7 days, respectively, in response to 100 μg or DNP-lys-Ficoll.

TABLE IV
Response of Lethally Irradiated CN × DN F_1 Mice to Poly I·C After Reconstitution with CN × DN F_1 Spleen Cells

Sex of recipient	[^{14}C]poly I·C binding*	
	Female donor	Male donor
	%	%
Female	56.1	4.7
	80.6	3.2
	76.9	0.2
	28.5	4.7
Male	21.0	1.7
	0.5	2.4
	14.4	0.2
	71.7	0.0
	21.1	0.0
	71.7	2.4
	50.9	0.0

* Recipient mice were lethally irradiated (1,000 R) and reconstituted with 50 × 10^6 F_1 male or female spleen cells given intravenously on the day of irradiation. They were immediately challenged with 100 μg of poly I·C in CFA and the percent [^{14}C]poly I·C binding was assayed on 25 μl of serum obtained 14 days after immunization.

recipients of F_1 female spleen cells. This included four of four F_1 female recipients and five of seven F_1 male recipients (Table IV). In contrast, none of the 11 F_1 mice (either male or female) which had received male F_1 spleen cells formed antibody to poly I·C.

Reconstitution of DNP-Lys-Ficoll Response in Lethally Irradiated CN × DN Mice with CN × DN Bone Marrow. The previous experiment demonstrated

that a population of female cells containing splenic B lymphocytes was able to transfer to irradiated F_1 males the ability to form antibody to poly I·C upon immediate immunization. In order to study the influence of environment on the maturation of these responsive cells, we reconstituted lethally irradiated male and female CN × DN F_1 mice with 10×10^6 bone marrow cells derived from either male or female CN × DN donors and waited 8 wk before immunizing with DNP-lys-Ficoll (Table V). The mice were sacrificed 4 days later and the numbers of nucleated spleen cells and direct TNP-SRBC PFC determined. Both male and female recipients of female bone marrow cells had substantially greater numbers of nucleated spleen cells than male and female recipients of male bone marrow cells. Indeed, the number of nucleated spleen cells in lethally irradiated recipients which received F_1 male cells resembled that of nonirradiated F_1 male animals, while the numbers of nucleated spleen cells in recipients of F_1 female cells resembled that of normal F_1 females (6). Furthermore, male and female

TABLE V

Response of Lethally Irradiated CN × DN F_1 Male and Female Mice to DNP-Lys-Ficoll after Reconstitution with 10×10^6 F_1 Male or Female Bone Marrow Cells

Sex of recipient	Sex of donor	No. of nucleated cells per spleen‡	TNP-SRBC*	
			PFC/spleen	PFC/10^6 cells
Male	Male	37.8 ± 4.2	50 ± 25	1.5 ± 1.2
	Female	90.3 ± 4.8	21,875 ± 709	243.7 ± 24.0
Female	Male	58.0 ± 7.5	1,575 ± 368	29.2 ± 9.8
	Female	112.0 ± 8.5	26,217 ± 3,397	210.3 ± 7.9

* Recipient mice were lethally irradiated (1,000 R) at 8 wk of age and reconstituted with 10×10^6 bone marrow cells. 8 wk after reconstitution, they were immunized with 100 μg of DNP-lys-Ficoll. IgM PFC were measured at 4 days.

‡ The number of nucleated cells per spleen ± SE (three animals per group).

recipients of F_1 female bone marrow cells made a vigorous response to DNP-lys-Ficoll while male and female recipients of male cells had very few TNP-SRBC PFC 4 days after immunization. Male recipients of male cells made essentially no response; female recipients of male cells mounted a small response, which can probably be ascribed to the contribution of F_1 female bone marrow cells that escaped or recovered from the effects of irradiation.

These experiments demonstrate that F_1 female spleen and bone marrow cells can transfer to lethally irradiated F_1 male recipients the ability to respond to T-independent antigens and imply that the maturation and function of F_1 female B cells can proceed normally in the environment of the irradiated abnormal host. Similarly, they show that cells from the abnormal donor do not develop normally even when placed in a normal (although irradiated) environment.

Reconstitution of Poly I·C and DNP-Lys-Ficoll Response in Nonirradiated CN × DN F_1 Male Mice. Transfers of spleen cells into irradiated recipients as described above do not rule out the possibility that an abnormal radiation-

sensitive regulatory mechanism operates in the F_1 male animals nor do they provide information about the cell type critical for restoring responsiveness upon transfer.

To approach these problems, we transferred cells from F_1 male and female donors to nonirradiated F_1 male and female recipients. In the experiment presented in Table VI, 50×10^6 male or female spleen cells were transferred to nonirradiated F_1 male recipients which were immunized on the same day with 100 μg of poly I·C. Recipients of F_1 female cells made substantial responses to poly I·C, indicating that female cells could function in the environment of an intact F_1 male mouse. When F_1 male cells were transferred to intact F_1 female recipients, no suppression of the anti-poly I·C response was obtained (data not shown) further indicating that abnormal T-cell regulation of B-cell activation was not responsible for the defect of the F_1 male.

TABLE VI

Response of CN × DN F_1 Male Mice to Poly I·C after the Administration of CN × DN F_1 Spleen Cells

Sex of recipient	[^{14}C]poly I·C binding*	
	Female donor	Male donor
	%	%
Male	38.5	0.0
	41.3	0.0
	58.7	13.0
	44.0	0.0
	72.3	0.0
	63.5	5.0
	51.6	2.4

* Recipient CN × DN F_1 male mice received 50×10^6 F_1 male or female spleen cells intravenously. They were immediately challenged with 100 μg of poly I·C in CFA and 14 days later the percent [^{14}C]poly I·C binding by 25 μl of serum was determined.

These results were confirmed and extended by transferring varying numbers of F_1 female spleen cells to F_1 male recipients which were immunized with 100 μg of DNP-lys-Ficoll immediately. As few as 1×10^6 female spleen cells allowed males to make a detectable response; 50×10^6 cells transferred a response comparable, in terms of PFC per 10^6 spleen cells, to that of intact F_1 female mice (Fig. 1). Moreover, treatment of F_1 female spleen cells with anti-θ and C before transfer had no effect on the ability of these cells to reconstitute the response of F_1 male recipients (Fig. 2). The effectiveness of treatment with anti-θ and C in removing T lymphocytes was demonstrated by the marked impairment of the responsiveness of such cells to concanavalin A (Con A). Thus, anti-θ- and C-treated cells gave a net incorporation of [^{3}H] thymidine of 3,081 cpm while cells treated with normal mouse serum and C incorporated 86,869 cpm in response to Con A. This result indicates that F_1 female T lymphocytes are probably not required to reconstitute the responsiveness of F_1 male mice. On the other hand, in vitro irradiation (1,000 R) of F_1 female spleen cells completely abolished the

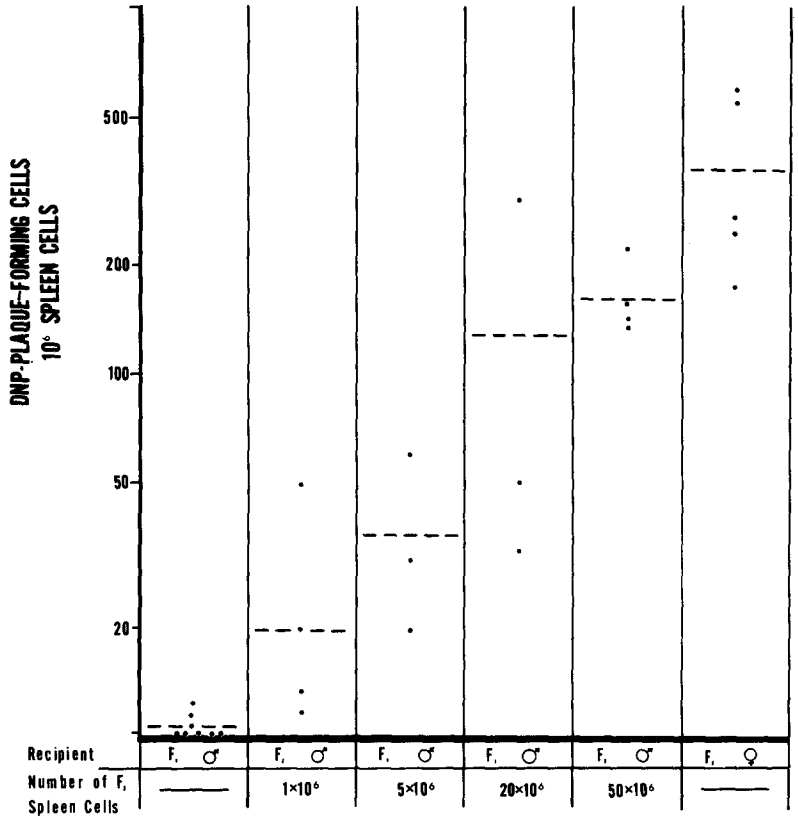

FIG. 1. The number of DNP-PFC/10^6 spleen cells in CN × DN F_1 males which were given different numbers of CN × DN F_1 female spleen cells intravenously and immunized with 100 μg of DNP-lys-Ficoll. The response to DNP-lys-Ficoll was measured on the 6th day after the administration of the female cells and immunization.

ability of these cells to transfer responsiveness to DNP-lys-Ficoll to intact F_1 male recipients. Thus, no PFC (less than background) were seen in recipients of 50×10^6 irradiated F_1 female cells while three F_1 male recipients of nonirradiated F_1 female cells had 275 ± 18 PFC/10^6 spleen cells after immunization with 100 μg of DNP-lys-Ficoll. Further investigation of this transfer model demonstrated that 3×10^6 F_1 female lymph node cells could also transfer responsiveness to DNP-lys-Ficoll to nonirradiated F_1 male recipients, whereas 50×10^6 F_1 female thymocytes were ineffective in initiating a response (Table VII).

Reconstitution of F_1 male mice with spleen cells from F_1 female donors not only allowed responses of the recipients to immediate challenge with DNP-lys-Ficoll but, as shown in separate experiments (Table VIII), allowed responses by the males to primary challenges administered as late as 42 days after cell transfer. This indicates that transferred F_1 female B lymphocytes survive in the recipient or that B lymphocytes develop from F_1 female stem cells present in the spleen cells used for transfer.

Lack of Thymic Influence on the Reconstitution of Responsiveness in Lethally Irradiated CN × DN F_1 Male Mice. The previous studies provide convincing

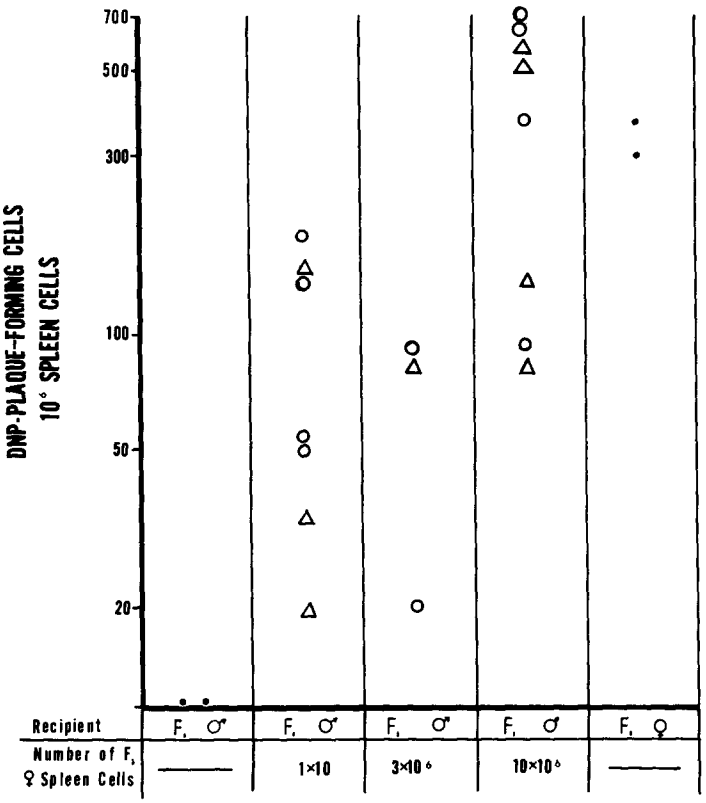

FIG. 2. The number of DNP-PFC/10^6 spleen cells in CN × DN F_1 males which were given different numbers of CN × DN F_1 female spleen cells which had been previously treated with anti-θ and C (△), or NMS and C (○). The response to DNP-lys-Ficoll was measured on the 6th day after the administration of the female cells and 100 μg of the antigen.

evidence that F_1 female cells can transfer to both intact and irradiated F_1 male recipients the capability to respond to T-independent antigens. Moreover, since anti-θ-treated spleen cells are effective, they provide strong evidence that the defect of the F_1 male is at the B-lymphocyte level. However, it could be argued that F_1 male B lymphocytes develop abnormally in the F_1 male because of an abnormality in the F_1 male T lymphocytes which, in some way, regulate B-cell development or, alternatively, because of an abnormal sensitivity of the F_1 male B cells to normal regulatory effects of F_1 male thymus or T lymphocytes. In order to investigate this point, F_1 male mice were thymectomized at 6 wk of age, irradiated (1,000 R) at 8 wk, and reconstituted with 10×10^6 anti-θ-treated male or female bone marrow cells 1 day later. They were held for 10 wk to allow repopulation of their lymphoid system and were then immunized with 100 μg of DNP-lys-Ficoll. Animals reconstituted with anti-θ-treated F_1 female bone marrow made excellent responses to DNP-lys-Ficoll, but those repopulated with anti-θ-treated F_1 male bone marrow were unresponsive to DNP-lys-Ficoll (Table IX). Spleen cells isolated from these F_1 male mice were cultured in the presence of Con A in order to determine if functional T lymphocytes were present. The proliferative response of these spleen cells were all less than 4% of normal F_1

TABLE VII

Response of CN × DN F_1 Male Mice to DNP-Lys-Ficoll after the Administration of CN × DN F_1 Female Spleen, Lymph Node, or Thymus Cells

F_1 male recipient	F_1 female donors		TNP-SRBC*	
	Cell source	No. of Cells	PFC/ spleen	PFC/10^6 cells
1	—	—	75	1.5
2	—	—	100	3.2
3	Spleen	3×10^6	10,950	267.0
4	Spleen	3×10^6	6,450	140.0
5	Spleen	10×10^6	11,050	254.0
6	Spleen	10×10^6	8,450	275.0
7	Lymph node	3×10^6	5,500	149.0
8	Lymph node	3×10^6	5,075	108.0
9	Lymph node	10×10^6	6,650	155.0
10	Lymph node	10×10^6	8,275	194.0
11	Thymus	50×10^6	75	1.2
12	Thymus	50×10^6	200	3.1
13	Thymus	50×10^6	150	2.2

* Recipient F_1 male mice were given varying numbers of F_1 female spleen, lymph node, or thymus cells intravenously. They were then challenged with 100 μg of DNP-lys-ficoll and IgM TNP-SRBC PFC were assayed on day 6.

TABLE VIII

Response of CN × DN F_1 Male Mice to DNP-Lys-Ficoll at Different Times after the Administration of F_1 Female Spleen Cells

Days after F_1 female cells	TNP-SRBC*	
	PFC/spleen	PFC/10^6 spleen
18	1,316 ± 719	35.2 ± 13.5
25	1,550 ± 123	38.5 ± 1.9
39	3,000 ± 177	57.4 ± 1.7
46	1,900 ± 50	45.8 ± 0.5

* Recipient F_1 male mice were given 50×10^6 F_1 female cells intravenously. At 14, 21, 35, and 42 days after the administration of these cells, three F_1 male mice were immunized with 100 μg of DNP-lys-Ficoll and their IgM TNP-SRBC PFC response was measured 4 days later. Data are expressed as the mean number of PFC of three mice ±SE.

male mice. Thus, it seems clear that the B-lymphocyte defect exhibited by F_1 male mice cannot be ascribed to development under the influence of abnormal T-dependent stimuli or to an abnormal response to normal T-dependent stimuli.

TABLE IX
Response of Thymectomized, Lethally Irradiated, Bone Marrow Reconstituted CN × DN F_1 Male Mice to DNP-Lys-Ficoll

Sex of recipient	Sex of donor	TNP-SRBC*	
		PFC/spleen	PFC/10^6 spleen
Female‡	—	60,450	530
Male	Female	51,000	362
Male	Female	27,927	338
Male	Male	25	0.9
Male	Male	75	1.1

* Recipient F_1 mice were thymectomized at 6 wk of age, lethally irradiated (1,000 R) at 8 wk and reconstituted with 10×10^6 anti-θ-treated bone marrow cells on the day after irradiation. At 18 wk of age, these mice were immunized with 100 μg of DNP-lys-Ficoll and their IgM TNP-SRBC PFC response was assayed 4 days later.

‡ Nonirradiated normal female immunized with 100 μg of DNP-lys-Ficoll.

Discussion

CN mice and male CN × DN F_1 mice have been previously shown to have a profound defect in immune responses to SIII, LPS, and poly I·C (1, 2). We show here that they are essentially unresponsive to DNP-lys-Ficoll. Moreover, in vitro studies confirm the unresponsiveness of F_1 male mice to DNP-lys-Ficoll while F_1 female mice mount vigorous responses.[3] Thus, CN mice fail to respond to four different T-independent antigens. Our results with DNP-lys-Ficoll are particularly impressive as this T-independent antigen yields very large numbers of IgM and IgG PFC in a variety of normal strains (8).

On the other hand, F_1 male CN × DN mice mount substantial responses to SRBC and TNP-SRBC. When considered in terms of PFC per unit of B lymphocytes (rather than as PFC per spleen), the IgM response of F_1 males is similar to that of F_1 females. This is so because of F_1 males generally have half as many nucleated cells per spleen as F_1 females (6). In addition, F_1 males have only 26% Ig-bearing cells among spleen lymphocytes, whereas 40% of spleen lymphocytes from F_1 females bear surface Ig (6). It should be noted that considering responses of F_1 males to DNP-lys-Ficoll on this basis does not result in such a normalization, as F_1 males make no net TNP-specific PFC as a result of immunization with this T-independent antigen. These results confirm our previous conclusion that the X-linked functional immune defect expressed by CN and male CN × DN F_1 mice is principally an inability to mount T-independent responses rather than a global defect in all humoral immune responses (6).

The finding that CN and CN × DN F_1 male mice fail to respond to T-independent antigens does not, by itself, establish that the defect of these

[3] Cohen, P., I. Scher, and D. Mosier. Manuscript submitted for publication.

animals resides in the B-lymphocyte pool. In order to study this problem, we evaluated the capacity of lymphoid cells obtained from F_1 female donors to transfer responsiveness to F_1 male recipients. Spleen cells from F_1 female mice were able to reconstitute an easily detectable antibody response to poly I·C or DNP-lys-Ficoll in F_1 males after immediate challenge. Moreover, both anti-θ-treated spleen cells and normal lymph node cells from F_1 female donors transferred responsiveness to F_1 males, while irradiated F_1 female spleen cells did not transfer responsiveness. The former indicates that mature T lymphocytes from the F_1 female are not required for the DNP-lys-Ficoll response; the latter experiments suggest that macrophages are not the limiting cell type, since macrophages are less frequent in lymph node cell populations than in spleen cell suspensions and are quite radiation resistant. Indeed, Mosier et al. (7) have shown that the in vitro response to DNP-lys-Ficoll is much less dependent on adherent cells than is the response to SRBC, further suggesting that macrophages are not likely to be the defective cell type in CN and male CN × DN F_1 mice.

Since cell populations rich in mature B lymphocytes from F_1 female mice will reconstitute the response of F_1 male mice, it is likely that the X-linked functional defect in these mice resides in the B-cell pool. Nonetheless, it could be proposed that the B-cell defect results from a defect in control of B-cell development or function based upon abnormal regulatory cells or abnormal sensitivity of B cells to external regulatory influences. Several of our experiments bear on these points. Firstly, mature F_1 female spleen cells function normally in intact or irradiated males, indicating that an excess of an acutely acting regulatory factor is not present in the F_1 male. Furthermore, nonirradiated F_1 males which have received F_1 female spleen cells mount anti-DNP-responses to DNP-lys-Ficoll administered up to 42 days after transfer, making it unlikely that a suppressive factor active only over a prolonged period limits responsiveness of mature B cells in the F_1 male. Also supporting this contention is the failure of F_1 male spleen cells to inhibit the response of intact F_1 females.

These data strongly indicate that mature B lymphocytes capable of responding to T-independent antigens are absent in CN and CN × DN F_1 mice. Such an absence could represent a defect in the differentiative potential of B-lymphocyte progenitors or, alternatively, a defect in the microenvironment in which B lymphocytes develop. Our data strongly points to the former rather than the latter. Thus, F_1 female bone marrow cells, when transferred to lethally irradiated F_1 male mice, allow the latter to respond to challenge with DNP-lys-Ficoll adminstered 8 wk later. This implies that female B-lymphocyte progenitors can develop normally in the environment of the F_1 male. On the other hand, F_1 male bone marrow cells transferred to lethally irradiated F_1 female mice do not reconstitute the response to DNP-lys-Ficoll of the recipients. The small response which these animals mount is equivalent to the number of background TNP-specific PFC in normal females and may represent a response on the part of surviving female B lymphocytes. Similarly, failure in development of male B cells responsive to DNP-lys-Ficoll cannot be ascribed to a regulatory influence of the male thymus or T lymphocytes since anti-θ-treated F_1 male bone marrow cells could not reconstitute responsiveness in thymectomized, lethally irradiated F_1 male recipients.

A final objection to the concept that a functional class of B lymphocytes is absent in CN and F_1 male mice could be raised, if it could be shown that the cells responding to poly I·C or DNP-lys-Ficoll in F_1 males which had received F_1 female cells were the defective F_1 male cells. According to this hypothesis, the presence of the F_1 female cells would allow the F_1 male cells to form antibody against these T-independent antigens. In order to study this problem, we have reconstituted F_1 males with T-lymphocyte-depleted DN spleen cells. In preliminary experiments, we have shown that the cell responding to DNP-lys-Ficoll was derived from the DN strain rather than the F_1 male.

Our data present convincing evidence that the X-linked defect in responsiveness to T-independent antigens by CN mice represents an intrinsic defect in the B-lymphocyte line rather than an abnormal extrinsic regulation of B-lymphocyte development and/or function. We cannot, as yet, determine whether these animals have a deletion in a single line of B lymphocytes (i.e., B cells responsive to T-independent antigens) or, alternatively, a defect in all lymphocytes which affects T-independent responses more profoundly than T-dependent responses. These mice provide a model system in which an analysis of the molecular mechanism of B-lymphocyte activation and a determination of the nature of the functional heterogeneity of such cells should be possible. In addition, they provide an excellent model for understanding the role of X-linked genes in the control of immune responses.

Summary

The mechanisms underlying the X-linked thymus-independent (B) lymphocyte functional defect in the CBA/N (CN) mice and their F_1 progeny were studied. Immune defective mice were unable to respond to the T-independent antigen 2,4-dinitrophenyl-lysyl-derivative of Ficoll (DNP-lys-Ficoll) but were able to form antibody against the highly cross-reactive hapten (trinitrophenyl) when it was coupled to an erythrocyte carrier. Immune defective CN × DBA/2N (DN) F_1 male mice, which do not normally respond to T-independent antigens, were able to respond to both polyribosinic-polyribocytidylic acid and DNP-lys-Ficoll after the adminstration of CN × DN F_1 female spleen cells even if these cells had been depleted of T lymphocytes.

In addition, it was shown that the inability of the CN mice and their F_1 progeny to respond to T-independent antigens was not due to an intrinsic abnormality of their microenvironment or the suppressive actions of a T lymphocyte. Our data present evidence that the X-linked defect in the CN mice is due to an intrinsic defect in B-lymphocyte development.

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