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

I. Studies of the Function and Composition of Spleen Cells*

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CBA/HN (CN)[1] mice are a subline of CBA mice with a marked defect in antibody-forming ability. Initially, it was shown that they failed to form specific antibody in response to immunization with type III pneumococcal polysaccharide (SIII) and bacterial lipopolysaccharide (LPS) (1). Subsequent studies of these animals have shown that they did not make a detectable antibody response to polyinosinic-polycytidylic acid (poly I·C) (2) or to the 2,4-dinitrophenyl-lysyl derivative of Ficoll (DNP-lys-Ficoll).[2] All of these antigens evoke vigorous antibody responses in all other mouse strains studied, even if these mice are deprived of thymus-dependent (T) lymphocytes (3–6). Thus, these antigens have been referred to as "T-independent" antigens. The response of CN mice to T-dependent antigens such as sheep red blood cells (SRBC) has been less well studied, but they have been shown to make easily detectable, although less than normal, responses to SRBC (1) and to other T-dependent antigens.[2] In addition, the levels of circulating IgM in the CN mice were shown to be very low (7). These data all suggest a defect in the T-independent antibody-forming system of CN mice (2).

Genetic studies of the immune response to SIII and poly I·C in the CN mice demonstrated that their inability to respond to these antigens was inherited as an X-linked recessive trait (1, 2). Thus, when CN females are crossed to normal male mice,

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[1] *Abbreviations used in this paper:* ADCC, antibody-dependent cell cytotoxicity; CJ, CBA/J; CN, CBA/HN; Con-A, concanavalin A; C6, C57BL/6; DN, DBA/2N; DNP-lys-Ficoll, 2,4-dinitrophenyl-lysyl-derivative of Ficoll; FCS, fetal calf serum; [^{3}H]TdR, [*methyl*-^{3}H]thymidine; KLH, keyhole limpet hemocyanin; LPS, bacterial lipopolysaccharide; MBLA, mouse B-lymphocyte antigen; NMS, normal mouse serum; NRS, normal rabbit serum; PHA, phytohemagglutinin; poly I·C, polyinosinic-polycytidylic acid; SIII, pneumococcal polysaccharide.

[2] Scher, I. and W. E. Paul. Manuscript in preparation.

the F_1 male progeny are unable to form antibody to SIII and poly I·C whereas the F_1 female progeny forms normal amounts of antibody.

CN mice provide an excellent system in which to evaluate the X-linked genetic control of immune responsiveness and may provide an important model for the study of the ontogeny, differentiation, and activation of bone marrow-derived (B) lymphocytes. In this communication, we present studies demonstrating that the numbers of B lymphocytes in the spleens of CN mice and of F_1 male progeny of crosses of CN females with DBA/2N (DN) male mice (CN × DN F_1 males) are diminished. In addition, we show that the response of lymphocytes of CN mice and CN × DN F_1 male mice to agents mitogenic for T-independent lymphocytes (B mitogens) is impaired and that they also have an impaired ability to participate in antibody-dependent cell-mediated cytotoxicity (ADCC). On the other hand, these mice display relatively normal T-lymphocyte function as judged by mitogen responses to concanavalin-A (Con-A) and phytohemagglutinin-P (PHA), by specific in vitro T-lymphocyte-mediated cytotoxicity and by skin graft rejection.

Materials and Methods

Animals. CN, DN, and C57BL/6 (C6) mice as well as F_1 and backcross animals derived from these strains were obtained from the Rodent and Rabbit Production Section of the National Institutes of Health, Bethesda, Md. CBA/J (CJ) mice were purchased from Jackson Laboratories, Bar Harbor, Maine. All mice were 6–12 wk of age at the time of their study. The CN mice are a distinct subline of CBA mice, and the history of their establishment has been described in detail elsewhere (1, 2). F_1 animals derived from the CN strain were produced by breeding a CN female with a DN male and are referred to as CN × DN F_1 males or females.

Cell Suspensions. Mice were killed by cervical dislocation and their spleens removed using aseptic technique. Spleen cells were obtained by gentle teasing with a rubber policeman into RPMI 1640 medium (Grand Island Biological Co., Grand Island, N. Y.). Cell aggregates were disrupted by passing the cell suspension through a 26-gauge needle. The single cell suspensions were then washed twice with RPMI 1640. Cell counts were determined with a Fisher Autocytometer (Fisher Scientific Co., Silver Spring, Md.).

Antisera. Rabbit antimouse B-lymphocyte antisera (anti-MBLA) was prepared according to the method of Raff et al. (8). AKR anti-Thy. 1.2 (anti-θ) was prepared by the method of Reif and Allen (9). Rabbit antimouse κ-serum was a gift of Dr. R. Mage, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, Md.; it was purified by absorption to and elution from an agarose affinity column to which mouse κ-type myeloma proteins of the γ_1- and γ_2-classes had been coupled. Mouse anti-Ia.8 antiserum was prepared by immunizing B10.A mice with lymphocytes from B10 mice (10) and was the gift of Dr. D. Sachs, National Cancer Institute, NIH, Bethesda, Md. This antisera recognizes an Ia antigen present in $H\text{-}2^q$ mice and expressed principally on B lymphocytes. All antisera were inactivated for 30 min at 56°C before use.

Immunofluorescence and Autoradiography. 5 million spleen cells were treated with Tris-buffered ammonium chloride, according to the method of Boyle (11), in order to remove red blood cells (RBC); they were then resuspended in 100 μl of RPMI 1640 with 10% fetal calf serum (FCS) (GIBCO) and 1% sodium azide (Calbiochem, San Diego, Calif.). This suspension was incubated at 4°C for 30 min with 50 μl of fluorescein-labeled rabbit anti-κ (prepared as previously described) (12). After incubation, the cells were recovered by centrifugation, washed three times in 5 ml of RPMI 1640, and examined in the living state with a fluorescence microscope equipped with an incident light ultraviolet illuminator. Small round cells were located by using a tungsten light source, and fluorescence of such cells was evaluated after switching to an ultraviolet source.

Autoradiography was performed as previously described (13), by adding 0.125 μg (10 μl) of ^{125}I-labeled anti-κ antibody (25–35 μCi/μg; radioiodinated by the chloramine-T method) to a

suspension of 5×10^6 spleen cells (in 100 μl of RPMI 1640, 10% FCS, and 1% sodium azide). This suspension was incubated at 4°C for 30 min and then washed three times by pelleting cells through 5 ml of FCS. The cell pellet was smeared on glass slides which were then fixed in glutaraldehyde, washed, and dipped in nuclear tract emulsion (NTB-2, Eastman Kodak Co., Rochester, N. Y.). Autoradiographs were exposed for 2–10 days. These cells were stained with methyl-green pyronin; small round nonpyroninophilic cells were scored for grains.

Complement (C)-Dependent Cytotoxicity. Spleen cells from CN × DN F_1 males or females were labeled with ^{51}Cr in order to assay the relative ability of anti-θ, anti-MBLA, anti-Ia.8, and anti-κ to kill these cells. 10 million spleen cells, treated to remove RBC, were suspended in 0.5 ml of 10% FCS in RPMI 1640 to which was added 0.2 mCi of sodium ^{51}Cr (1 mCi/μl, Amersham/Searle Corp., Arlington Heights, Ill.). The mixture was incubated for 30 min at 37°C. The cells were washed three times in 20 vol of RPMI 1640 and brought to 1×10^6 cells/ml. 50 μl of this suspension was incubated in triplicate with 50 μl of various dilutions of antisera at room temperature for 15 min. Rabbit serum (50 μl of a 1:4 dilution) was added as a source of C and the mixture incubated for an additional 30 min at 37°C. After sedimentation of the cells by centrifugation at 200 *g* for 10 min, 100 μl of the supernatant fluid was removed and the radioactivity measured in a gamma ray spectrometer. Total releasable radioactivity was determined by freeze-thawing samples containing labeled cells and medium. Control samples contained labeled cells, normal mouse serum (NMS) or normal rabbit serum (NRS), and C. The percent lysis was determined by the following formula:

$$\% \text{ Net chromium released} = \frac{\text{radioactivity released by antisera and C} - \text{radioactivity released by NMS (NRS) and C}}{\text{radioactivity released by freeze-thawing} - \text{radioactivity released by NMS (NRS) and C}} \times 100.$$

All lots of C were absorbed with 0.1 vol of mouse liver powder (Miles Laboratories, Inc., Miles Research Div., Kankakee, Ill.) for 1 h at 4°C.

Stimulation by Mitogenic Agents. The mitogens employed in these studies were used at levels determined by preliminary experiments to give optimum stimulation with DN spleen cells. RPMI 1640 with *N*-2-hydroxyethyl-piperazine-*N'*-2-ethane sulfonic acid buffer (25 mM; Calbiochem), penicillin (100 U/ml), streptomycin (100 μg/ml), and L-glutamine (2 mM) was used as medium for the mitogen experiments. PHA-P (Difco Laboratories, Detroit, Mich.) was used at 0.1%; LPS *Escherichia coli* 0111:B4 (Difco Laboratories) was used at 50 μg/ml; poly I·C (P-L Biochemicals Inc., Milwaukee, Wis.) was used at 250 μg/ml; and Con-A (Calbiochem) was used at 1.25 μg/ml.

Cultures were established in sterile tissue culture microtiter plates (no. 3040, Falcon Plastics, Division of BioQuest, Oxnard, Calif.). 100 μl of 6×10^6 spleen cells/ml in medium (6×10^5 cells/culture) and 50 μl of medium containing the mitogen were added. In some experiments, FCS was added to yield a 10% solution and in certain other experiments, the number of cells added to each culture was varied from 2×10^5 to 1×10^6. These cultures were covered with sterile raised lids (Limbro no. 55, Bellco Glass, Inc., Vineland, N. J.) and cultured for 72 h, unless otherwise noted, in a humidified atmosphere of 95% air, 5% CO_2 at 37°C. 18 h before harvesting, 20 μl of media containing 1 μCi of [*methyl*-^{3}H]thymidine ([^{3}H]TdR) 1.9 Ci/mM (Schwarz/Mann Div., Becton, Dickinson & Co., Orangeburg, N. Y.) was added to each well. Cultures were harvested using the multiple automated sample harvester (14), transferred to 1-dram vials, and 3 ml of Liquiflor (New England Nuclear, Boston, Mass.), were added. The samples were counted in a liquid scintillation spectrometer and data, counts per minute, are reported as the mean of triplicate cultures, plus or minus the standard error (±SE), unless otherwise indicated.

Skin Grafting. Skin grafts from C6 mice to CN × DN F_1 males and females were made according to the technique of Billingham and Medawar (15). The casts were removed after 8 days and the site of transplantation was macroscopically examined daily for signs of rejection. The data are expressed as the time required for rejection. Statistical analysis of the data was analyzed using the rank sum method.

Lymphocyte-Mediated Cytotoxicity. 21 days after receiving C6 skin grafts CN × DN F_1 males and females were given 50×10^6 C6 spleen cells by intraperitoneal injection. 10 days later, they were bled from the retro-orbital sinus and sacrificed. Spleen cells from these animals were treated to

remove RBC and suspended in RPMI 1640 with 10% FCS at 6×10^6 cells/ml. 100 μl of these cells were added to a microtiter well in triplicate along with 100 μl of washed EL-4 tumor cells at 6×10^4 cells/ml which had been labeled with sodium ^{51}C as noted previously. This mixture was allowed to incubate under 95% air, 5% CO_2 at 37°C for 16 h; 100 μl of the supernatant fluid was collected after centrifugation at 200 *g* for 10 min and assayed for radioactivity. The percent lysis was calculated as noted previously.

ADCC. 6×10^4 ^{51}Cr-labeled EL-4 tumor cells in 1 ml of RPMI 1640 were incubated for 1 h at 37°C with 0.25 ml of antiserum produced by the CN × DN F_1 mice described in the previous section. The cells were washed in 4 vol of RPMI 1640 and resuspended in 1 ml of RPMI 1640. 100 μl of these sensitized labeled cells were added to microtiter wells. In addition, 100 μl of a suspension of spleen cells from unsensitized mice of various strains, in RPMI 1640 medium with 10% FCS at 6×10^6 cells/ml, were added to each well in triplicate. The cells were incubated for 4 h in the same manner as in the lymphocyte-mediated cytotoxicity experiments. After centrifugation at 200 *g* for 10 min, 100 μl of the supernatant fluid was collected and assayed for radioactivity. The percent lysis was calculated as noted previously.

Treatment of Spleen Cell to Enrich for B Lymphocytes. Spleen cells obtained from either CN × DN F_1 males or females were depleted of adherent cells and T lymphocytes by the following techniques. The spleen cells were suspended in RPMI 1640 (5% FCS) at 25×10^6 cells/ml and incubated at 37°C for 60 min in 100-mm plastic culture dishes (Falcon Plastics, Div. of BioQuest). The nonadherent cells that were recovered after two 60-min incubations were then treated with anti-θ sera and C and dead cells were removed by Ficoll-Hypaque density centrifugation as described in a previous report (16).

Results

Numbers of Nucleated Cells per Spleen in C6 × DN F_1, CJ, CN × DN F_1, and CN Mice. The number of nucleated cells per spleen of CJ, CN, CN × DN, and C6 × DN males and females is shown in Fig. 1. These data were gathered over a period of 6 mo on animals which were between 6 and 10 wk of age. The CN male and female mice have approximately 60×10^6 nucleated cells/spleen vs. the CJ inbred strain or the C6 × DN F_1 mice which have approximately 120×10^6 nucleated cells/spleen. There is little difference between the numbers of nucleated spleen cells in the males or females of the C6 × DN F_1 mice. However, the numbers of spleen cells in the CN × DN F_1 females is significantly greater than in the F_1 males ($92 \times 10^6 \pm 5.0$ vs. $51 \times 10^6 \pm 4.1$, $P < 0.001$). Thus, the CN × DN F_1 females have considerably more nucleated cells per spleen than their F_1 male littermates although somewhat fewer than a similar F_1 strain or the CJ strain. The CN males and females resemble the CN × DN F_1 males in the numbers of nucleated cells per spleen.

Percentage of Kappa-Bearing Cells per Spleen. Spleen cells from male and female CN, DN, and CN × DN F_1 mice were treated with anti-κ which had been labeled with either fluorescein or ^{125}I. Approximately 50% of both male and female DN spleen cells were labeled as is shown in Fig. 2. There was no statistical difference between the males and females in the DN mice or the CN mice. However, the CN mice had only approximately 22% positive cells. The CN × DN F_1 males had a mean of 26% κ-bearing spleen cells and thus resembled the CN mice whereas the CN × DN F_1 females had an intermediate value with a mean of 39.9%, a highly significant difference ($P < 0.005$) from that of the F_1 males.

Percent Chromium Released by Antisera-Treated Spleen Cells. Fig. 3 *a* shows the percent net chromium released by CN × DN male and female cells treated with anti-θ and C. There was little difference in the chromium released

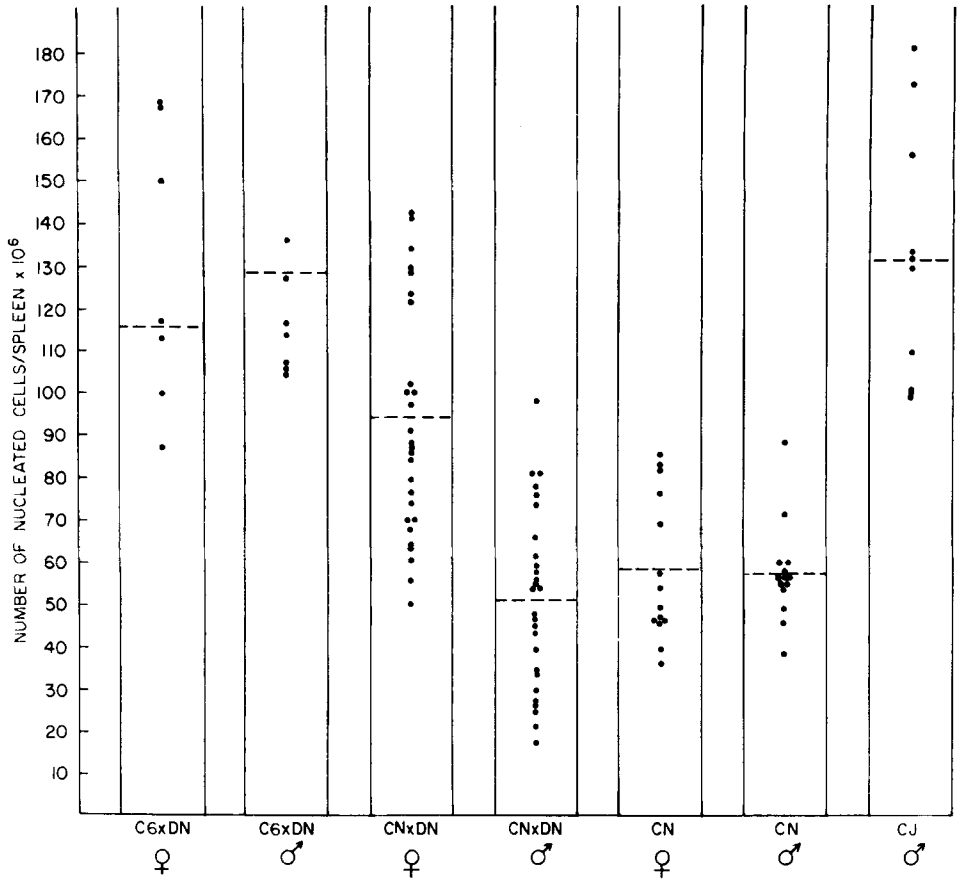

FIG. 1. Number of nucleated cells in the spleens of C6 × DN F_1 males and females, CN × DN F_1 males and females, CN males and females, and CJ males. Each point represents data from an individual animal and the mean of each group is shown by the horizontal dashed line.

from male or female cells treated in this manner. When anti-κ (Fig. 3 *b*), anti-Ia.8 (Fig. 3 *c*), or anti-MBLA (Fig. 3 *d*) and C were used to treat CN × DN F_1 spleen cells, considerable differences in the net chromium released were noted between the male and female cells. At the highest concentration of antisera used, the amount of chromium released by the F_1 male cells was approximately 1/3 of the chromium released by the F_1 female cells (0.34, 0.27, and 0.32 for the anti-κ, anti-Ia.8, and anti-MBLA, respectively).

Response of Spleen Cells to Nonspecific Mitogens. Individually cultured spleen cells from male and female CN, CN × DN, and C6 × DN mice which were incubated for 3 days in the absence of mitogens and FCS incorporated little [^{3}H]TdR as is shown in Fig. 4. However, there was significantly more incorporation by the unstimulated CN × DN F_1 female cells than the unstimulated F_1 male cells ($P < 0.001$). The [^{3}H]TdR incorporated by unstimulated F_1 male spleen cells was similar to that of cells from CN mice whereas the incorporation of [^{3}H]TdR by F_1 female spleen cells was similar to C6 × DN cells. The T-cell

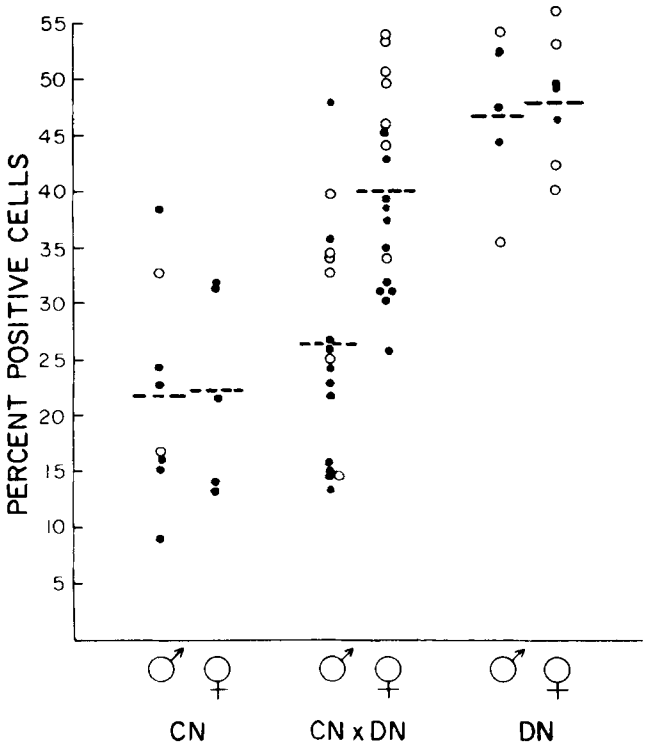

FIG. 2. Percent positive small round cells stained with fluorescein-labeled (●) or ^{125}I-labeled (○) rabbit antimouse-κ, in spleen cell suspensions from male and female CN, CN × DN, and DN mice. Each point represents data from an individual animal and the mean of each group is shown by the horizontal dashed line.

mitogens PHA and Con-A induced an excellent response in the absence of FCS in spleen cells from both male and female mice of all strains studied (Fig. 4). However, the response of the spleen cells from CN males and females and CN × DN F_1 males, under similar conditions, to the B-cell mitogens LPS and poly I·C was quite low (<4,000 cpm and <3,500 cpm, respectively). This low response persisted in the spleen cells of these animals when they were stimulated with a concentration of LPS from 25 to 50 μg/ml or poly I·C from 50 to 250 μg/ml in the presence of 10% FCS (Fig. 5). When CN × DN F_1 females and C6 × DN males or females were stimulated with LPS or poly I·C, an excellent response was seen in the absence (Fig. 4) or presence (Fig. 5) of 10% FCS. The response of spleen cells from a pool of four CN × DN F_1 males and four F_1 females to poly I·C (250 μg/ml) in 10% FCS at 24, 48, 72, and 96 h is shown in Fig. 6. Although the maximum response to poly I·C of cells from F_1 males occurred slightly later than that of F_1 female cells, at no time did the response of the F_1 male cells approach that of the F_1 female cells.

In order to determine the influence of increasing the number of spleen cells per culture on the response seen with LPS or poly I·C, experiments were run with from 2×10^5 to 8×10^5 spleen cells/culture in the presence of 5% FCS (Fig. 7). The response induced by these B-cell mitogens on both CN × DN F_1 male and

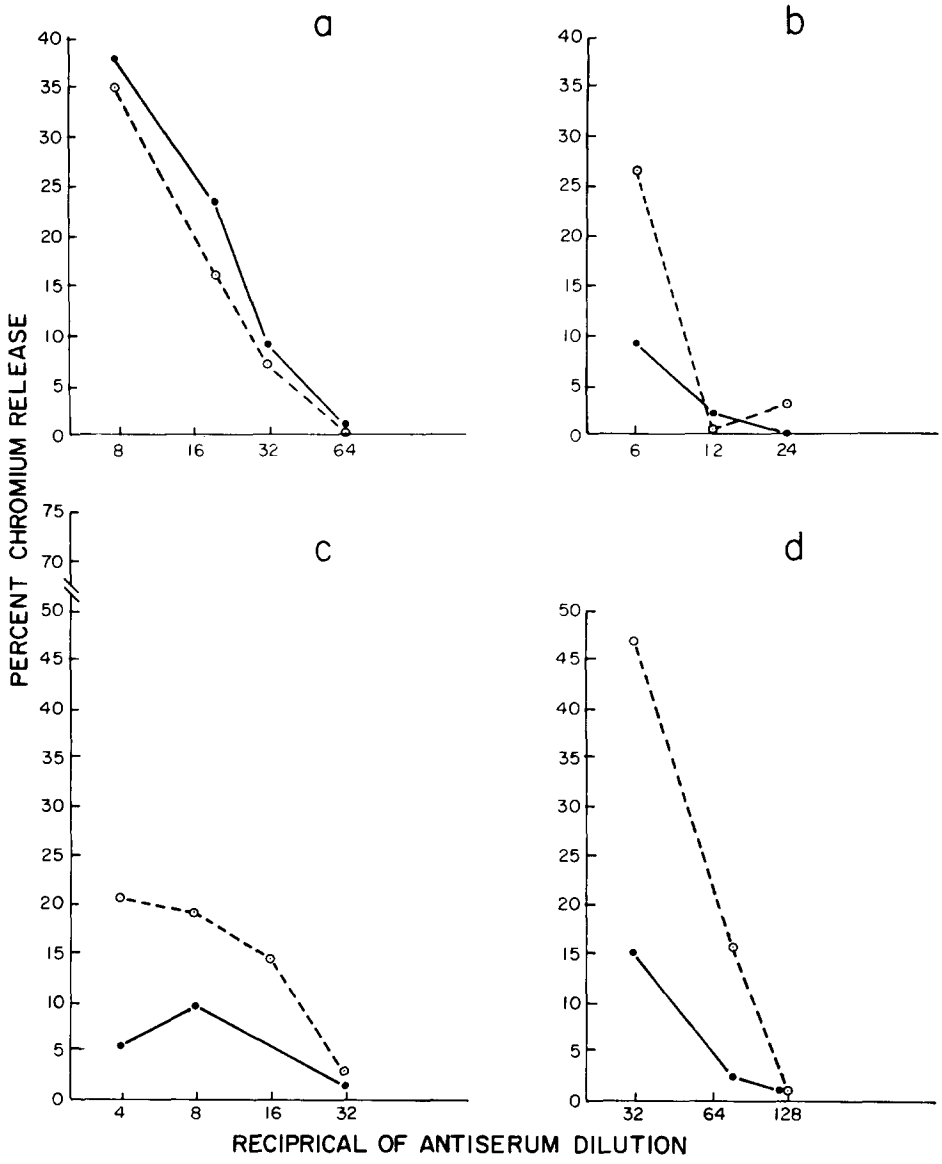

FIG. 3. Percent ^{51}Cr released from spleen cells from CN × DN F_1 males (●—●) or females (O---O) treated with (*a*) anti-θ, (*b*) anti-κ, (*c*) anti-Ia.8, or (*d*) anti-MBLA and C.

female spleen cells increased as the number of cells per culture was increased. However, the response of 8×10^5 male cells was significantly lower than the response of 4×10^5 female cells to both LPS and poly I·C. In a similar experiment, varying numbers of spleen cells depleted of both adherent cells and T lymphocytes were cultured in the presence of LPS or poly I·C (Fig. 8). The response to LPS of 1×10^6 male cells was similar to 4×10^5 female cells and the response to poly I·C of 1×10^6 male cells was less than that of 6×10^5 female cells.

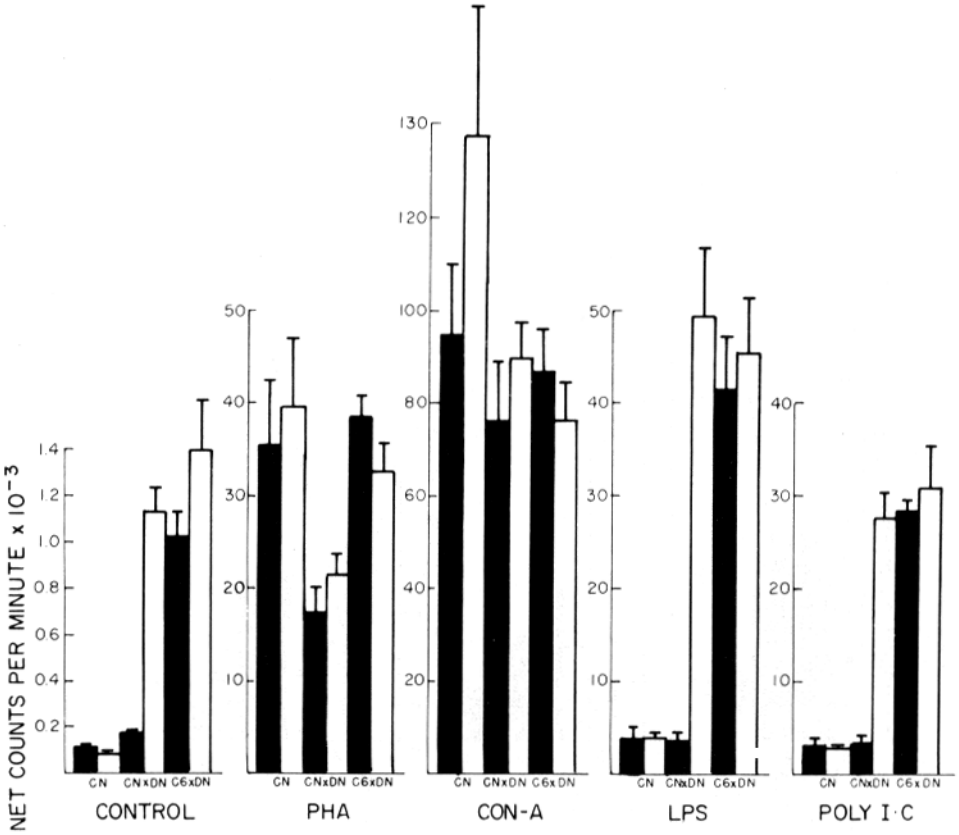

FIG. 4. Mean uptake of [^{3}H]TdR by spleen cells from male (closed bars) or female (open bars) CN, CN × DN, or C6 × DN mice in response to media alone (control), PHA (0.1%), Con-A (1.25 μg/ml), LPS (50 μg/ml), or poly I·C (250 μg/ml). The horizontal connected lines represent the SE obtained using three individual animals. Cultures were run in the absence of FCS.

Skin Graft Rejection and Antibody- and Nonantibody-Mediated Cell Cytotoxicity. Eight CN × DN males and eight females (*H-2*$^{k/d}$) were grafted with C6 (*H-2*^{b}) skin and the rejection time measured. There was no significant difference (rank sum test) between the mean rejection time for the males (11.0 days, SE ± 0.6) vs. the females (10.2 days, SE ± 0.6) as shown in Table I.

These mice were boosted with 50 × 10^6 C6 spleen cells after graft rejection as noted in the Materials and Methods. The ability of these sensitized CN × DN F_1 male and female spleen cells to lyse EL-4 (*H-2*^{b}) tumor cells was then assayed. There were no differences in the degree of chromium released from EL-4 tumor cells as a result of incubation with spleen cells from sensitized CN × DN F_1 males or females (Table II).

However, when the EL-4 tumor cells were treated with an anti-*H-2*^{b} antiserum, spleen cells from unsensitized CN × DN F_1 females caused the release of three times as much chromium as did unsensitized F_1 male cells (Table III). Spleen cells from CN males and females responded as the CN × DN F_1 males

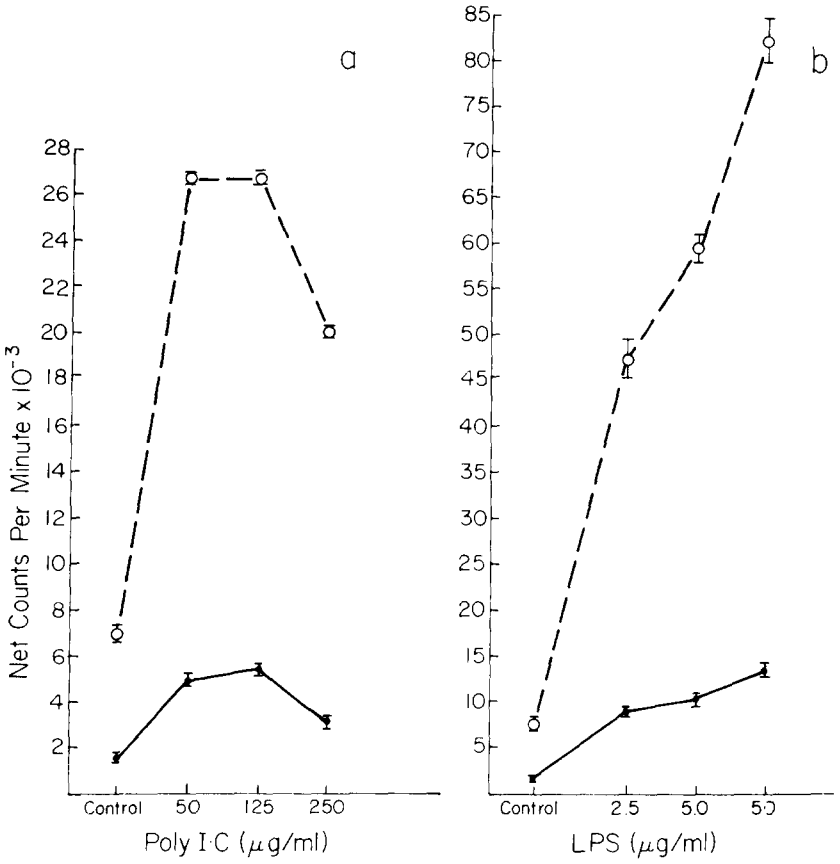

FIG. 5. Uptake of [^{3}H]TdR by spleen cells from male (●—●) or female (O---O) CN × DN mice in response to media alone (control), (*a*) poly I·C (50–250 μg/ml) or (*b*) LPS (2.5–50 μg/ml). Cultures were run in the presence of 10% FCS.

while cells from C6 × DN males and females responded as the CN × DN F_1 females.

Discussion

In this paper we have studied the composition and functional characteristics of the lymphoid system of the immune-deficient CN mice and their F_1 progeny. Considerably fewer nucleated cells per spleen were noted in the immune-deficient CN × DN F_1 males when compared to the apparently immunologically normal F_1 females. In addition to a difference in the total number of nucleated spleen cells between the F_1 males and females, a substantial decrease in the percentage of B lymphocytes in the spleens of CN mice and F_1 males was also seen. Thus, spleen cells from a normal strain (DN) had approximately 50% immunoglobulin-bearing lymphocytes whereas CN males and females and CN × DN males had values which were similar to each other (22%, 22%, and 26%) but approximately half that of DN strain. Nonetheless, some individual CN mice and

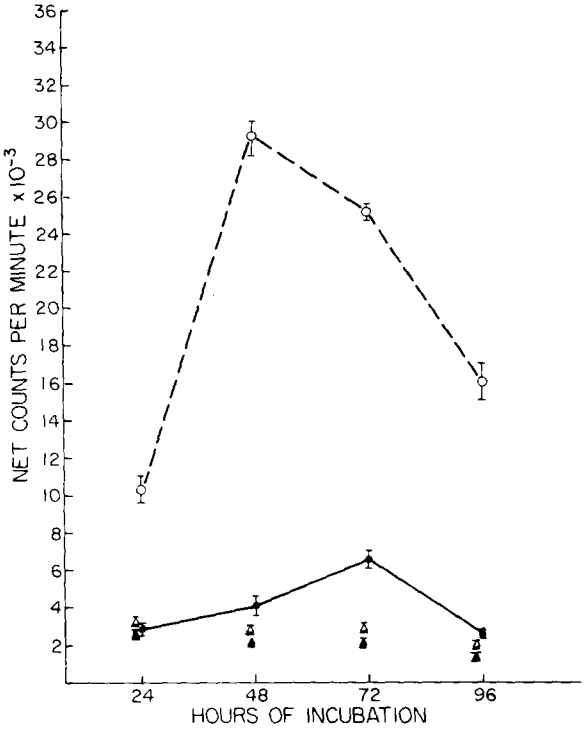

FIG. 6. Uptake of [^{3}H]TdR by male CN × DN spleen cells exposed to media (▲—▲) or poly I·C [250 μg/ml (●—●)] and by female CN × DN spleen cells exposed to media (Δ---Δ) or poly I·C [250 μg/ml (O---O)] for different lengths of time. Cultures were run in the presence of 10% FCS.

CN × DN F_1 males had more than 35% immunoglobulin-bearing lymphocytes, indicating that their immunologic defect is not simply secondary to a decrease in the number of these cells.

The level of C-dependent lysis induced by antisera directed against three markers with predominant or exclusive expression on mouse B lymphocytes, MBLA, κ, and Ia.8 supports the data obtained with the direct staining with labeled anti-κ. It would appear, therefore, that the decrease in the number of B lymphocytes in the CN × DN F_1 males (and by inference in CN males and females) is not due to a selective absence of B lymphocytes bearing either the κ, Ia.8, or MBLA markers, since the representation of cells bearing these markers appears to be diminished to the same degree.

Studies of the response of spleen cells to nonspecific mitogens provide a method of evaluating T- or B-lymphocyte cell activation (16). There were no significant differences in the response of spleen cells from CN × DN F_1 males vs. that of the females to either Con-A or PHA. Indeed, the response to Con-A and PHA by all the animals examined was quite comparable. Furthermore, the fraction of θ-bearing cells in the spleen cell population of F_1 male and female mice was similar, as determined by an in vitro cytotoxicity assay.

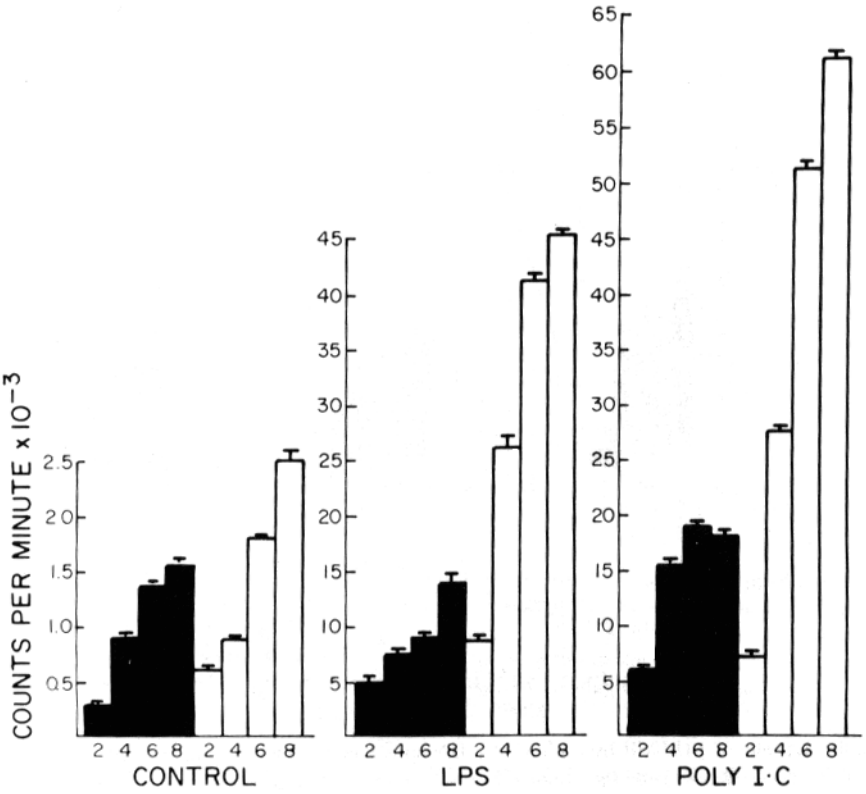

FIG. 7. Uptake of [^{3}H]TdR by increasing numbers of male (closed bars) or female (open bars) CN × DN F_1 spleen cells in response to media alone (control), LPS (50 μg/ml), or poly I·C (250 μg/ml). The numbers under the bars represent the number of cells per culture × 10^{-5}. Cultures were run in the presence of 5% FCS.

The response of the C6 × DN F_1 mice and CN × DN F_1 female mice to the B-cell mitogens LPS and poly I·C were equivalent and similar to that of several other strains we have studied. However, both a very low-resting level of [^{3}H]TdR incorporation and a low response to LPS and poly I·C were regularly obtained with spleen cells of CN and CN × DN F_1 male mice. Although the fraction of B lymphocytes in spleen cell suspensions of CN and CN × DN F_1 male mice is less than that of the other strains studied, this does not by itself explain the poor in vitro reactivity of these cells to LPS and poly I·C. Thus, the response of 8×10^5 F_1 male spleen cells to LPS or poly I·C is less than the response of 4×10^5 F_1 female spleen cells whereas the number of B lymphocytes in these cultures should be similar (Fig. 7). Although it is likely that these results are secondary to a qualitative abnormality in the F_1 male B lymphocytes, it is possible that different ratios of adherent cells and/or T lymphocytes contribute to these results. However, as is shown in Fig. 8, even with equivalent numbers of adherent and T-lymphocyte-depleted spleen cells per culture, the F_1 male response to LPS and poly I·C is considerably less than that of the F_1 female response. These data suggest that the low response to LPS and poly I·C seen with the CN × DN

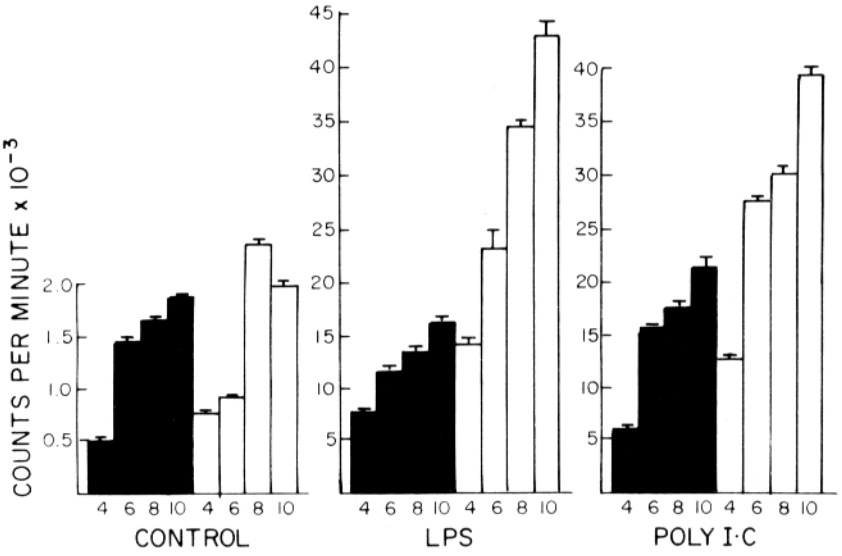

FIG. 8. Uptake of [^{3}H]TdR by increasing numbers of male (closed bars) or female (open bars) CN × DN F_1 spleen cells, depleted of adherent cells and T lymphocytes, in response to media alone (control), LPS (50 μg/ml), or poly I·C (250 μg/ml). The numbers under the bars represent the number of cells per culture × 10^{-5}. Cultures were run in the presence of 5% FCS. The response of these cells to PHA and Con-A was less than 7% of control cultures.

TABLE I

Skin Rejection Day of C6 Grafts by CN × DN Males and Females

Strain	Sex	No. of animals	Day of rejection ± SEM
CN × DN	M	8	11.0 ± 0.57
CN × DN	F	8	10.2 ± 0.59

F_1 male spleen cells is secondary to both a decreased absolute number of B lymphocytes per culture and a qualitative abnormality in the ability of these B lymphocytes to respond to these mitogens.

In order to further evaluate the functional capabilities of the T and B lymphocytes of the CN and CN × DN F_1 males, we studied skin allograft rejection, lymphocyte-mediated cytotoxicity, and ADCC. Allograft rejection was equivalent in the CN × DN F_1 males and females as was the ability of spleen cells from sensitized F_1 males and females to kill EL-4 tumor cells. In contrast, spleen cells of unsensitized CN × DN F_1 females showed an increased ability to lyse antibody-coated EL-4 tumor cells when compared to their F_1 male littermates. Thus, two immunologic functions clearly associated with T cells (17, 18) appear normal in the immunodeficient F_1 males while one function, ADCC, which is independent of T lymphocytes (19, 20) is abnormal. The decreased antibody-dependent cytotoxicity seen with the F_1 male mice could be explained by either

TABLE II

Percent Chromium Released by EL-4 Tumor Cells after Incubation with Sensitized CN × DN Male or Female Cells

Strain	Sex	No. of animals	Mean percent chromium release ± SEM
CN × DN	M	5	20.6 ± 0.74
CN × DN	F	5	20.6 ± 0.52

TABLE III

Percent Lysis of Antibody-Coated EL-4 Tumor Cells After Incubation with Unsensitized CN, CN × DN, or C6 × DN Male and Female Cells

Strain	Sex	No. of animals	Mean percent chromium release ± SEM
CN	M	3	23.0 ± 1.19
CN	F	3	23.0 ± 2.24
CN × DN	M	8	19.8 ± 1.03
CN × DN	F	8	56.9 ± 1.54
C6 × DN	M	3	56.4 ± 0.67
C6 × DN	F	3	58.4 ± 2.33

low numbers of B lymphocytes with altered ratios of target to effector cells or to a functional abnormality of the effector cells (17).

Our studies have not detected any abnormality in the function of T lymphocytes in CN mice, whereas they provide strong evidence for a defect in certain B-lymphocyte functions. Although a quantitative decrease in the number of κ-bearing cells is seen in the CN mice, as discussed above, it is unlikely that this decrease can, by itself, account for the abnormal immune function seen in these animals. Thus, the proportion of immunoglobulin-bearing cells in the spleens of CN mice and CN × DN F_1 males is approximately half normal and the total number of spleen B lymphocytes approximately 25% of normal; their response to the T-independent antigens SIII (1) and DNP-lys-Ficoll[2] is undetectable under conditions in which a response of less than 5% of normal could easily be measured. Moreover, the immune responses of CN × DN F_1 male mice to SRBC,[2] DNP-keyhole limpet hemocyanin (KLH),[2] and poly I·C complexed to methylated bovine gamma globulin (2) are all easily measured, although diminished to variable degrees when compared to F_1 females. In addition, Barthold and Janeway (personal communication) have shown that priming CN mice with DNP-KLH generates a substantial number of primed DNP-specific precursors of antibody-forming cells, which can be detected in transfer experiments.

These results could be explained in two general ways. On the one hand, they could be accounted for by a general defect in all members of the B-lymphocyte class such that some functions were completely lost while others were less severely affected or were normal. Alternatively, one might postulate the existence of two (or more) independent sublines of B lymphocytes with distinct functions. In this case, the CN mice might have a normal representation of one subline and either absent or defective B lymphocytes of the other subline. Although we cannot yet reach a dicision regarding these two possibilities, both the

finding that the frequency of cells bearing three distinct B markers and the observation that many (? all) B-lymphocyte functions are at least partially impaired is more consistent with the first possibility.

A direct influence of the X chromosome on immune function in man has been shown by the association of IgM levels to the X chromosome (21, 22) and by the X-linked inheritance of a number of immunodeficiency diseases. These diseases include infantile X-linked immunodeficiency with hyper-IgM, immunodeficiency with thrombocytopenia and eczema (Wiskott-Aldrich syndrome), and X-linked severe combined immunodeficiency (23). In all these diseases and the X-linked immune defect seen in the CN mouse strain, the suggested cellular defect involves B lymphocytes, supporting the conclusion that the X chromosome is critical to the functional development of the B-lymphocyte portion of the immune system. There is an interesting similarity between several features of the immune abnormalities of CN mice and of patients with the Wiskott-Aldrich syndrome. In both of these disorders, very low concentrations of serum IgM occur (7, 24, 25), and the immune response to polysaccharide antigens is either very low or absent. Lymphocytes of both the CN mice and patients with the Wiskott-Aldrich syndrome are able to respond to T-lymphocyte phytomitogens (26). While the T-lymphocyte function in CN mice appears to be normal, there is some evidence that it is impaired in the Wiskott-Aldrich syndrome (24, 26). However, it has been suggested that this abnormality in T-cell function, based primarily on the decreased skin reactivity seen in these patients, is a secondary rather than a primary phenomenon (24). Further studies of the CN mouse strain may provide a model to study both B-lymphocyte differentiation and activation and to aid in the evaluation of the cellular deficiency in at least some forms of human immune deficiency diseases.

Summary

A study of the composition and functional properties of spleen cells from the immune deficient CBA/HN mice and their F_1 progeny is reported. While abnormalities were seen in both the numbers and function of thymus-independent (B) lymphocytes, all studies involving thymus-dependent (T) lymphocytes were normal. The X-linked nature of the immune defect in these mice was therefore attributed to abnormal or absent B lymphocytes. The possible nature of this defect and the similarity of the immune defect in these mice to certain human X-linked immunodeficiency diseases are discussed.

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