ONTOGENY OF B CELLS IN CBA/N MICE

Evidence for a Stage of Responsiveness to Thymus-independent Antigens during Development*

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CBA/N is a mutant mouse strain derived from CBA with an x-linked, recessive immune defect at the B-cell level. The defective mice are unable to raise antibodies to a number of thymus-independent (TI)[1] antigens like lipopolysaccharide (LPS), pneumococcal polysaccharide type III (1), and polyinosinic-polycytidylic acid (2) whereas the antibody response to thymus-dependent (TD) antigens such as sheep erythrocytes (SRBC) is normal (1). It has also been shown that the percentage of Ig-bearing spleen cells is lower in these mice compared to normal mice (3). The distribution of Ig-determinants on the individual B cell is abnormal (4). A number of T-cell functions, such as mitogen responses to phytohemagglutinin and concanavalin A, specific in vitro T-lymphocyte-mediated cytotoxicity and graft rejection are normal (1). Several reports clearly show that the immune defect of CBA/N cannot be explained by an increased amount of suppressor T cells (5–7). A major question is whether CBA/N suffer from an arrest in the maturation of their B-cell line thus representing an immature B-cell state or if their B cells have deviated during development. A prerequisite for the latter hypothesis is that there exist two different mature subgroups of B cells, one that responds to TI antigens and another that responds to TD antigens (for review see reference 8). If a deviation of development has occurred in the CBA/N mouse it would indeed have mature B cells but only the kind that make antibodies to TD antigens. The subgroup of B cells responsible for antibody production to TI antigens would be lacking. In a previous paper we have shown that mature B cells from normal, adult mice respond to polyvinyl pyrrolidone (PVP), a TI antigen without T-cell help, whereas immature B cells require T cells as helpers to respond to PVP (9). This concept of T dependency in immature B cells seems to be general as similar results have been obtained by others using a different TI antigen, LPS (10). Immature B cells are defined as B cells from mice up to 2 wk of age, or B cells from lethally irradiated and bone marrow reconstituted adult mice up to 2 wk after reconstitution. We thus have a tool for studying the characteristics of the immature B cell.

The present paper is an attempt to analyze whether an arrest or a deviation has occurred in the development of the B-cell lineage in the CBA/N mouse, using the

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[1] *Abbreviations used in this paper*: BSS, balanced salt solution; DNP, dinitrophenyl; HRBC, horse erythrocytes; LPS, lipopolysaccharide; OA, ovalbumin; PVP, polyvinyl pyrrolidone; SRBC, sheep erythrocytes; TD, thymus dependent; TI, thymus independent.

above mentioned system. We have used lethally irradiated and bone marrow reconstituted adult mice which are challenged with antigen at the time of reconstitution. Antibody titers have been determined at different times after immunization. The present results show that the B cells of CBA/N mice, like B cells from normal mice, pass through a stage of maturation where they can be triggered by TI antigens and helper T cells. In contrast, adult CBA/N mice cannot be triggered by TI antigens and helper T cells. Thus, it seems as if CBA/N mice, at an early stage of development, have normal B cells but that these immature B cells only develop into the TD subgroup of B cells, indicating a deviation of development.

Materials and Methods

Mice. CBA/N mice were obtained from National Institutes of Health Rodent and Rabbit Production Section, Bethesda, Maryland. A/Sn mice were taken from our animal department. (A/Sn ♂ × CBA/N ♀)F_1 mice were used. The males of these hybrids are immunodeficient whereas the females can be used as normal controls. A/Sn mice possess a dominant gene(s) that determines high response to PVP (11). BALB/c and nude mice on a BALB/c genetic background were obtained from BOM Ltd., Denmark.

Antigens and Immunization. PVP with mol wt 10,000 and 360,000 were obtained from Fluka AG, Switzerland. Immunization was performed with 1 μg of the 360,000 preparation given either dissolved in balanced salt solution (BSS) intraperitoneally or in Freund's complete adjuvant (Difco Laboratories, Detroit, Mich.), subcutaneously. In cell transfer experiments, 0.1 μg was given dissolved in BSS intravenously together with the cells. Dinitrophenyl (DNP)-ovalbumin (OA) was prepared according to Little and Eisen (12) and for immunization 50 μg were given in Freund's complete adjuvant subcutaneously. *Escherichia coli* LPS 055:B5 was obtained from Difco Laboratories. For immunization, 1 μg dissolved in BSS was given intraperitoneally. In cell transfer experiments, 1 μg was given dissolved in BSS intravenously together with the cells. Horse erythrocytes (HRBC) were given intraperitoneally at a dose of 2×10^8 for immunization.

Serologic Tests. Determination of hemolytic antibody to HRBC, PVP-labeled sheep erythrocytes and LPS-coated sheep erythrocytes as well as antigen-binding capacity of antisera using I 125-PVP was performed as previously described (13). I125-labeled hydroxyphenacetyl-DNP-lysin (14) was also used in antigen-binding assays.

Irradiation of Mice and Preparation of Cell Suspensions. Performed as previously described (13).

Results

The Antibody Response to PVP, LPS, DNP-OA, and HRBC. Defective (A × CBA/N)F_1 male mice and their normal female littermates were immunized with the TI antigens, PVP and LPS, and the TD antigens DNP-OA and HRBC. In parallel, nude mice were immunized as a control for the T dependency. A normal unrelated mouse strain, BALB/c, was also included. As can be seen in Tables I, II, and III (A × CBA/N)F_1 ♂ are unable to raise antibody responses to PVP and LPS, as expected, measured both by hemolysis and antigen binding capacity. The responses to DNP-OA and HRBC are quite normal, the antibody titers being equivalent to those in (A × CBA/N)F_1 ♀ and BALB/c mice.

Response of Maturing B Cells from the Bone Marrow of (A × CBA/N)F_1 Transferred to Normal Syngeneic Mice. To examine whether the B cells during their maturation pass through the same early stage in defective (A × CBA/N)F_1 ♂ as in normal (A × CBA/N)F_1 ♀, the following experiment was performed. Bone marrow cells from the defective and control mice were transplanted to lethally irradiated normal recipients either together with antigen alone or together with antigen and thymus cells from previously immunized (A × CBA/N)F_1 ♀. Fig. 1 shows the results obtained in the PVP-system.

TABLE I

Antigen-binding Antibody Response to PVP and DNP-OA in T- and B-Cell-deficient Mouse Strains

Mice	Antibody response day 14 Log_{10}ABC ± SE*	
	PVP	DNP
B-cell deficient (A × CBA/N)F_1 ♂	0.08 ± 0.19	1.35 ± 0.15
Normal control (A × CBA/N)F_1 ♀	2.87 ± 0.06	1.42 ± 0.19
T-cell deficient nude	2.95 ± 0.05	<−1.0
Normal control BALB/c	2.56 ± 0.21	1.71 ± 0.02

* Mean of five to eight mice. Nanograms of antigen bound per milliliter of serum.

TABLE II

Hemolytic Antibody Response to PVP and HRBC in (A × CBA/N)F_1 Mice

Mice	Antibody response day 17 log_3 hemolytic titer*	
	PVP	HRBC
(A × CBA/N)F_1 ♂	<1	5.1
(A × CBA/N)F_1 ♀	3.1	6.0

* Mean of seven to nine mice.

TABLE III

Hemolytic Antibody Response to LPS in (A × CBA/N)F_1 Mice

Mice	Antibody response day 7 Log_3 hemolytic titer* LPS
(A × CBA/N)F_1 ♂	<1.0
(A × CBA/N)F_1 ♀	6.0

* Mean of three to five mice.

The control animals given only bone marrow cells show a rather poor antibody response to PVP during the first 2 wk of development, but, gradually develop a normal response to PVP. Adding immune thymus cells greatly enhances the antibody response. This is in accordance with results shown in an earlier paper (9). The defective animals, that is, lethally irradiated A × CBA/N ♀ mice reconstituted with ♂ bone marrow cells, show exactly the same pattern. It is evident that the immature (A × CBA/N)F_1 ♂ B cells can be triggered to an antibody response with the help of immune T cells. The result is confirmed in the LPS-system (Fig. 2), where also immune T cells were able to help the maturing B cells from the defective mice to produce antibody to LPS. Thus, immature B cells from defective A × CBA/N ♂ mice behave similarly as normal, immature B cells in the antibody response to two different TI antigens.

The Antibody Response to PVP, LPS, and HRBC after Addition of Normal or Immune Thymus Cells to Adult (A × CBA/N)F_1 Mice. To investigate whether an antibody response could be rasied in adult intact (A × CBA/N)F_1 ♂ mice after addition of thymus cells from the normal (A × CBA)F_1 animals the following experiment was done. (A × CBA/N)F_1 ♂ mice received an intravenous injection of thymus cells from normal unimmunized (A × CBA)F_1 mice and were then immunized with PVP. As can be

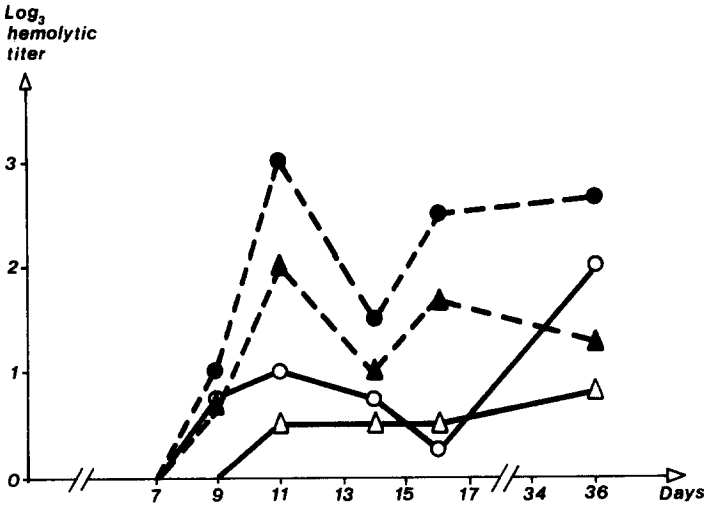

FIG. 1. Effect of immune thymus cells on hemolytic antibody response to PVP in lethally irradiated and bone marrow reconstituted (A × CBA)F_1 mice. Mean of three to four mice. 2×10^7 bone marrow cells were given intravenously. 1×10^7 thymus cells, taken from (A × CBA/N)F_1 ♀ immunized 10 wk earlier with PVP, were given intravenously. Bone marrow donor: △,(A × CBA/N)F_1 ♂; ○, (A × CBA/N)F_1 ♀; ▲, (A × CBA/N)F_1 ♂ + immune thymus cells; ●, (A × CBA/N)F_1 ♀ + immune thymus cells.

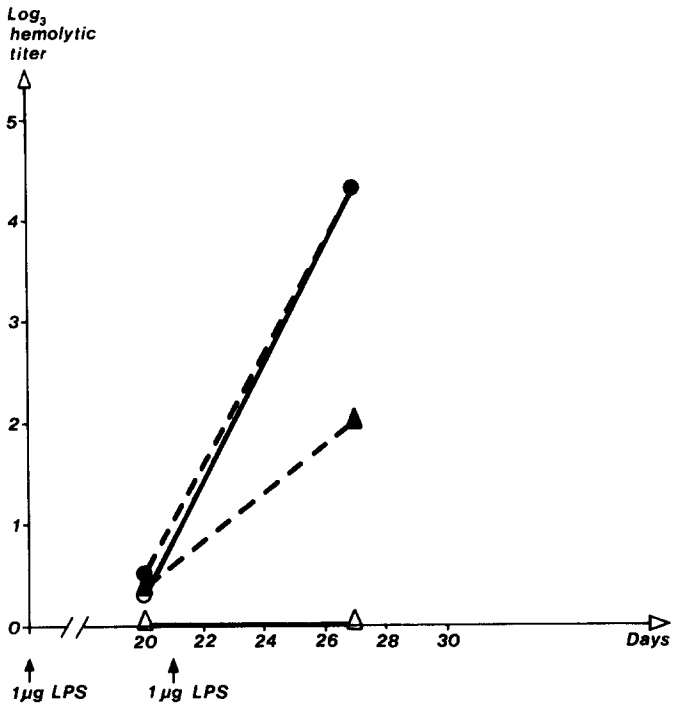

FIG. 2. Effect of immune thymus cells on hemolytic antibody response to LPS in lethally irradiated and bone marrow reconstituted (A × CBA)F_1 mice. Mean of seven to eight mice. 2×10^7 bone marrow cells were given i.v. 2×10^6 thymus cells, taken from (A × CBA/N)F_1 ♀ immunized 7 days earlier with LPS, were given intravenously. Bone marrow donor: △, (A × CBA/N)F_1 ♂; ○, (A × CBA/N)F_1 ♀; ▲, (A × CBA/N)F_1 ♂ + immune thymus cells; ●, (A × CBA/N)F_1 ♀ + immune thymus cells.

TABLE IV

Hemolytic Antibody Response to PVP and HRBC after Transfer of Normal or Immune Thymus Cells to Intact (A × CBA/N)F_1 Mice

Thymus cells	Antibody response day 16 Log_3 hemolytic titer*			
	PVP		HRBC	
	(A × CBA/N)F_1			
	♂	♀	♂	♀
None	<1.0	1.40	2.20	4.60
None immune‡	<1.0	2.25	3.50	6.00
Immune§	<1.0	1.80	3.40	5.00

* Mean of four to five mice.

‡ 1.3×10^7 thymus cells were given intravenously.

§ 1.0×10^7 thymus cells, taken from (A × CBA)F_1 mice immunized 13 d earlier with a mixture of PVP and HRBC, were given intravenously.

TABLE V

Hemolytic Antibody Response to LPS after Transfer of Immune Thymus Cells to Intact (A × CBA/N)F_1 Mice

Thymus cells	Antibody response day 7 Log_3 hemolytic titer*	
	LPS	
	(A × CBA/N)F_1 ♂	(A × CBA/N)F_1 ♀
None	1.0	8.1
1×10^7 thymus cells‡	1.0	8.3
2×10^6 thymus cells‡	0.8	9.0

* Mean of three to eight mice.

‡ Thymus cells were taken from (A × CBA/N)F_1 ♀ immunized 7 d earlier with LPS intraperitoneally.

seen in Table IV, (A × CBA/N)F_1 ♂ were still not able to produce antibodies to PVP. In an additional experiment, thymus cells from (A × CBA)F_1 animals previously immunized with PVP and HRBC were transferred and the mice were immunized with PVP. Even after this procedure, the defective mice were not able to respond to PVP. A similar experiment was done with LPS as an antigen. Also in this case, the males were unable to respond properly to LPS after immunization as can be seen in Table V. Clearly, the B cells from adult CBA/N mice are not just arrested at an early stage of development because at an early stage, B cells from these mice respond normally i.e., they are able to make antibodies to TI antigens after addition of normal or immune T cells, whereas the adult B cells are not triggered by those antigens.

Discussion

These studies deal with the CBA/N mice that have a genetic defect in their B-cell lineage, making them unable to respond to a variety of TI antigens. This defect is evident only in the adult, mature mice because we demonstrate that CBA/N B-cells pass through an early stage of maturation, where they can respond to TI antigens like immature B cells from normal mice. The B-cells of CBA/N adult mice are thus not arrested in their maturation at the immature stage of young mice, but rather a deviation of development has occurred. This is evident from the finding that adult

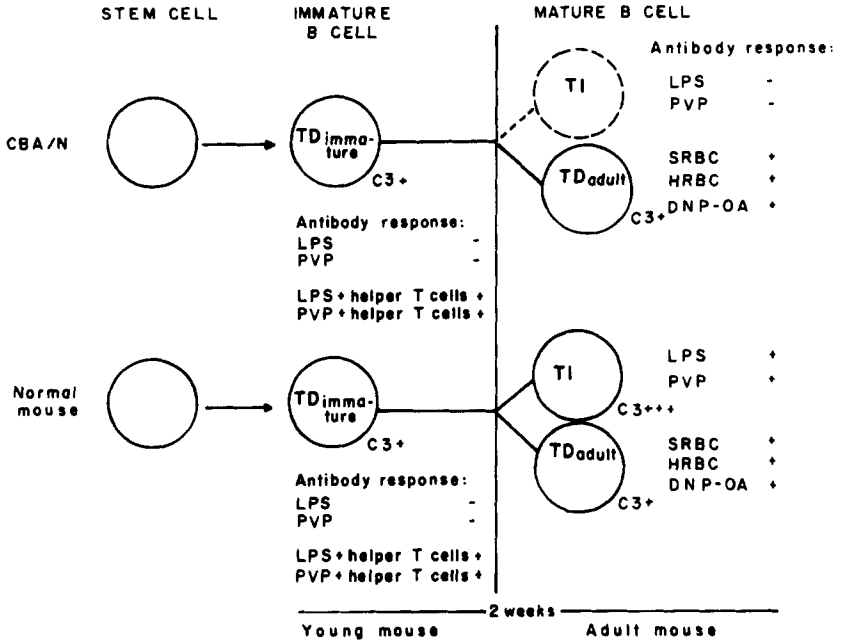

FIG. 3. Illustration of the ontogeny of B cells in normal and CBA/N mice.

CBA/N mice, in spite of being supplemented with immune T-cells, are quite unable to respond to the TI antigens which the immature CBA/N B-cells do, if provided with T-cell help. This experiment also shows that the antibody production is really performed by the immature cells and not by contaminating B cells present in the transferred thymus cell preparation.

The ontogeny of B cells in normal and CBA/N mice can, according to our hypothesis, be visualized as seen in Fig. 3. In the normal mouse there is, during embryonical life, an omnipotent stem cell which gives rise to among others an immature B cell. This immature B cell can be detected in the young mouse up to 2 wk of age after which it gradually disappears. The immature B cell is totally T dependent, that is even with the so-called TI antigens, helper T cells are required for antibody production. From this, immature B-cell maturation goes along two lines giving rise to two different subgroups of B cells. Accordingly, in the adult mouse there is one subgroup of B cells which is restricted to making antibodies to TI antigens, like PVP and LPS and another one which, with the help of T cells, make antibodies to TD antigens only, such as SRBC, HRBC, and DNP-OA.

In the CBA/N mouse, the development of B cells passes through the same stage in the young mouse where we find the immature TD B cell which after 2 wk of age development deviates and only goes along the line that leads to the adult TD B cell. It has to be pointed out that the immature TD B cell is not identical to the adult TD B cell. The former is totally T dependent, that is it can not make antibodies to any kind of antigen unless provided with T-cell help. The latter is a specialized subgroup of the adult B-cell population responding only to true TD antigens. Another explanation to the immune defect in CBA/N mice would be an arrest in maturation at the stage of the 2-wk mouse, with the immature TD B cell prevailing in the adult mouse.

This is clearly not the case as we can not make the defective adult mouse respond to TI antigens after addition of helper T cells.

Preliminary data to be published in a following paper indicate that the receptor for complement factor 3 (C3) is of crucial importance as a developmental surface marker. It has previously been shown that the percentage of C3-receptor positive cells is lower in the spleens of newborn normal mice (9) and this also seems to be the case in newborn as well as in adult CBA/N mice. Thus, the adult CBA/N mice seem to lack a subgroup of B-cells with high concentration of the C3-receptor and which are responsible for the antibody response to TI antigens (Fig. 3). Again, it has to be stressed that the immature TD B cell is not identical to the adult TD B cell, although they seem to share surface characteristics with respect to C3-receptor density.

Summary

This paper deals with the CBA/N mice, a strain bearing a genetic defect in their B-cell compartment. By using a previously described system we have been able to show that the immature cells of CBA/N mice are functionally indistinguishable from normal immature cells, in that both can be triggered to respond to thymus-independent (TI) antigens, provided they are supplied with helper T cells. When the maturation is completed, CBA/N B cells are unable to respond to TI antigens (like lipopolysaccharide and polyvinyl pyrrolidine) irrespective of the presence of helper T cells, whereas normal mature B cells have grown able to respond without any help.

These data allow us to reject the hypothesis that CBA/N mice are arrested at an immature stage and clearly support the idea that they have deviated during development so that only thymus-dependent B cells develop.

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