Defects in the Immune Response of NZC Mice[1] (36653)

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(Introduced by Z. Ovary)
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It is generally held that a common hematopoietic stem cell differentiates to form both the erythroid and myeloid pathways and the immunocyte series including lymphoid cells and antibody-secreting cells. Previous studies on mice having genetic defects affecting either hematopoietic stem cells (1), or the environment necessary for their differentiation (2), have not shown accompanying defects in the ability of these animals to mount an immune response (3).

In studies on hematopoietic stem cell activity of several inbred New Zealand mouse strains it was shown that the NZB and NZC strains both have excessively high endogenous spleen colony-forming activity (4), and the NZC strain also shows a marked *reduction* in spleen hematopoietic colony formation in syngeneic transplantation experiments (4). As young NZB mice have been shown to be hyperreactive to antigenic stimuation (5), the present study was undertaken to evaluate the immune response of NZC mice in conventional immunization schemes, in adoptive transfer studies with spleen cells and in *in vitro* experiments.

Materials and Methods. NZC mice from a colony maintained at the Hall Institute were used in all experiments (4). Age- and sex-matched controls of (BALB/c $\times$ NZB)F_1 (BNF_1) mice, were used in the adoptive transfer experiments. Various other strains were used as age- and sex-matched controls for the *in vitro* and *in vivo* studies, and were all obtained from Hall Institute colonies.

In direct immunization studies mice were given 0.2 ml of a 5% suspension of washed sheep erythrocytes (SRBC) and 0.1 ml of a 0.20 dilution of Brucella ring test antigen (C.S.L., Melbourne). The antigens were injected intraperitoneally.

In the adoptive transfer experiments, recipient mice first received 800 rad total body irradiation at the rate of 160 rad/min. Following irradiation and cell-transfer, mice were kept 4–6 to a cage and given food and water *ad libitum*.

Spleen cell suspensions were prepared by gently teasing donor spleens through a stainless steel sieve. Cells were then washed in Eisen's balanced salt solution (EBSS), which contains 0.01 *M* Tris; 0.001 *M* $MgSO_4$; 0.002 *M* $CaCl_2$; 0.006 *M* KCl; 0.12 *M* NaCl; at pH 7.4, and after resuspension in EBSS a viability count was performed. The appropriate dilution of cells was made and 0.2 ml of the dilution injected intravenously. At the time of cell injection, animals received 0.1 ml of a 10% solution of SRBC intraperitoneally.

Phytohemagglutinin (PHA) was obtained from Difco Laboratories (Detroit). Pokeweed mitogen was obtained from Grand Island Biological Corporation (Grand Island, N.Y.).

Direct plaque-forming cells (PFC) to SRBC were detected by a modification of the Jerne technique (6). Cells stimulated by PHA and pokeweed were harvested at 48 hr and pulsed with tritiated thymidine by a method previously described (7). *In vitro* studies on the immune response to SRBC were performed using the tissue culture of Diener and Armstrong (8). The assay for antigen-sensitive cells was performed as de-

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TABLE I. Antibody Responses *In Vivo* to Sheep Erythrocytes and Brucella.

	Serum antibody response[b]					
	SRBC			Brucella		
Strain[a]	6[c]	14	28	6	14	28
NZC	7.9 ± 1.4	6.3 ± 1.1	8.9 ± 0.8	8.8 ± 0.9	9.5 ± 0.8	9.3 ± 0.6
BALB	6.8 ± 1.0	6.6 ± 1.6	8.2 ± 0.7	7.5 ± 1.1	10.0 ± 1.2	9.2 ± 0.8
C57Bl/Wehi	7.8 ± 1.3	7.9 ± 1.6	9.1 ± 0.9	8.5 ± 1.0	6.9 ± 1.8	10.0 ± 0.5

[a] All mice between 10 and 12 months of age. Thirteen mice per group.

[b] Log 2 reciprocal titer + SD [(Hemagglutination assay in microtiter trays) as previously described (19)].

[c] Day postimmunization.

scribed by Kennedy et al. (9), and involved the injection of normal spleen cells and SRBC into lethally irradiated recipients. The recipient spleens were assayed 7 days later for the presence of foci of anti-SRBC activity. The content of PFC in samples of the spleen was also determined, and from the ratio of total spleen PFC/total spleen ASU an estimate of the proliferation rate of PFC within the ASU focus was made.

Cell seeding studies were made with spleen cell suspensions incubated with 100 μCi [^{51}Cr] sodium chromate for 2 hr at room temperature, washed three times and then 10^7 cells with known radioactivity were injected intravenously into normal recipients of various strains. Two hours later recipient spleens and livers were removed and ^{51}Cr activity determined in a gamma scintillation counter.

Results. Antibody production in vivo. Three groups of 10–12 month old mice of different strains were immunized intraperitoneally with SRBC and Brucella. Serum samples were taken at 6, 14, and 28 days later. The antibody titers of these sera are given in Table I, and show that NZC mice give normal humoral antibody responses under these conditions. Similar results were obtained in another experiment using 5 month old mice of NZC, C57, BALB/c, and CSW strains.

In order to determine whether young adult NZC mice give normal numbers of plaque-forming cells on immunization with sheep erythrocytes, NZC, (CBA × C57Bl)F_1 and (BALB/c × NZB)F_1 mice (of approximately 3 months of age) were immunized with SRBC and their spleens taken for PFC 5 days later. The mean results respectively

TABLE II. Transfer of Antibody-Forming Capacity with Unprimed Spleen Cells into Syngeneic and Allogeneic Recipients.

			Mean direct PFC ± SE[c]				
Experiment[a]	Spleen cell[b] dose	Day of PFC test	$F_1 \rightarrow F_1$	NZC → NZC	NZC → BNF_1	BNF_1 → BNF_1	BNF_1 → NZC
A	2×10^7	5	2254 ± 395	192 ± 33	—[d]	—	—
B	2×10^7	5	—	560 ± 107	350 ± 82	1792 ± 1096	1708 ± 822
C	10^7	7	—	240 ± 67	475 ± 165	2275 ± 213	3975 ± 886
D	10^7	7	—	245 ± 60	299 ± 38	954 ± 198	—
E	10^7	11	—	52 ± 17	190 ± 83	1008 ± 367	1820 ± 717

[a] Five separate experiments were performed.

[b] Number of donor strain spleen cells transfused intravenously.

[c] Mean number of direct PFC per recipient spleen in the donor host combination indicated. F_1 mice are (CBA × C57Bl)F_1.

[d] Dash indicates not tested.

were 108,000; 103,133; and 91,500 PFC per spleen for three mice each of the 3 strains. Again, a normal anti-SRBC response was therefore observed in NZC mice.

Spleen cell transfer of anti-SRBC response. A series of transfer experiments were then carried out with varying numbers of normal spleen cells being transferred into allogeneic or syngeneic irradiated recipient mice also given SRBC. The PFC response was determined 5–11 days later.

The results given in Table II uniformly show that transfer of NZC cells into either allogeneic *or syngeneic* recipients resulted in a low response, approximately 10–30% of the BNF_1 into BNF_1 response, whereas BNF_1 cells transfused into NZC recipients also responded like the syngeneic BNF_1 into BNF_1.

Uptake of transfused ^{51}Cr labeled spleen cells. Spleen cell suspensions of NZC and BNF_1 mice were labeled with ^{51}Cr and 10^7 cells of known radioactivity were injected into syngeneic and allogeneic mice. Uptake of cells in the recipient spleen and liver was determined from radioactivity counting of known amounts of tissue and is expressed in terms of a constant inoculum and recipient organ size. The results (Table III) show virtually identical proportions of injected cells settling in spleen and liver regardless of the donor–host strain combination used. A similar amount of radioactivity per mg of recipient spleens was found, and the ratio of spleen to liver uptake per gm was also similar. The results are also expressed as the *total* radioactivity in the recipient spleen as a percentage of the total radioactivity injected and again similar values were found, the greatest difference being between the two syngeneic groups, with the NZC transfer being 71% that of the BNF_1 transfer.

Antigen sensitive cells in NZC mice. Unprimed spleen cells (5×10^6) from NZC and BNF_1 mice were injected into irradiated BNF_1 recipients, and the recipient spleens were assayed 7 days later for antigen sensitive units (ASU) and PFC. The results given in Table IV show that with NZC donor cells, only 14% of the BNF_1 ASU response was observed. The ratio of PFC/ASU was however quite similar, indicating a normal proliferation of those ASU that had reached the spleen.

In vitro *response of NZC cells to antigen, PHA, and pokeweed.* Spleen cell suspensions of NZC, CBA and BNF_1 were cultured *in vitro* (15×10^6 cells) with SRBC. The development of an immune response was then assessed by the number of direct PFC in the culture 4 days later. Although CBA and BNF_1 cells gave the expected degree of response, NZC cells entirely failed to respond (Table V). Similarly, the *in vitro* response to PHA was markedly depressed, being only 20% of the BNF_1 response. The NZC response to pokeweed was only marginally depressed, being 66% that of the BNF_1 response.

Discussion. Although immunization of

TABLE III. Uptake of ^{51}Cr-labeled Spleen Cells in Recipient Mice.

Donor ^{51}Cr cells	Recipient strain	# Mice	Mean splenic[a] uptake	Mean liver[b] uptake	Percent[c] uptake in spleen	Spleen[d] liver ratio
NZC	NZC	3	6020 ± 728	6860 ± 301	13.8	8.4
NZC	BNF_1	4	5940 ± 785	8350 ± 958	16.7	7.1
BNF_1	NZC	4	6140 ± 735	6410 ± 550	14.0	9.5
BNF_1	BNF_1	4	7590 ± 1334	7420 ± 623	19.6	10.2

[a] Mean radioactivity (cpm) in recipient spleens (± SE) per 26,000 cpm of injected cells and per 100 mgm recipient spleen.

[b] Mean radioactivity (cpm) in recipient livers (± SE) per 26,000 cpm of injected cells and per gram recipient liver.

[c] Percent of total injected radioactivity found in total recipient spleen.

[d] Ratio of spleen to liver uptake per mg of each tissue.

TABLE IV. Assay for Antigen Sensitive Cells.

Donor spleen cells	Recipient strain	# Mice	ASU[a]	PFC[b]	PFC/ASU
NZC	BNF_1	6	1.2	60	50
BNF_1	BNF_1	5	8.4	198	23.5

[a] Antigen sensitive units (9).
[b] Direct plaque forming cells.

NZC mice *in vivo* leads to normal levels of humoral antibody responses, *in vitro* and cell transfer studies indicate that NZC immune responsiveness is markedly suboptimal. The apparent contrast between results from standard *in vivo* immunizations, and *in vitro* or transfer observations, may be explicable in terms of the number of available cells that are required for the particular response. It has frequently been noted that an *in vivo* immunization may not activate the entire available population of potentially reactive cells (11) and thus a large reserve of cells exist. In cell transfer studies with limiting numbers of cells it is possible, however, that a difference in numbers of available reactive cells would lead to a detectable difference in the level of the immune response.

In cell transfer studies it was observed that NZC spleen cells were markedly inferior to BNF_1 cells in transferring anti-SRBC responses to either NZC or BNF_1 mice. Stem cell studies have indicated histocompatibility between these strains, and it appears that NZC mice are probably of H-2^d type (like BNF_1 mice). These studies therefore imply that the defect lies with the donor NZC spleen population, rather than with the recipient animal. Furthermore, similar depressed activity was observed directly with NZC cells *in vitro*.

Four possible explanations might be considered: (1) There is a reduced number of all types of precursor antigen reactive cells in NZC lymphoid tissues. (2) There is a normal number of such cells but they do not settle in recipient spleens. (3) There is a normal number which do settle, but are defective in their susceptibility to activation or proliferative rates. (4) There is a reduced number of cells of a specific subpopulation that is required for the induction of the response.

Studies with transfer of ^{51}Cr-labeled cells suggest that the defect is not due to the inability of cells to settle in the spleen (mechanism (2) above). The normal proliferative capacity of the ASU that did reach the spleen (Table IV) also suggests that mechanism (3) is not the explanation.

It is suggestive at present that the defect may be associated with a reduction in the

TABLE V. *In Vitro* Response to Antigen and Mitogens.

Spleen cells	*In vitro* stimulant	Mean PFC ± SE	DPM ± SE	Ratio to background[a]
CBA	SRBC	2033 ± 109	—	—
NZC	SRBC	23 ± 3	—	—
BNF_1	SRBC	1353 ± 91	—	—
NZC	nil	—	8,616 ± 1,026	—
NZC	PHA	—	19,198 ± 1,230	2.2
NZC	PWM	—	33,849 ± 6,730	4.0
BNF_1	nil	—	10,519 ± 884	—
BNF_1	PHA	—	109,479 ± 8,813	10.4
BNF_1	PWM	—	63,836 ±18,461	6.0

[a] Ratio of disintegrations per minute (DPM) for stimulated culture to nonstimulated culture.

size of one, or several, cell populations. Recent data has clearly shown that at least three cell types are involved in the anti-SRBC response, namely macrophages (12), thymic dependant lymphocytes (T cells) and nonthymic dependant lymphocytes (B cells) (13). In cell transfer studies, it might be expected that the macrophage component could be provided by the host, although there is data to indicate radiosensitivity of macrophages in at least some immune responses (14).

Recent studies (15) with mitogen stimulation of T and B lymphocyte populations have suggested that PHA reactive cells are solely T cells, whereas both T and B cell populations can be stimulated by pokeweed. In view of the much greater depression of PHA responsiveness than PWM responses in NZC mice we might therefore provisionally suggest that the defect lies at the T cell level. Further studies with purified cell populations, other antigens, and cell replacement studies are in progress to elucidate this possibility, and also to determine whether this defect is related to any of the known immune response genes (16).

Finally, as it has been suggested (17, 18) that both the differentiated cells of the hematopoietic system and of the immune system are derived from a common stem cell, it will be of considerable interest to determine whether the defect in NZC mice is ultimately related to the defects known to exist in the stem cell compartment (4). For example, it may be that there is an altered differentiation pathway that does not lead to the usual number of mature T cells, thereby leading to reduced immunocompetence.

Summary. The NZC strain of mouse is known to have a defect involving the transplantation of hematopoietic stem cells. Attempts to test the immune system of this strain show a normal antibody response *in vivo*. However, in adoptive transfer systems, NZC cells were shown to be minimally responsive on transfer, although (Balb × NZB)F_1 cells could respond in the NZC environment. *In vitro* studies of the response of NZC cells to SRBC, phytohemagglutinin and pokeweed all show reduced responses. Evidence is presented which suggests that there is a defect in the number of progenitor cells for the immune system, possibly involving only one component cell population.

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