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BRIEF COMMUNICATION

Hyperactive T-cell function in young NZB mice; Increased proliferative responses to allogenic cells

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SUMMARY

The one-way mixed lymphocyte reaction was employed to study proliferative responses to antigens by mature, immunocompetent T cells from NZB mice 3 weeks to 4 months old. Compared to cells from control mice of the same H-2 type, thymus, spleen and lymph node cells from NZB mice were hyperactive in this response. The results are discussed in relation to possible effects of chronic stimulation by endogenous type C leukaemia virus upon differentiation of functional T cells or upon regulation by T cells of other T-cell functions, including augmentation of antibody responses.

INTRODUCTION

New Zealand Black (NZB) mice are a model of autoimmune disease associated with type C leukaemia virus. They spontaneously develop lymphoid hyperplasia, autoimmune haemolytic anaemia, antinuclear antibodies, immune complex glomerulonephritis and sometimes lymphoreticular malignancies. Numerous reports have suggested that cellular immune deficiency plays a central role in development of disease, and that failure of negative regulation by T cells leads to excessive synthesis of autoantibodies by B cells (Talal & Steinberg, 1974).

Decrease of T-cell numbers and effector functions is indeed characteristic of older NZB mice. Younger NZB mice, however, have normal numbers of recirculating and theta-bearing lymphocytes and function normally in rejection of allogeneic skin grafts, induction of graft-versus-host disease by spleen cells and in proliferative responses of thymus and spleen cells to T-cell mitogens (Zatz, Mellors & Lance, 1971; Stobo, Talal & Paul, 1972a, b; Stutman, 1972; Gelfand & Steinberg, 1973; Stutman, Yunis & Good, 1968; Leventhal & Talal, 1970). Some T-cell functions are actually increased in young NZB mice. They develop the ability to regress tumours induced by murine sarcoma virus at a younger age than five control strains (Gazdar, Beitzel & Talal, 1971). They also show increased augmentation of the antibody response to sheep erythrocytes after *in vivo* sensitization, and heightened cross-reactions in antibody responses to erythrocytes from various species, both abnormalities occurring at the level of the T cell (Playfair, 1968, 1972; Playfair & Marshall-Clarke, 1973).

To further investigate T-cell activity in young NZB mice, we chose the one-way mixed lymphocyte proliferative reaction (MLR) because it is a classic *in vitro* response of mature, immunocompetent T cells (Konda, Nakao & Smith, 1972). Our studies demonstrate that this response is consistently increased in NZB mice from 3 weeks to 4 months of age.

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MATERIALS AND METHODS

NZB mice descended from NIH stock were obtained from the Vivarium at the University of California, San Francisco (NZB/SF) or from Jackson Laboratories, Bar Harbor, Maine. (NZB/J). Control mice matched at the major histocompatibility locus (H-2^d) were obtained from Jackson Laboratories (DBA/2J, C57BL/KsJ) as were allogeneic (H-2^b) mice (C57Bl/6J) used as the source of stimulating cells.

Three to twelve mice from each experimental group were sacrificed by cervical dislocation. Responding thymus, spleen or lymph node cells were collected through number 60 wire mesh and 25-gauge needles, and washed twice by centrifugation at 200 *g*. Allogeneic stimulating cells were exposed to 25 μg/ml mitomycin C (number 8232, Nutritional Biochemical Corporation, Cleveland, Ohio) for 30 min at 37°C and washed three times. The concentration of viable mononuclear cells was determined by counting in a haemocytometer with 0·2% trypan blue. Total leucocytes were counted with a Coulter counter after lysis of erythrocytes with Zap-isoton (Coulter Electronics, Hialeah, Florida). Using RPMI 1640 medium with 10% heat-inactivated (56°C for 30 min) fresh (frozen) human serum, Hepes buffer (10 mm) and 1% antibiotic-antimycotic (Gibco, Grand Island, New York), cultures containing 10^6 responding cells and 10^6 mitomycin-blocked stimulating cells in 0·2 ml were established in microtitre plates (IS-FB-96-TC, Limbro Chemical Company, New Haven, Connecticut) and maintained at 37°C in 5% CO_2.

On days 2–5, four replicate cultures from each group were pulse-labelled for 8 hr with 1 μCi of tritiated thymidine ([^{3}H]Tdr, 3 Ci/mmol, Schwartz-Mann, Orangeburg, New York) and harvested by suction onto glass fibre filters (Hartzman *et al.*, 1972). Radioactivity was determined in a Packard liquid scintillation counter.

Kinetic curves of the means of the logarithms ($\overline{\log}$) to base 10 of the counts per minute (ct/min) were linear at least through day 4; data from that day are presented. Stimulation by alloantigens is taken as allogeneic $\overline{\log}$ (A) minus syngeneic $\overline{\log}$ (S) for experimental (e) and control (c) cultures. P values were obtained from

$$t = \frac{[(Ae-Se)-(Ac-Sc)]}{\sqrt{SE_{Ae}^2+SE_{Se}^2+SE_{Ac}^2+SE_{Sc}^2}}.$$

RESULTS

The proliferative response of NZB (H-2^d) lymphoid cells to mitomycin-blocked, allogeneic spleen cells was studied in several different experiments (Table 1). In each experiment, responding cells pooled from NZB mice were compared with responding cells pooled from appropriately matched H-2^d control strain mice. The response to syngeneic cells was used as a basis for evaluating the allogeneic response.

TABLE 1. One way mixed lymphocyte reaction* of NZB and control strain lymphoid cells

Source of responding cells			Incorporation of ^{3}H-Tdr ($\overline{\text{ct/min}}$/10^6 cells)		Stimulated increase in $\overline{\log}$	P
Organ	Age and inbred strain		Syngeneic	Allogeneic		
Thymus	3 weeks	C57Bl/KsJ	577	3150	0·74	<0·0001
		NZB/J	1230	16,900	1·14	
	12 weeks	C57Bl/KsJ	642	3070	0·67	<0·001
		NZB/J	1370	13,700	1·00	
	16 weeks	DBA/2J	871	5490	0·81	<0·0001
		NZB/SF	194	12,000	1·80	
Spleen	3 weeks	C57Bl/KsJ	5990	17,600	0·47	<0·001
		NZB/J	7340	43,600	0·77	
	12–20 weeks	DBA/2J	6620	19,900	0·48	<0·0001
		NZB/SF	2900	37,800	1·12	

* NZB and control H-2^d mice were stimulated in mixed lymphocyte cultures by mitomycin-blocked, H-2^b spleen cells from C57Bl/6J mice.

The incorporation of [^{3}H]Tdr into DNA by allogeneic cultures was consistently greater for NZB mice than for control mice, whether expressed as mean counts per minute per 10^6 responding cells or as mean log ct/min per total cells recovered from a lymphoid organ. Moreover, the stimulated increment of the allogeneic over the syngeneic proliferative response, expressed as the change in $\overline{\log}$ ct/min, was consistently greater for NZB cells than for controls. Comparison of logarithmic increments by a *t*-test showed that the difference between NZB and control stimulation was in all cases significant ($P<0{\cdot}001$).

The increased response to alloantigens was found in thymus, spleen and lymph node of NZB mice supplied from two different vivariums when compared with two control strains matched for age, sex, and H-2 type. It was observed in animals from 3 weeks to 4 months of age and in both sexes.

DISCUSSION

The MLR, a proliferative response of T cells capable of primary reactions with antigens, was found to be increased in young NZB mice. These *in vitro* results are supported by adoptive transfer experiments in which irradiated NZB mice reconstituted with syngeneic bone marrow were able to regenerate proliferative responses of thymus cells faster than were control mice (Dauphinée, Palmer & Talal, 1975). The observed increase might be attributed to accelerated differentiation of responsive T cells from immature precursors (Smith *et al.*, 1974; Konda, Nakao & Smith, 1973). Alternatively, responses of a normally differentiated population of mature T cells might be subject to abnormal regulation by other T cells (Rich & Rich, 1974).

There are two situations where increased antibody formation by NZB mice is attributable to an abnormal co-operative role of T cells. These are *in vivo* sensitization for the plaque-forming cell response to sheep erythrocytes and resistance to induction of tolerance (Playfair, 1968, 1972; Talal & Steinberg, 1974). It is uncertain whether the abnormality involves loss of suppressor function, augmentation of helper function, or both. However, differentiation of T cells responsive to alloantigens is associated with differentiation of helper T cells (Mosier & Pierce, 1972). Furthermore, T cells activated by allogeneic cells produce soluble factors which stimulate rather than inhibit B cells (Schimpl & Wecker, 1972). Accelerated maturation of these cells is therefore consistent with generation of helper activity. Normal mice infected with a type C leukaemia virus develop a thymus-dependent plasma factor which non-specifically enhances the antibody response to sheep erythrocytes (Rubin & Cerny, 1975). A type C leukaemia virus is endogenous to NZB mice and produced by them in high titres (Levy, 1974).

Our studies showing increased responsiveness to alloantigens *in vitro* and following adoptive transfer reveal that T-cell function in young NZB mice is at least in part hyperactive. In this respect it resembles T-cell function in various models of chronic stimulation by adjuvants or membrane-related antigens (Allan, Crampton & Jenkins, 1975; Smith *et al.*, 1974; Konda *et al.*, 1973). Adjuvant-induced potentiation of antibody formation, a T-cell dependent phenomenon (Allison & Davies, 1971), appears to be present spontaneously in NZB/NZW F1 mice (Jacobs *et al.*, 1972). A variety of infections and malignancies can lead to autoimmunity or immune-complex disease (Nelson, 1974; Oldstone & Dixon, 1971). In NZB mice, type C leukaemia virus may lead to enhanced MLR reactivity, augmented humoral immunity and autoantibody formation by stimulating differentiation of active T cells or by interfering with T-cell regulation of other T-cell functions.

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