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Immunological responses of the BB rat colony in Edinburgh

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SUMMARY

Several immunological responses of the spontaneously diabetic BB rat colony in Edinburgh designated (BB/E) have been studied. The proliferative responses to Con A and LPS, ability to make IL-2 and to show NK activity have been studied using diabetic and non-diabetic BB/E rats and normal Wistar rats. Our data suggest that the diabetic animals in the BB/E colony do not have marked deficiencies in any of these parameters. Lymphopenia and depressed T-cell responses do not appear to be a prerequisite for the development of diabetes in the BB/E colony.

INTRODUCTION

BB rats are a partially inbred line of rats that have a high incidence of development of insulin-dependent diabetes mellitus (see Review by Yale & Marliss, 1984). This disease has an autoimmune involvement and makes the BB rat a good animal model for human Type 1 (insulin-dependent) diabetes mellitus. Several workers have demonstrated immunological deficiencies in the diabetic BB rat (Bellgrau *et al.*, 1982; Maclaren *et al.*, 1983; Prud'Homme *et al.*, 1984; Guttmann *et al.*, 1983), and Colle, Guttmann & Seemayer (1981) have shown lymphopenia and diabetes to be genetically linked in the BB rat. However, this has not been a uniform finding, and since we are attempting to dissect those factors that determine whether an animal progresses towards the development of diabetes, it was important for us to ascertain the immunological status of the BB colony in Edinburgh. We describe in this paper our comparative studies of some immunological parameters in the diabetic BB/E rat.

MATERIALS AND METHODS

Animals

The Edinburgh colony of BB rats (BB/E) was derived from a nucleus which was kindly donated in 1982 by Dr P. Thibert, Animal Resources Division of Canada, Ottawa. The BB/E colony has been selectively inbred to generate two lines: the high incidence, diabetes-prone animals (incidence of diabetes is 60–70% with the mean age of onset at 96 days) and the non-diabetic subline (now in its eighth generation with a very low incidence of diabetes, $<4\%$). Wistar rats used in these experiments were obtained from Olac 1976 Ltd, Bicester, Oxon.

The animals used in these experiments were established diabetic rats, non-diabetic subline rats and Wistar rats that were all age-matched.

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Proliferation assays

Spleen cells from individual rats were washed three times in BSS and cultured at 2×10^5 cells per well in 96-well, flat-bottomed microtitre plates (Sterilin, Teddington, Middlesex) in RPMI-1640 (Flow Laboratories, Irvine, Ayrshire) supplemented with 5% (v/v) fetal calf serum (FCS), 2 mM L-glutamine, 5×10^{-5} M 2-mercaptoethanol, 100 U/ml penicillin G and 100 μg/ml streptomycin (final volume 200 μl). The mitogens used were concanavalin A (Con A) from ICN Biomedicals (High Wycombe, Bucks) used at doses indicated in the experiment, and lipopolysaccharide (LPS) from Difco Laboratories (West Molesey, Surrey) used at a final concentration of 20 μg/ml. After 48 hr at 37° in a humidified atmosphere of 5% CO_2 in air, the cultures were pulsed with 0·5 μCi ^{125}I-deoxyuridine (Amersham International, Amersham, Bucks) in 50 μl RPMI. Eighteen hours later, the cultures were harvested onto glass fibre discs using a Titertek cell harvester (Flow Laboratories) and incorporated radiolabel assessed by gamma counting.

IL-2 release assay

Spleen cells were cultured as above with 2 μg/ml Con A. After 24 hr 100 μl of supernatant were removed from each well, and IL-2 was measured by its ability to maintain the growth of CTL-L cells (Kendal Smith, Dartmouth Medical School, Dartmouth, New Hampshire) at 2×10^4 cells per well (assessed in a 24-hr proliferation assay).

NK assay

The NK-sensitive cell line YAC-1 was grown in RPMI-1640 with 10% (v/v) FCS and supplements as above. ^{51}Cr-labelled YAC-1 were plated out at 10^4 cells per well into flat-bottomed 96-well microtitre plates with fresh spleen cells (effector cell ratios of 12·5 : 1, 25 : 1, 50 : 1 and 100 : 1) in a final volume of 200 μl in RPMI plus 10% FCS and supplements as above. After 4 hr of incubation at 37°, 100 μl of supernatant were removed

from each well and counted on a gamma counter. The percentage specific lysis was calculated by:

$$\% \text{ specific lysis} = \frac{\text{c.p.m. experimental release} - \text{c.p.m. spontaneous release}}{\text{c.p.m. maximum release} - \text{spontaneous release}} \times 100.$$

RESULTS

Response to Con A

Responses of BB/E (diabetic and non-diabetic) and age-matched Wistar rat spleen cells are shown in Fig. 1. There was considerable variation in the responses of spleen cells from diabetic animals to Con A (Fig. 1b) but these responses were significantly lower than those found with cells from Wistar rats (Fig. 1a). However, this lowered response to Con A did not correlate with the diabetic status of the animals since spleen cells from the non-diabetic subline gave markedly poorer responses (Fig. 1c) than those from either Wistar or diabetic animals.

Ability of Con A-stimulated spleen cells to make IL-2

From Table 1 it can be seen that spleen cells from diabetic animals produce normal levels of IL-2 following stimulation with Con A. Both Wistar and diabetic rat spleen cells produced more IL-2 than those from the non-diabetic subline. It is interesting to note that one animal in the diabetic group appeared to be spontaneously releasing IL-2, which probably reflects the presence of activated T cells *in vivo*.

Response to LPS

The ability of rat spleen cells to respond to LPS was assessed and the data are presented in Table 1. Spleen cells from all these rat strains responded significantly but poorly to LPS, no difference being detected between those spleen cells derived from diabetic or normal age-matched control animals.

NK activity

Previous work has suggested that the numbers of NK cells may be elevated in diabetic rats (Marliss, Gross & Yale, 1985; Rabinovitch, Mackay & Boulton, 1985; Woda *et al.*, 1986. The NK activity in spleen cells from the BB/E (diabetic and non-diabetic) and Wistar rats was therefore measured using YAC-1 cells as targets. From Fig. 2 and Table 2 it can be seen that no significant difference in NK activity at any effector to target ratio was demonstrated using spleen cells from diabetic and non-diabetic BB/E and normal rats.

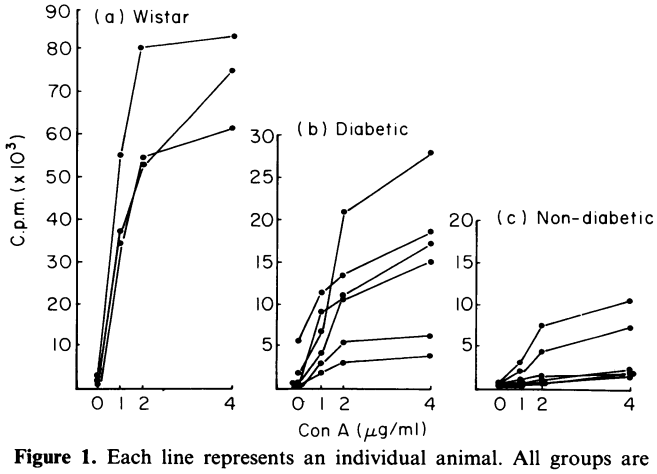

Figure 1. Each line represents an individual animal. All groups are significantly different from each other ($P < 0.01$).

Table 1. IL-2 release and LPS responses

Rat number	Response measured			
	IL-2 release*		LPS (20 μg/ml)†	
	−Con A	+Con A	−LPS	+LPS
Wistar				
1	368±103	10,377±760	2758±226	4840±349
2	1511±96	7826±138	1415±314	4180±168
3	240±48	14,652±1020	557±40	1354±164
Non-diabetic (BB/E)				
1	296±29	4422±89	791±213	1429±717
2	354±79	5051±269	418±19	1357±223
3	405±55	4514±1421	636±201	1451±517
4	640±157	2460±162	581±102	1742±618
5	190±18	4528±581	431±83	1252±383
6	227±46	2450±181	300±18	1080±204
Diabetic (BB/E)				
1	573±120	7266±320	455±44	1368±112
2	516±122	9269±3046	5889±42	7261±1130
3	686±89	7113±297	1876±356	7397±123
4	2067±158	5884±371	741±36	2086±505
5	13,578±662	14,812±251	527±29	1461±239
6	2188±961	6873±105	476±56	1453±34

Twenty-four hour supernatants were used at 50% in the assay.

* Twenty-four hour assay using the CTL-L line at 2×10^4/well. Wistar and diabetic were not significantly different, but both were different from the non-diabetic group ($P < 0.001$).

† Seventy-two hour assay with 2×10^5 spleen cells/well. Wistar and diabetic were not significantly different but both were different from the non-diabetic group ($P < 0.001$).

DISCUSSION

Studies on Con A responses in BB rats (Jackson *et al.*, 1983; Yale & Marliss, 1984) have produced conflicting data. The findings that responses to Con A were low in rats with insulitis but normal in rats without insulitis (Rossini *et al.*, 1979) could provide one explanation for the discrepancies. Bellgrau (1982) found normal responses in BB diabetic and non-diabetic animals to Con A, but allogeneic responses were not found in mixed lymphocyte cultures. Prud'Homme *et al.* (1984) attributed this defect in the response of BB spleen cells to Con A to suppressor macrophages. Our results suggest that BB/E diabetic rats can make a significant response to Con A and do not manifest any defect related to diabetic status.

In our studies, normal levels of IL-2 could be demonstrated in supernatants from diabetic spleen cells following Con A

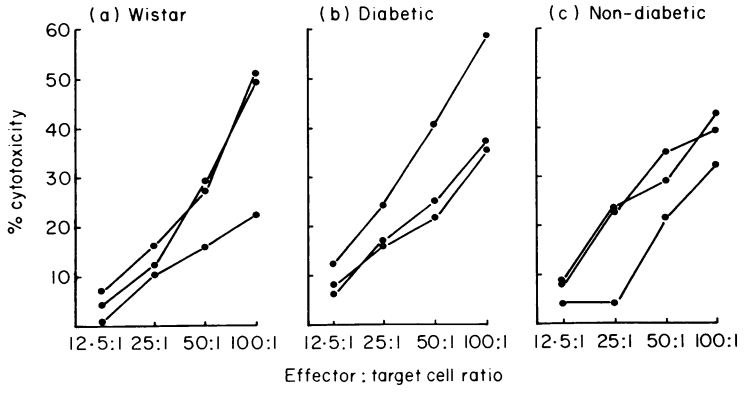

Figure 2. Each line represents an indivudal animal assayed at various effector: target ratios. There is no significant difference between any of the groups.

Table 2. NK assay

Rat number	% cytotoxicity
Wistar	
1	49·5±2·7
2	51·6±2·6
3	22·7±1·7
Non-diabetic	
1	32·2±3·3
2	39·5±0·9
3	42·5±5·2
Diabetic	
1	38·6±3·7
2	36·7±4·2
3	35·7±1·8
4	18·1±4·5
5	57·9±1·8
6	58·9±1·4
7	36·4±9·6
8	37·9±3·5

Four hour ^{51}Cr-release assay, with an effector: target ratio of 100 : 1.

Groups are not significantly different

stimulation. This confirms the data of Maclaren *et al.* (1983) and Elder & Maclaren (1983) but conflicts with those of Prud'-Homme *et al.* (1984) who found a defect in IL-2 release. While assaying for IL-2 release following Con A stimulation, spontaneous IL-2 release following simple culture of spleen cells was ascertained. One of the diabetic animals spontaneously produced large amounts of IL-2, suggesting the presence *in vivo* of preactivated T cells. Francfort *et al.* (1985) have shown activated T lymphocytes in pre-diabetic BB (diabetes-prone) rats, the presence of which peaks before onset of diabetes and declines with onset. These authors conclude that elevated Ia-positive T lymphocytes may be a marker for susceptibility to diabetes. Since all the diabetes-prone rats used were established diabetics, we may have missed the activated T-cell response in the majority of animals used in this study and only observed one animal with endogenously activated T cells.

The cell-surface marker studies of Marliss, Grose & Yale (1985) showed an increased population of cells (possibly NK cells) in diabetic animals, which was not present in non-diabetes-prone BB rats. Recently Woda *et al.* (1986) showed an increased number of $OX8^+$, $OX19^-$ (NK) cells in diabetes-prone and acute diabetic rats. The results from our study show comparable levels of NK activity in all three types of rats, although slight variations between individual spleens were observed. We conclude that NK cell activity does not appear to be deficient or elevated in the spleens of diabetic BB/E rats.

Our data suggest that the diabetic animals in the BB/E colony, unlike those in other BB rat colonies, do not manifest marked deficiencies in their ability to respond to T-cell or B-cell mitogens, to make IL-2 or to generate NK activity. Lymphopenia and depressed T-cell reponses do not therefore appear to be a prerequisite for the development of diabetes in the BB/E colony. Yale & Marliss (1984) have suggested that the immunological disorder is more severe in BB rats when compared to humans, since man does not have lymphopenia or functional T-cell defects. Thus, the BB/E colony may resemble human Type 1 diabetes mellitus more closely than other colonies previously described.

ACKNOWLEDGMENTS

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